PILOT FERTILIZATION OF THE NECHAKO RIVER II: NITROGEN-LIMITED PERIPHYTON PRODUCTION AND WATER QUALITY STUDIES DURING TREATMENT OF THE UPPER RIVER

NECHAKO FISHERIES CONSERVATION PROGRAM
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ABSTRACT

As a continuation of fertilization trials in the upper Nechako River for conservation of chinook salmon (*Oncorhynchus tshawytscha*), detailed monitoring of fertilization of the upper river was conducted coincidentally with an experiment designed to examine changes in periphyton biomass over a gradient of N additions at surplus P.

The fertilizer was a blend of 12-51-0 and 34-0-0. It was added continuously from six independently controlled dispensers located near the Swanson Creek confluence to increase inorganic N and P concentrations to 20 and 5 $\mu g \cdot L^{-1}$ respectively in the Nechako River mainstem. Chemical binders associated with the fertilizers also resulted in small additions of micronutrients.

Concentrations of nitrate (NO_3), ammonium (NH_4), and soluble reactive phosphorus (SRP) were mostly undetectable in weekly water samples collected at distances of 1 (T1), 5 (T5), 10 (T10), 20 (T20), and 50 km (T50) downstream of the fertilizer dispensers. A simple simulation model suggested that complete assimilation of the added N and P would be expected at all times within 5 km of the dispensers, and after the first month of treatment within the first kilometre.

The periphyton community downstream of the dispensers was dominated by diatoms and the biomass increased to a maximum of 133 mg chlorophyll $a \cdot m^{-2}$ from control levels of 15.6 mg chlorophyll $a \cdot m^{-2}$. The peak biomass (PB) was reached within a month at T1 and T5, but subsequently collapsed and then increased to a second peak of 68 mg chlorophyll $a \cdot m^{-2}$ before declining again at the end of the study period. Unlike these responses, areal biomass at T10 through T50 increased at a slower rate, not reaching PB until the end of the monitoring period in early July. The differences in areal biomass between sites was explained by the role of algal-bacteria relationships, downstream spiralling of added nutrients through mineralization processes, and the possibility of confounding nutrient inputs from allochthonous sources.

The increased levels of areal biomass had no impact on potential trihalomethane production at Fort Fraser or on dissolved oxygen concentrations in gravels at T1. There was also no evidence of accumulation of fertilizer-derived metals in periphyton tissue.

The N addition experiment indicated strong co-limitation of N and P, and by comparison with data from 1988 indicated that very small differences in P concentrations (less than detection limits in water), the presence of ammonium, and changes in current velocity may be important in bioassay procedures. Ammonium in particular may be a preferred N source for the periphyton since biomass responses were greater with ammonium present compared to those when only nitrate was present.

The response of areal biomass to changes in N concentration indicated that marginally lower responses could be achieved at half the concentrations used during the river fertilization. By comparison of the data with results from other experiments, it was also shown that the nutrient-limited biomass curve could be used as a generalized response curve for periphyton limited by a single resource. By combining resource-limitation curves from this and other experiments it was evident that 60-70% of PB in the Nechako River could be produced at N and P levels of 10 and 1 $\mu g \cdot L^{-1}$. The curves were also suitable for determining a biomass response relative to a maximum possible response at a gradient of N and P additions.

INTRODUCTION

As part of stage 1 of the Nechako Fisheries Conservation Program (NFCP) remedial measures, fertilization trials of the upper Nechako River in 1988 demonstrated an autotrophic response to nutrient addition (Perrin 1993). Additions of inorganic nitrogen (N) and phosphorus (P) to achieve *in situ* concentrations of 40 $\mu g \cdot L^{-1}$ and 10 $\mu g \cdot L^{-1}$ respectively, increased the areal biomass of periphyton by ten fold, reaching levels up to 200 $mg \cdot m^{-2}$ of chlorophyll *a* on artificial substrata. An accompanying periphyton bioassay indicated that production of algae was limited by N, but when N was added, the accumulation of areal biomass was limited by P. These data supported an hypothesis that autotrophic production in the Nechako River could be increased by controlled additions of N and P.

Testing the hypothesis that nutrient addition increases the growth rates of juvenile chinook (*Oncorhynchus tshawytscha*) (concept discussed by Perrin 1993) is a more complex process, requiring consideration of trophic interactions, efficiency of the assimilation of added nutrients, transport of added nutrients, and effects on downstream ecosystems and water users. There are inherent ecological and water quality interactions that must be understood before nutrient addition can be considered an acceptable technique for salmonid enhancement. Although nutrient addition has been shown to increase the mean size of juvenile coho (*Oncorhynchus kisutch*) and steelhead (*Oncorhynchus mykiss*) in coastal rivers (Johnston et al. 1990), the evidence of a similar response for juvenile chinook in the Nechako River is undefined. Also, the coincidental evidence that the production of fish-food-organisms increases as a function of increased production of benthic algae has not been established.

An objective of the NFCP is to examine the viability of fertilization as a remedial measure. The first trials (Perrin 1993) dealt specifically with the periphyton responses and indicated that N and P additions as low as 20 $\mu g \cdot L^{-1}$ and 5 $\mu g \cdot L^{-1}$, respectively could produce a desirable algal response for trophic enhancement. The next phase of the work, that started in 1989 was to examine changes in growth rates and abundance of chinook to habitat improvement with and without the effects of fertilization. This experiment involved continuous fertilization of the mainstem Nechako River over a minimum of 5 km during the early part of the growing season.

The work in 1989 also addressed questions related to the longitudinal extent of nutrient transport and changes in water quality in the mainstem, periphyton responses within and downstream of the experimental area, and minimum levels of N and P addition required for the periphyton responses. This report deals with results from these investigations. Specific objectives were to:

1. Measure changes in nutrient concentrations and areal biomass of periphyton downstream of the fertilizer addition;
2. Measure changes in dissolved oxygen concentrations and trihalomethane production associated with increased periphyton production;
3. Determine if metals contained in the fertilizers accumulated in periphyton biomass;
4. Determine if the N-deficiency found in 1988 was consistent in 1989; and
5. Describe changes in the areal biomass of periphyton over a gradient of N additions at surplus P.

METHODS

Study Site

The study area included 50 km of the Nechako River between the Swanson Creek confluence and Fort Fraser (Figure 1). This area included reaches numbered 2 through 4 in the NFCP reach designations. The fertilizer was added to the mainstem immediately downstream of the confluence with Swanson Creek, 29 km downstream of the Kenney Dam, in upper Reach 2. Access to the site was from the Big Valley Ranch located on the south shore, 27 km downstream of the Kenney Dam. The mainstem had a wetted width at the fertilization site of 84 m, current velocities ranged from 0.1 $m \cdot s^{-1}$ near the margins to 1.7 $m \cdot s^{-1}$ in the middle of the channel, and water depths were less than 0.8 m.

Five tributary streams discharged into the Nechako River within the study reach. All were small streams having wetted widths of 3-4 m. The Swanson Creek confluence was immediately upstream of the fertilization site and it drained ranch land from the south. It was an ephemeral stream that was flowing at about 1 $m^3 \cdot s^{-1}$ in early May, but was dry by late June. Targe Creek (km 30.5) drained a small forested basin to the north and was flowing at 0.5 $m^3 \cdot s^{-1}$ for the study

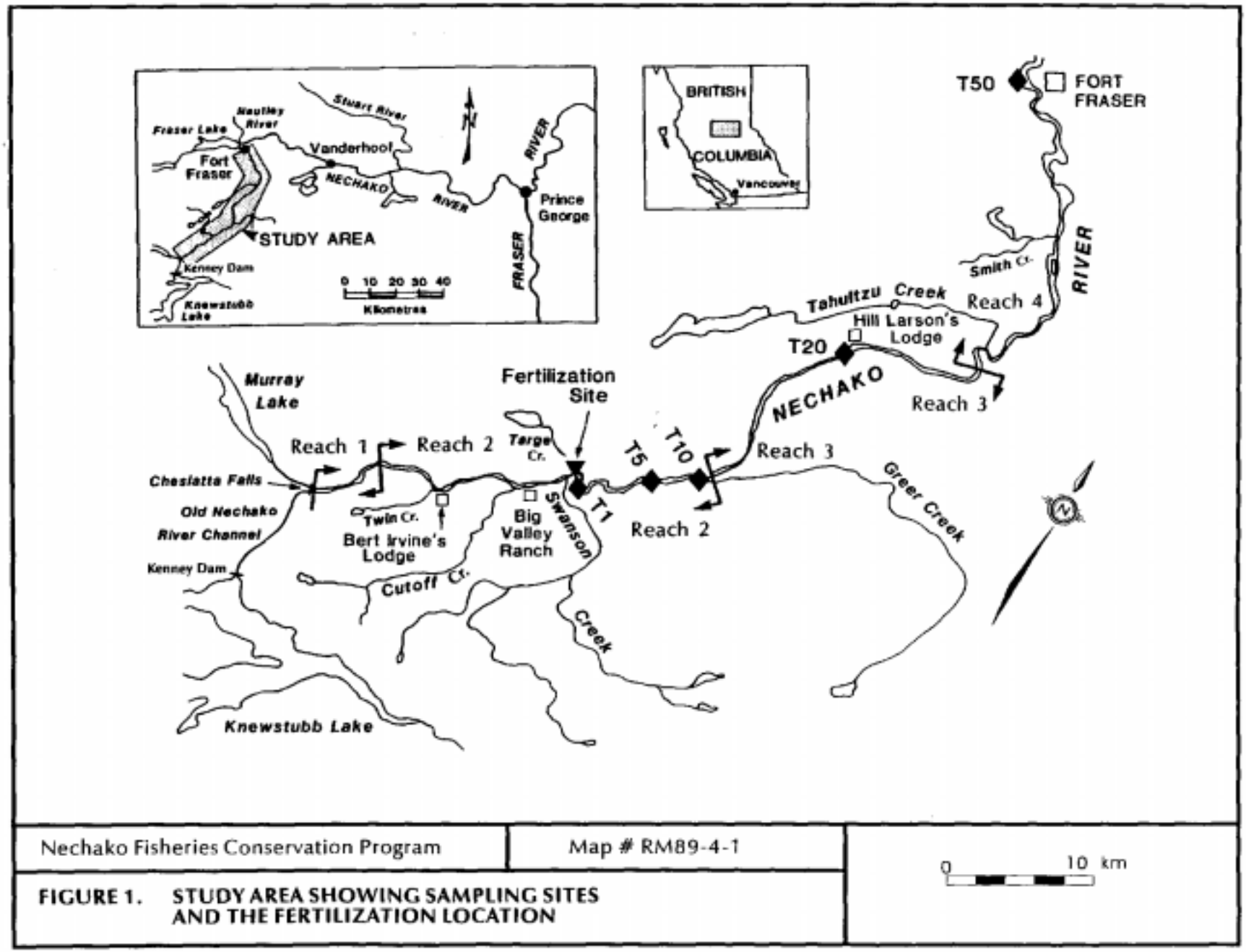


FIGURE 1. STUDY AREA SHOWING SAMPLING SITES AND THE FERTILIZATION LOCATION

duration. Greer Creek (km 43) was a larger stream having flows of about 3 $m^3 \cdot s^{-1}$ in May, but they declined to about 0.5 $m^3 \cdot s^{-1}$ by early July. The two additional streams, Tahultzu Creek (km 69.3) and Smith Creek (km 82.7) drained forested land north of the Nechako River, but were not surveyed during the study. All streams are known to support salmonids (P. Slaney, NFCP Technical Committee).

Periodic water sampling showed that contributions of inorganic N and P from Swanson Creek, Targe Creek, and Greer Creek were very low despite nitrogen fertilization of grazing lands close to Swanson Creek and Greer Creek (Appendix 1).

Salmonid habitat in the study area included pools, glides, riffles, and flats (Table 1).

Table 1
Proportions (%) of Salmonid Habitat Types in the Study Area
Data reproduced from Slaney et al. (1984) with permission.

Reach #	Pools	Glides	Riffles	Flats
2	44.2	30.6	25.3	0.0
3a	25.4	13.9	3.8	56.9
3b	11.9	44.7	34.6	8.8
4	8.8	59.8	24.3	7.0

The river substrata was primarily gravel (2 - 64 mm) and cobble (64 - 250 mm) although sand (<2 mm) was most abundant in reaches close to Fort Fraser.

Physical Data

Mean daily temperatures and instantaneous daily flows for the study area were obtained from data files of the Department of Fisheries and Oceans and Water Survey of Canada, respectively. The temperature recorder was located at Bert Irvine's Lodge on the south shore of the river (Figure 1). The instrumentation included a thermistor probe that was linked to the Water Survey of Canada data collection platform. Hourly measurements were routinely down-loaded to a computerized data base via satellite. Flows were measured at the Water Survey of Canada gauging station #08JA017 located below Cheslatta Falls.

Fertilizer Addition

A fertilizer blend of 34-0-0 and 12-51-0 (as N:P:K) was introduced to the river to achieve final concentrations of 20 $\mu g \cdot L^{-1}$ of dissolved inorganic N (NO_3+NO_2+NH_4-N) and 5 $\mu g \cdot L^{-1}$ of PO_4-P at full mixing. Fertilization started on May 4 and continued until July 10, 1989.

The fertilizer was dispensed from six Tessomat automatic feeders, two having a hopper capacity of 100 L and four having a 200 L capacity. They were placed across the wetted width to provide an even distribution of fertilizer. All feeders were equipped with spreaders that sprayed the fertilizer over a 5 m diameter. The feeders were suspended from welded aluminum A-frame supports that were anchored to the river bottom with reinforcing bar. 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 System 108 control unit (Modus Elektronic AB, Sweden) was programmed to activate the feeders for a specified duration every five minutes, 24 hours per day. Each feeder was activated from independent channels in the control unit, thus allowing the feeders to be operated in offset intervals. This timing prevented the dumping of fertilizer in large pulses.

Because dust in fertilizer acts like a desiccant and can cement to surfaces in the presence of water or humidity, exposed parts of the feeders required regular cleaning to maintain the desired output.

Sampling

Sampling Design

Several variables were measured to examine the effect of fertilizer addition to the river. Dissolved nutrient concentrations, the accrual of periphyton biomass, and the taxonomic composition of periphyton were each measured at a control site (C) upstream of the fertilizer dispensers and at distances of 1 (T1), 5 (T5), 10 (T10), 20 (T20), and 50 km (T50) downstream of the feeders (Figure 1). The town of Fort Fraser was located at the 50 km point. Dissolved oxygen concentrations in gravels and the metals content in the periphytic algae was measured at the control and at T1, one of the sites which had greatest accumulations of periphyton downstream of the feeders. The production of trihalomethanes was compared between the control and T50.

Measurements of the variables were replicated within the upstream control and downstream treatment sites. Since there were no replicate reaches, the design contained psuedoreplication (Stewart-Oaten et al. 1986, Hurlbert 1984). Although this design was statistically weak, the large autotrophic responses that are associated with nutrient addition (Bothwell 1989, Perrin 1993, Perrin et al. 1987, Peterson et al. 1983) are usually convincing without the use of statistics. Problems in drawing conclusions from psuedoreplicated designs are generally a non-issue with these data.

Water

Water samples were collected from all six sites on one date before fertilization started, approximately weekly after May 4, and once every two weeks after June 10. Final samples were collected on July 4. The samples were filtered in the field through 0.45 µm membrane filters and shipped to Cantest laboratories in Vancouver on the day of collection for analysis of NO_3^-+NO_2^--N, NH_4^+-N, soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP). The sum of the concentrations of NO_3^-+NO_2^--N and NH_4^+-N represents dissolved inorganic nitrogen (DIN). All analyses were performed according to procedures in APHA (1985). In this report, NO_3^-+NO_2^--N is simplified to NO_3. Total ammonia (NH_4^++NH_3) is shown as NH_4.

Dissolved oxygen (DO) concentrations were measured at the end of treatment on July 11, 1989 with a YSI model 57 oxygen meter, that was calibrated on the day of use. At six random sites in each of the control and T1 sites, both surface and intergravel (depth of 15 cm) DO was measured. Surface water was collected as grab samples in a 1000 mL glass Erlenmeyer flask. Intergravel water was extracted using a stainless steel tube that was inserted into the gravel to a depth of 15 cm. The water was drawn into an Erlenmeyer flask with a hand pump, taking care not to create turbulence.

The potential formation of trihalomethanes (THM) was determined on triplicate water samples collected upstream of the dispensers and near the water intake for Fort Fraser. Since there was no chlorine source at the control site and uncertain levels of chlorine in the Fort Fraser water supply, the samples were injected with chlorine to produce 0.2 $mg \cdot L^{-1}$ of residual chlorine (excess chlorine that was not used in the formation of THM) using chlorine demand procedures described in APHA (1985). The concentration of residual chlorine was determined using the colorimetric procedure in APHA (1985). To saturate the formation of the THM, the samples were allowed to stand at room temperature for 30 minutes after injection of the chlorine. The residual chlorine measurements were done after this incubation period. The concentrations of THM's were then determined by the purge and trap method (USEPA method 501.1, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio) where the sum of chloroform, bromoform, dichlorobromo-methane, and dibromochloro-methane was considered to represent total THM.

Dissolved metals scans were determined by Inductively Coupled Plasma Spectrographic Analysis (ICP, APHA 1985). Since the ammonium phosphate fertilizer may have high levels of cadmium (McIsaac, DFO, West Vancouver, B.C.; pers. comm.), Cd was singled out for graphite furnace analyses (APHA 1985) to obtain a detection limit that was lower than could be achieved using ICP.

Periphyton

Periphyton biomass was measured from samples collected on styrofoam-DB (Snowfoam Inc., El Monte, California) substrata (30 cm x 30 cm x 0.6 cm) that were installed onto submerged concrete patio blocks. Triplicate substrata were installed at mid-channel locations at each site on May 12. In weekly intervals up to July 8, 2 cm diameter cores of the styrofoam and the adhering biomass were removed, frozen at -15°C, and shipped on dry ice to Cantest Ltd. in Vancouver for analysis of chlorophyll *a* concentrations. The fluorometric analysis described in APHA (1985) was used for all samples after chlorophyll extraction in 90% acetone. Instrumentation was calibrated using fresh standards purchased from Sigma Chemicals Ltd.

On June 1 and July 4, an additional core was extracted from each substrata and preserved in Lugol's solution for later taxonomic analysis of the attached community. The relative proportion by volume of each algal taxa was determined from counts taken at 500x magnification along transects of subsamples that were allowed to settle in Utermohl chambers (Northcote et al. 1975). Communities between sites were qualitatively compared in terms of presence, absence, and relative proportion of sample volume occupied by dominant species (those occupying

greater than 10% of the sample volume).

On July 8, another core was taken from each substrata, packed on dry ice and shipped to Cantest Ltd. for analysis of metal content in the algal biomass. Significantly higher levels of metals in the algae in the fertilized areas would indicate the possibility of bioconcentration and adsorption effects if those same metals were also found in metals scans of the fertilizer. The periphyton samples were digested using a combination of pure trace metal grade nitric and hydrochloric acids. The resulting solutions were scanned for trace metals using ICP. Cadmium was determined by graphite furnace to obtain a lower detection limit. Results were expressed on an areal basis. Reagent blanks and blank styrofoam cores were digested and analysed concurrently.

Bioassay

The bioassay experiment involved measurements of changes in periphyton biomass through time as a function of seven levels of N at surplus P (5 $\mu g \cdot L^{-1}$ based on experiments from 1988, Perrin 1993). The experiment also tested periphyton responses to additions of surplus N alone, surplus P alone, and the combination of surplus N and P. Surplus N was assumed to be an addition of 100 $\mu g \cdot L^{-1}$ With a control of no nutrient additions, the experiment involved nine treatments:

Treatment ($\mu g \cdot L^{-1}$)

	A	B	C	D	E	F	G	H	I
N addition	0	0	50	5	10	20	35	50	100
P addition	0	5	0	5	5	5	5	5	5

Peak biomass, determined as the mean value on the day that highest chlorophyll *a* concentrations were measured, was always used as the index of periphyton response because it is strongly correlated with specific growth rate (Bothwell 1989) and is simple to determine from sampling at a remote field site.

With the exception of the dimensions of the flow-through chambers, the bioassay apparatus was identical to that used in the 1988 experiment (Perrin 1993). The chambers were fabricated from plexiglass and had inside dimensions of 102 mm x 27 mm x 1230 mm. The chambers were suspended under the water surface from a floating frame. The apparatus was anchored upstream of the fertilizer dispensers in non-turbulent laminar flow. Sodium fluorescein dye injections to the chambers showed that the current velocity across the substrata was 52.5 $cm \cdot s^{-1}$, about double the flows used in 1988. Water flow in each chamber was 0.8 $L \cdot s^{-1}$. A styrofoam substratum was secured to a removable tray in each chamber and provided a surface from which the attached periphyton could be sampled.

Nutrient solutions were introduced to the upstream end of each chamber by siphoning concentrated solutions of $NaNO_3$, and KH_2PO_4 from 2 L polyethylene Mariotte bottles through microbore tubing. $NaNO_3$ was used in preference to NH_4NO_3 used in the 1988 studies (Perrin 1993) because uptake of NO_3 by the periphyton may be inhibited in the presence of NH_4, thus introducing some uncertainty as to what the actual concentration of available DIN was in each treatment when both forms of N were used. The nutrient solutions were prepared from reagent grade chemicals and distilled water. Drip rates into the chambers were calibrated daily.

With the exception of water collection methods, samples were processed using similar procedures to those described for the *in situ* sampling. A suction tube was inserted into each chamber and water was drawn into a glass collection flask with a hand pump. Weekly collections were filtered in the field and shipped to Cantest for analysis of NO_3, NH_4, and SRP. Duplicate cores of the styrofoam with the attached biomass were removed once per week for four weeks and processed as described above for the analysis of chlorophyll *a* concentrations. The bioassay was started on June 10 and it ended on July 8.

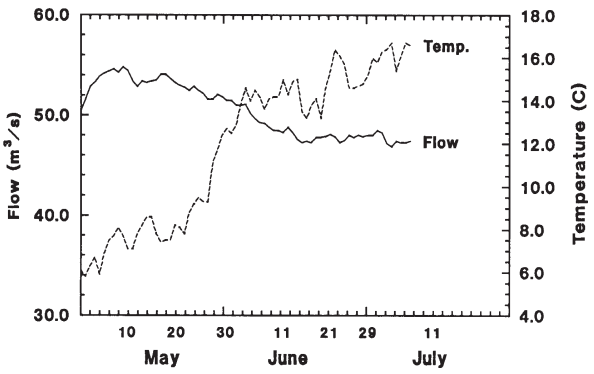
RESULTS

Physical

Mean daily temperature increased from 6°C at the start of fertilization to 16°C by July 10 (Figure 2). During the bioassay, the temperatures were between 14°C and 16°C.

Flow of the Nechako River generally declined from a peak flow of 54.8 $m^3 \cdot s^{-1}$ on May 10 to a minimum of 46.9 $m^3 \cdot s^{-1}$ on July 6 (Figure 2). Since discharge at the Skins Lake spillway was held constant during the study period, the changing flows were due to inflow

Figure 2
Instantaneous Daily Flow Downstream of Cheslatta Falls and Mean Daily Temperature of the Nechako River near Bert Irvine's Lodge



variation to Cheslatta and Murray Lakes.

Fertilizer Additions

A total of 20.96 tonnes of 34-0-0 (7.13 tonnes of N) and 8.4 tonnes of 12-51-0 (1.85 tonnes of P) were added to the river from May 4 through July 10. The 12-51-0 contained several micro-nutrients in concentrations shown in Table 2. Loads of micronutrients ranged from undetectable levels of Cd, Cu, and Mo, up to 73 kg of Fe, 63 kg of Al, 60.5 kg of Zn, and 57.1 kg of SiO_2. The 34-0-0 was a relatively pure product having only SiO_2 as part of the binder matrix.

By rating the daily input of N and P to river flows, the average calculated concentration of N and P was 23.5 $\mu g \cdot L^{-1}$ and 6.1 $\mu g \cdot L^{-1}$ respectively. The predicted mean daily concentration of N and P(Figure 3) varied according to periodic calibrations, and changes in river flows.

Measured Nutrient Concentrations

Concentrations of N and P in water samples were less than the calculated levels (Table 3). NO_3-N concentrations were above the detection limit only within the first two weeks of treatment, and in that time, the concentrations were usually less than calculated levels. With the exception of one sampling date (May 14), NO_3 was only detectable within 5 km downstream of the dispensers. NH_4 and SRP concentrations were generally less than 10 and 1 $\mu g \cdot L^{-1}$ respectively for the duration of the project at all sites. A few exceptions were only marginally greater than these detection limits. Before fertilization, TDP was undetectable, but thereafter, concentrations were between 2 and 32 $\mu g \cdot L^{-1}$ at the control site, with no apparent temporal trend. There were relatively high TDP concentrations at T50 from May 18 to June 1.

On two separate dates, NO_3, NH_4, and TDP concentrations were measured in short time intervals at T1 to determine the magnitude of any nutrient pulsing from the dispensers. On May 9, samples were collected every 15 minutes for one hour at three evenly spaced locations across the wetted width. Pulsing of NO_3-N and NH_4-N concentrations was undetectable and with the exception of one NH_4 value and one NO_3 value, all the inorganic N concentrations were below detection limits (Table 4). Some variation was found in the TDP, levels but this was similar to that found in the control samples and could not be attributed to nutrient pulsing from the fertilizer additions. To examine the possibility that the sampling interval was too long and that pulsing may have been missed, additional samples were collected every 5 minutes for half an hour on June 9 (Table 4). Again, pulses of inorganic N were undetectable, but variation in TDP was more evident. However, the variation did not exceed that found at the control site (Table 3).

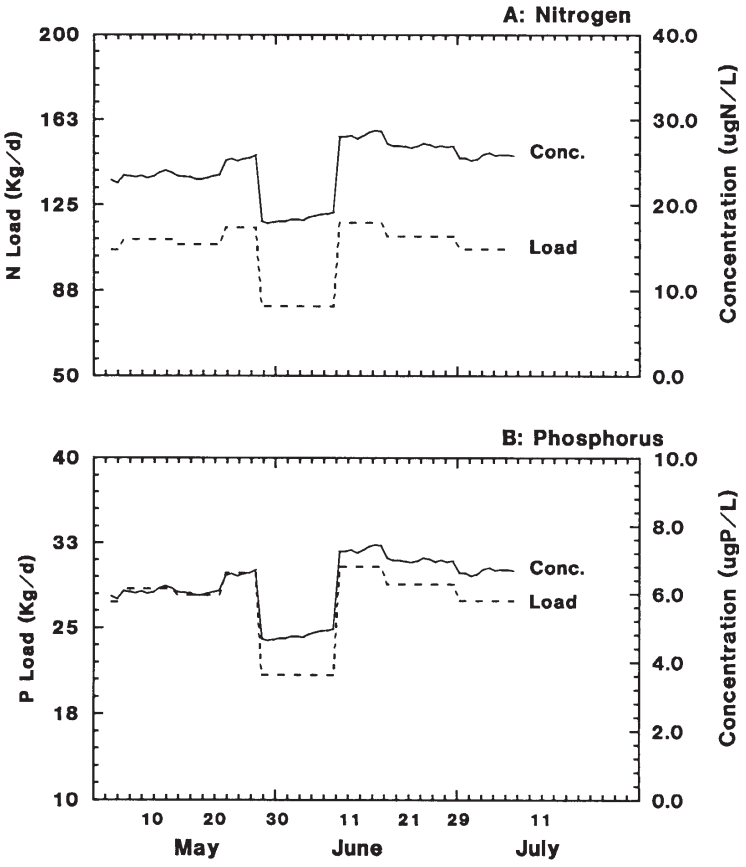
Table 2
Concentrations of Micronutrients in 34-0-0 and 12-51-0 and Total Loads of Micronutrients Added to the Nechako River During Fertilization, 1989

Element		12-51-0 Component (%)	12-51-0 Total Loading (kg)	34-0-0 Component (%)
Aluminum	Al	0.75	63.0	<0.01
Calcium	Ca	0.26	22.8	<0.005
Chromium	Cr	0.05	4.2	<0.005
Copper	Cu	<0.005		<0.005
Iron	Fe	0.87	73.1	<0.005
Magnesium	Mg	0.44	37.0	<0.005
Molybdenum	Mo	<0.005		<0.005
Vanadium	V	0.03	2.5	<0.005
Zinc	Zn	0.72	60.5	<0.005
Potassium	K_2O	0.11	9.2	<0.005
Cadmium	Cd	<0.0035		<0.0035
Boron	B	0.006	0.5	<0.001
Cobalt	Co	<0.002		<0.002
Lead	Pb	<0.008		<0.008
Manganese	Mn	0.05	4.2	<0.005
Sodium	Na	0.05	4.2	<0.01
Silica	SiO_2	0.68	57.1	0.04

Assuming that uptake by biota was mainly responsible for the removal of nutrients from solution, the rate of nutrient removal can be estimated by expanding nutrient uptake rates determined in related studies to the amount of periphyton biomass covering the wetted surface area. A comparison of this potential nutrient uptake rate to the input rates of N and P within specific reaches was used in Table 5 to estimate the potential of the lotic community to control nutrient transport.

In mid-May, the areal biomass had not yet increased greatly from the nutrient addition and calculations suggest that at T1 the substrata assimilation of P was slightly less than half the input rate. The differences between N uptake and input rates were even closer. At these differences, the concentrations of inorganic N and P would be close to detectable limits. At T5, the potential uptake rate was greater than the inputs by a substantial margin which would result in undetectable concentrations of SRP, NO_3, and NH_4. For the simulation in June, biomass levels were higher, but uptake rates were set lower because of the possibility that diffusion processes limited the availability of nutrients to all cells in a dense algal mat. With these changes, input rates remained less than uptake rates, again indicating that the periphyton community had the potential to sequester most, if not all the added N and P within the first kilometre downstream of the dispensers.

Figure 3
Total Daily Loading and Predicted Concentrations at Full Mixing of Nitrogen (A) and Phosphorus (B) from Fertilizer Additions



Periphyton

Taxonomy

The periphyton included diatoms, chlorophytes, and cyanophytes on both sampling dates (Table 6).

On June 1, the chlorophytes comprised less than 7% of the communities at all sites except at T1 where their relative abundance was 16%. At all sites, the chlorophytes were filamentous and included *Ulothrix sp.*, *Zygnema sp.*, *Oedogonium sp.*, and *Spirogyra sp.*. The cyanophyte, *Anabaena sp.* was found in very low abundance and only at T20. In the July samples, the relative abundance of chlorophytes declined to less than 5% at all sites and included *Cosmarium sp.* at the control and a combination of *Stigeoclonium sp.* and *Cosmarium sp.* at treatment sites. The chlorophytes were replaced by cyanophytes in July which were found in up to 15% of the community close to the nutrient source but also increased to 8% of the community in the control. They included *Oscillatoria sp.* and *Anabaena sp.*.

Diatoms comprised more than 80% of the communities, but the species composition changed between sampling dates. On June 1, *Synedra sp.* and *Fragilaria sp.* were most important at the control, but *Gomphonema herculeanum* dominated at T1 and T5. Further from the nutrient input, the relative abundance of *G. herculeanum* declined and *Synedra sp.* and *Fragilaria sp.* regained their importance. At T1 and T5, *Fragilaria sp.* and *Synedra sp.* tended to form long chains which in combination with the chlorophytes contributed to a filamentous appearance on the substrata. *G.herculeanum* formed a mucilaginous layer

Table 3
Nutrient Concentrations at Nechako River Mainstem Sites During Fertilization, 1989
"<" indicates concentrations were less than the detectable limit. Units are $\mu g \cdot L^{-1}$

	NO_3-N						NH_4-N					
Date	**C**	**T1**	**T5**	**T10**	**T20**	**T50**	**C**	**T1**	**T5**	**T10**	**T20**	**T50**
April 25	< 5	< 5	< 5	< 5	< 5	< 5	<10	<10	<10	<10	<10	<10
May 3	12	10	14	<10	<10	<10	<10	<10	<10	<10	<10	<10
May 14	< 5	17	25	10	13	< 5	<10	<10	<10	<10	<10	<10
May 18	< 5	10	< 5	< 5	< 5	< 5	<10	<10	<10	<10	<10	<10
May 24	< 5	< 5	< 5	< 5	< 5	< 5	17	14	<10	10	14	12
June 1	< 5	< 5	< 5	< 5	< 5	< 5	<10	<10	<10	<10	<10	<10
June 10	< 5	< 5	< 5	< 5	< 5	< 5	15	17	<10	<10	14	<10
June 21	< 5	< 5	< 5	< 5	< 5	< 5	<10	<10	<10	<10	<10	<10
July 4	< 5	< 5	< 5	< 5	< 5	< 5	<10	<10	<10	<10	<10	<10

	SRP						TDP					
Date	**C**	**T1**	**T5**	**T10**	**T20**	**T50**	**C**	**T1**	**T5**	**T10**	**T20**	**T50**
April 25	<1	<1	<1	<1	<1	<1	< 1	< 1	< 1	< 1	< 1	< 1
May 3	<1	<1	<1	<1	<1	<1	6	8	9	8	23	19
May 14	<1	<1	<1	<1	<1	<1	10	19	19	10	18	16
May 18	<1	<1	<1	<1	<1	<1	8	16	19	12	19	54
May 24	<1	<1	<1	<1	<1	<1	5	10	8	9	15	43
June 1	<1	<1	<1	<1	<1	<1	5	8	11	10	12	37
June 10	<1	2	3	2	1	1	32	12	12	12	13	2
June 21	<1	2	3	3	3	2	15	18	5	2	59	2
July 4	<1	<1	<1	<1	<1	<1	2	2	6	3	3	4

underlying the filamentous mat. On July 4, the *Synedra-Fragilaria* community was replaced by a community dominated by *Epithemia turgida* at all sites. *Cocconeis placentula*, *Rhopolodia sp.* and others occurred in fewer numbers and the communities at the control and treatment sites were very similar.

Biomass

The time-course changes in chlorophyll *a* concentrations differed according to location in the river (Figure 4). Upstream of the dispensers, chlorophyll *a* levels increased slowly, reaching a peak of 15.6 $mg \cdot m^{-2}$ on July 4. In contrast, the peak concentration at T1 was about 8 times greater than that at the control, and at T5, it was about 6 times greater (Figure 4, Table 7). At both T1 and T5, algal biomass increased rapidly, reaching these peak levels within the first two weeks after fertilization started. In the following four weeks, the biomass declined

Table 4
Nutrient Concentrations Measured at Station T1 Over Short Time Periods
All units are $\mu g \cdot L^{-1}$.

Series 1: May 9, 1989

Time	NO_3-N			NH_4-N			TDP		
	A[1]	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0	<10	<10	<10	28	<10	<10	11	13	9
15	<10	<10	<10	<10	<10	<10	13	17	7
30	<10	<10	21	<10	<10	<10	27	15	7
45	<10	<10	<10	<10	<10	<10	27	11	10
60	<10	<10	<10	<10	<10	<10	15	9	8

Series 2:June 9, 1989[2]

Time (min)	NO_3-N	NH_4-N	TDP
5	<5	12	10
10	<5	<10	10
15	<5	<10	27
20	<5	11	2
25	<5	13	13
30	<5	13	2

[1]A, B, and C are locations evenly spaced across the width profile at station T1. Site "A" was closest to the south shore.
[2]Series 2 samples were collected from mid-channel flow.

by more than 4.5 times at T1 and 3.3 times at T5 despite continued fertilization. Beginning in mid-June, the biomass increased again, reaching a second peak of about 69 $mg \cdot m^{-2}$ at T5 and 64 $mg \cdot m^{-2}$ at T1 before declining again at the end of the sampling period.

In contrast to responses at the upstream sites, the areal biomass at T10 through T50 increased logarithmically (Figure 4). Biomass was higher at T10 and T20 than at the control and T50 in the first few weeks of sampling, but it never reached the very high levels found at T1 and T5 at that time. Levels actually declined slightly at the time that peaks were being measured at the upstream sites. As the biomass declined at T1 and T5, exponential increases were measured downstream, which resulted in peak levels of biomass of 122.4 $mg \cdot m^{-2}$ at T50, 104.1 $mg \cdot m^{-2}$ at T20 and 70.9 $mg \cdot m^{-2}$ at T10 all occurring on July 4, the 52nd day after the substrata were installed.

Table 5
A Comparison of the Uptake Rate of N and P by Periphyton Algae in the Upper Nechako River Determined from Selected Literature, to the Known Input Rates of N and P from Fertilization

1. Mid-May	**T1 (km=1)**	**T5 (km=5)**
Substrata surface area (m^2)[1]	80,000.0	400,000.0
P V_{max} ($\mu gP \cdot \mu gChl\text{-}a^{-1} \cdot h^{-1}$)[2]	0.4	0.4
N V_{max} ($\mu gN \cdot \mu gChl\text{-}a^{-1} \cdot h^{-1}$)[3]	2.52	2.52
Areal biomass ($mgChl\text{-}a \cdot m^{-2}$)[4]	19.0	19.0
P Uptake rate ($kg \cdot h^{-1}$)	0.61	3.04
N Uptake rate ($kg \cdot h^{-1}$)	3.83	19.15
P input ($kg \cdot h^{-1}$)[5]	1.17	1.17
N input ($kg \cdot h^{-1}$)	4.58	4.58

2. June 9	**T1 (km=1)**	**T5 (km=5)**
Substrata surface area (m^2)	80,000.0	400,000.0
P V_{max} ($\mu gP \cdot \mu gChl\text{-}a^{-1} \cdot h^{-1}$)	0.1	0.1
N V_{max} ($\mu gN \cdot \mu gChl\text{-}a^{-1} \cdot h^{-1}$)	1.64	1.64
Areal biomass ($mgChl\text{-}a \cdot m^{-2}$)	114.0	114.0
P Uptake rate ($kg \cdot h^{-1}$)	0.91	4.56
N Uptake rate ($kg \cdot h^{-1}$)	14.96	74.78
P input ($kg \cdot h^{-1}$)	0.87	0.87
N input ($kg \cdot h^{-1}$)	3.38	3.38

[1]Approximate surface areas were determined from air photo mosaics.
[2]P uptake rates were those of a nutrient replete diatom-dominated community in the Thompson River (Bothwell 1985).
[3]N uptake rates were those of N replete marine diatoms (Price et al. 1985).
[4]Areal biomass was that measured at T1 on May 19 and June 7 for the first and second simulations respectively.
[5]N and P input rates were known from dispenser calibrations (Figure 3).

Trihalomethanes

Of the four trihalomethanes found in fresh water, chloroform and bromodichloro-methane were detected following the chlorination of water collected from both the control and Fort Fraser sites (Table 8). Total potential THM, determined as the sum of all forms, was less than 12 $\mu g \cdot L^{-1}$ at both sites. This level was 29 times lower than critical levels for drinking water criteria established by CCREM (1987). The test suggested that the increase in areal biomass of periphyton did not affect potential THM production.

Dissolved Oxygen

Dissolved oxygen concentrations in gravels at T1 were not significantly different from those in the control gravels (p<0.05) (Table 9). Surface water concentrations were close to 9.0 $mg \cdot L^{-1}$ and the lowest average concentration at a depth of 15 cm in the gravel was 5.97 $mg \cdot L^{-1}$.

Metals and Micronutrients in Periphyton Biomass

Seven metals that were detected in the fertilizer were also found in the periphyton, but with the exception of manganese, there was no evidence of higher concentrations downstream of the fertilizer dispensers compared to those upstream (Table 10). Iron and manganese undergo very similar transformations in surface water although the concentrations of iron are substantially higher (Stumm and Morgan 1970). Both are mostly in suspension occurring mainly as insoluble forms at circumneutral pH and at high dissolved oxygen concentrations. It is likely that the amounts introduced from the fertilizer were in these insoluble forms once introduced to the river, and they would have adsorbed or settled onto the periphyton plates. Both elements can, however, be tied up in organic complexes (Stumm and Morgan 1970) which were common in both the control and treatment reaches.

Table 6
The Relative Abundance of Periphyton Taxa Collected
in the Nechako River in Fertilized and Control Areas

A. CLASSES AND PHYLA

1. June 1, 1989

Location	% Diatom	% Chlorophytes	% Cyanophytes
Control	94	6	0
T1 (1 km)	84	16	0
T5 (5 km)	97	3	0
T10 (10 km)	97	3	0
T20 (20 km)	98	2	1
T50 (50 km)	100	0	0

2. July 4, 1989

Control	91	1	8
T1 (1 km)	84	5	11
T5 (5 km)	84	1	15
T10 (10 km)	95	0	5
T20 (20 km)	95	1	4
T50 (50 km)	94	4	2

B. DIATOMS

Location	June 1	July 4
Control	*Synedra sp.* (23%) *Fragilaria sp.* (19%) *Cymbella sp.* (14%)	*Epithemia turgida* (25%) *Rhopolodia sp.* (20%) *Cocconeis placentula* (15%)
T1	*Gomphonema herculeanum* (39%) *Synedra sp.* (18%) *Fragilaria sp.* (10%)	*Epithemia turgida* (23%) *Cocconeis placentula* (22%) *Rhopolodia sp.* (12%)
T5	*Gomphonema herculeanum* (30%) *Synedra sp.* (25%) *Fragilaria sp.* (20%)	*Epithemia turgida* (24%) *Cocconeis placentula* (21%) *Fragilaria spp.* (13%)
T10	*Synedra sp.* (35%) *Fragilaria sp.* (28%) *Gomphonema herculeanum* (15%)	*Epithemia turgida* (25%) *Cocconeis placentula* (24%) *Fragilaria sp.* (14%)
T20	*Synedra sp.* (36%) *Fragilaria sp.* (30%) *Hannaea arcus* (11%)	*Epithemia turgida* (28%) *Rhopolodia sp.* (22%) *Cocconeis placentula* (17%)
T50	*Synedra sp.* (37%) *Fragilaria sp.* (26%) *Cymbella sp.* (15%)	*Epithemia turgida* (42%) *Rhopolodia sp.* (22%) *Epithemia sorex* (13%)

Like iron, aluminum is a dominant element that is conserved in large concentrations from basic weathering processes and is always found in high concentrations in surface solution. Aluminum silicate is also an important component of the binders associated with the 12-51-0, fertilizer but it is being introduced to a milieu of aluminum complexes which existed in large amounts in ambient solution. The other metals would have occurred either bound in organic complexes which could settle onto substrata, or as less complex compounds that were adsorbed onto algal tissue, and subsequently were available for direct uptake by the periphytic algae and bacteria (e.g. Lemmons and Kennington 1983, Sigel 1986, Starodub et al. 1987, Stokes 1983, Whitton 1984, Wong et al. 1982). Physico-chemical conditions in the Nechako River and amounts of metals introduced from the fertilizer did not favour accumulation of metals in the periphyton community within the two month period between the start of fertilization and the time of sampling for metals content.

Figure 4
Time Course Changes in Chlorophyll *a* Concentrations on Styrofoam Substrata Within the First 5 km (Top Curve) and 10 through 50 km (Bottom Curve) Downstream of the Fertilizer Addition

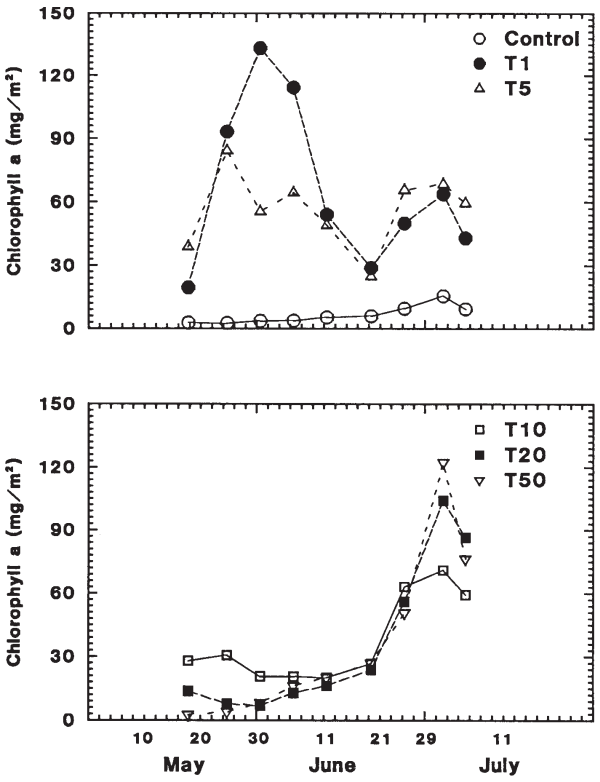


Table 7
Peak Chlorophyll *a* Concentrations on Artificial Substrata at Control and Treatment Sites

Location	Peak Biomass (mg chl *a* • m^{-2})	Date of Peak biomass
Control	15.6	July 4
T1	133.2	June 1
T5	84.2	May 26
T10	70.9	July 4
T20	104.1	July 4
T50	122.4	July 4

Table 8
Trihalomethane (THM) Concentrations (± SE) Determined as the Formation of THM's after Chlorine Injection to Water Samples from the Nechako River, 1989

Sampling	Trihalomethane (µg · L^{-1})				
Site	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	Total
Control	11.51	0.303	<0.5	<1.0	11.81
	(3.38)	(0.08)			
Fort Fraser	11.5	0.26	<0.5	<1.0	11.76
	(0.9)	(0.03)			
Water Quality Criteria (CCREM, 1987)					350.0

Table 9
Dissolved Oxygen Concentrations (mg · L^{-1}) in Surface and Subsurface Water at the Control and T1 Sites on July 11, 1989

Depth	Control	T1
Surface	9.1 ± 0.4	8.8 ± 0.5
15 cm	6.6 ± 0.5	6.0 ± 0.5

Bioassay

Nutrient Additions

Nutrient analyses of water samples indicated that the nutrient additions approximated the intended levels (Table 11), but the average values had coefficients of variation of greater than 50%. Large variation was also found in water sampling of the bioassay chambers used in 1988 and that was attributed to differences in the timing of sample collections and drips of the nutrient solution into the chambers. Some drip frequencies were as slow as one drip every few seconds and samples may have been collected at the time of a drip or between drips which would have introduced variation in the nutrient concentrations in water samples. Consequently, in 1989, nutrient levels in the water samples were considered unreliable as an indicator of the actual treatment levels. More useful evidence was the daily monitoring of flow rates through the chambers, flow calibrations of the microbore tubing and triple checking of calculations used in mixing the stock nutrient solutions. With these procedures, there was no doubt that the average nutrient concentrations inside the chambers were at the intended levels.

Taxonomy

The relative abundance of diatoms was greater than 80% for all treatments except where N additions were greater than 35 $\mu g \cdot L^{-1}$ (Table 12). At the higher N levels, diatoms were 74 to 76% of the community. Cyanophytes followed in importance ranging from 2% in treatment C to 23% in treatment I. The greater abundance of cyanophytes occurred at the highest N additions. Chlorophyte abundance was $\leq$3% of the communities at all treatment levels.

The diatom taxonomy (Table 12) was similar to that found on the mainstem substrata. At all nutrient levels, *Cocconeis placentula*, and *Fragilaria sp.* were common, but at higher N additions, *Achnanthes minutissima* became more abundant. *Epithemia sp.* and *Rhopolodia sp.* were found only at the lowest nutrient levels. At the highest P concentration and N levels greater than 35 $\mu g \cdot L^{-1}$, *Gomphonema sp.* was common.

Biomass Curves

The time-course curves of change in chlorophyll *a* concentrations were separated into two analyses. One was to determine if N or P or both (co-limitation) limited the accrual of periphyton. The second was to examine the response of peak biomass to a gradient of N additions at surplus P.

Separate N and P additions produced no significant difference in biomass at any point through time from changes in the control chamber (repeated-measures ANOVA; $p<0.05$) but the combination of N and P additions increased the areal biomass by up to seven times of that in the control (Figure 5). These changes in biomass were a clear indication of co-limitation by N and P. The addition of N alone drove the community into limitation by P and vice versa, the addition of P drove the community into N limitation. The areal biomass increased only with the addition of both nutrients.

Table 10
Concentration of Metals[1] ($mg \cdot m^{-2}$) in Periphyton at the Control and Treatment Sites on July 8, 1989
The elements listed are only the metals that were also detected in the ICP scan of fertilizer (Table 2).

Element	Control	T1	T5	T10	T20	T50
Al	1603.5	1134.5	2132.2ns	823.4	1997.1ns	1652.0ns
B	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
Cr	2.4		2.3		4.5	2.1
Fe	5579.0	3543.9	7093.6ns	1601.2	5625.7ns	2197.7
V	16.4	10.3	19.7ns	5.1	15.8	7.0
Zn	6.8	6.1	11.5ns	3.2	8.1	4.9
Mn	143.2	146.0ns	193.0ns	202.5ns	302.9*	301.2*

[1]Values were corrected for background levels determined from analyses of blank styrofoam discs.
[2]Where a treatment mean was greater than the control mean, a one way ANOVA was run to test the significance of the difference: ns infers no difference at $P<0.05$;
* infers a significant difference at $P<0.05$.

Table 11
Comparison of Mean N and P Concentrations Measured in Samples Collected From Inside the Bioassay Chambers with the Target Levels of N and P Addition

Chamber Label	A	B	C	D	E	F	G	H	I
N/P Added ($\mu g \cdot L^{-1}$)	0/0	0/5	50/0	5/5	10/5	20/5	35/5	50/5	100/5
Measured NO_3-N	<5	<5	41	4.7	7.7	17.3	28.3	53.7	96
Measured SRP	<1	4.3	<1	5.0	7.0	6.0	4.6	5.5	5.0

Table 12
Dominant Periphyton Algae in the Bioassay Chambers

Treatment	Diatoms (%)	Chlorophytes (%)	Cyanophytes (%)
A: N = 0, P = 0	94	2	4
B: N = 0, P = 5	91	2	7
C: N = 50, P = 0	96	2	2
D: N = 5, P = 5	83	2	15
E: N = 10, P = 5	90	2	8
F: N = 20, P = 5	90	2	8
G: N = 35, P = 5	84	2	14
H: N = 50, P = 5	76	2	22
I: N = 100, P = 5	74	3	23
J: Micronutrients	84	2	14

Treatment	Diatoms		Treatment	Diatoms	
A:	*Cocconeis placentula*	(27%)	F:	*Epithemia turgida*	(15%)
	Epithemia turgida	(15%)		*Fragilaria sp.*	(15%)
	Fragilaria sp.	(11%)		*Cocconeis placentula*	(14%)
				Synedra sp.	(14%)
B:	*Epithemia turgida*	(35%)	G:	*Gomphonema sp.*	(15%)
	Rhopolodia sp.	(15%)		*Fragilaria sp.*	(15%)
	Cocconeis placentula	(15%)		*Cocconeis placentula*	(15%)
C:	*Achnanthes minutissima*	(85%)	H:	*Achnanthes sp.*	(25%)
	Fragilaria sp.	(5%)		*Gomphonema sp.*	(25%)
				Cocconeis placentula	(15%)
				Fragilaria sp.	(15%)
D:	*Epithemia turgida*	(35%)	I:	*Achnanthes sp.*	(25%)
	Rhopolodia sp.	(17%)		*Gomphonema sp.*	(25%)
	Cocconeis placentula	(15%)		*Cocconeis placentula*	(15%)
				Fragilaria sp.	(13%)
E:	*Epithemia turgida*	(20%)	J:	*Cocconeis placentula*	(25%)
	Cocconeis placentula	(17%)		*Fragilaria sp.*	(18%)
	Fragilaria sp.	(17%)		*Gomphonema sp.*	(15%)

Figure 5
Time Course Changes in Chlorophyll *a* Concentrations with Additions of P Alone (5 µg · L^{-1}), N Alone (50 µg · L^{-1}), and N plus P in the Flow-Through Chambers

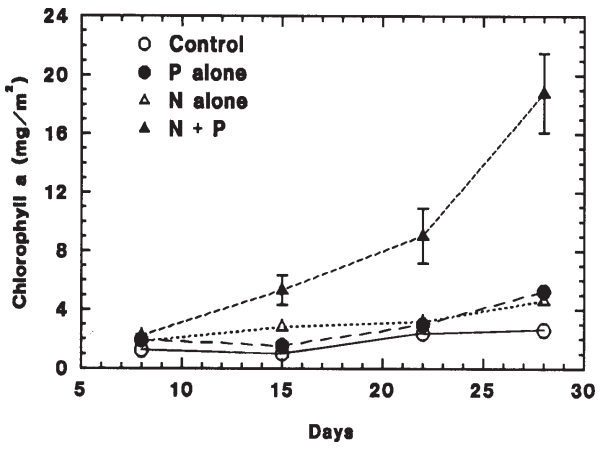
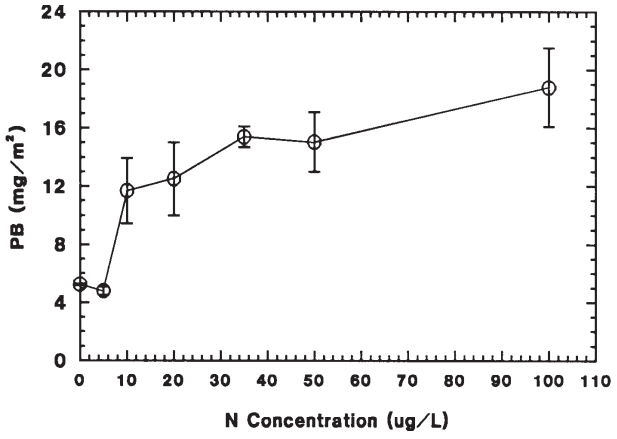


Figure 6
Peak Areal Biomass as a Function of N Additions at Surplus P (5 µg · L^{-1}) Measured in Flow-Through Chambers
The data are means ± SE.



The peak biomass (PB) resulting from the additions of surplus P (5 $\mu g \cdot L^{-1}$, determined by Perrin (1993)) and N ranging from 0 to 100 $\mu g \cdot L^{-1}$ over seven treatments provided data for the calculation of a curve showing PB as a function of N concentration (Figure 6). The curve showed a steep increase in PB up to about 10 $\mu g \cdot L^{-1}$ additions of N followed by continued, but smaller increases in areal biomass per unit N added up to the 100 $\mu g \cdot L^{-1}$ addition. The maximum response of 67% was achieved at an addition of only 10 $\mu g \cdot L^{-1}$. At an addition of 35 $\mu g \cdot L^{-1}$, 83% of the response was achieved. Clearly, large changes in areal biomass occurred at very small additions of N at surplus P.

DISCUSSION

This study has further developed a strategy for continuous low-level nutrient addition, provided an improved understanding of periphyton responses to nutrient addition over long distances downstream of an enrichment source, and improved the accuracy in selecting target concentrations for fertilization in the Nechako River. These findings will assist in establishing guidelines for operational fertilization, should the technique be accepted as a remedial measure.

Fertilizer Addition Strategy

The approach for nutrient addition in this project followed recommendations that a minimum of four point sources across a 75 m width of the Nechako River would be required to ensure that fertilizer would not become concentrated in certain areas of the channel (Perrin 1993). The high frequency activation of six dispensers operating on independent and offset schedules, did provide complete coverage of the wetted width of the river and prevented large pulses of nutrients from occurring downstream of the dispensers. This strategy eliminated any possibility of toxic effects particularly from ammonia pulses which were of greatest concern (Perrin 1993).

Although the use of granular fertilizer was manageable for this small scale treatment, this fertilizer would not be appropriate for larger applications (i.e. whole river) where costs must be minimized. Adjustments and cleaning of the equipment was required almost daily to maintain the fertilizer output, and on a large scale, the associated costs would likely be prohibitive. Operating costs could be reduced with alternate methods of fertilizer addition. Addition of liquid fertilizers by continuous injection from a reservoir tank, for example, would facilitate accurate control of nutrient discharge and maintenance would be reduced to periodic checks of valve settings and regular fertilizer deliveries by tanker truck directly to an injection site. Other methods including alternate hardware for dispensing conventional granular fertilizer may also be cost efficient and clearly should be included in a review of methods that would be required at the time that fertilization may be implemented on the river.

Temporal and Spatial Periphyton Responses

The changes in areal biomass of periphyton in the mainstem (Figure 4) may be separated into two general responses. The first involved an immediate increase within 5 km downstream of the dispensers that resulted in peak levels being reached within three weeks after nutrient addition started (T1 and T5 in Figure 4). The high level of biomass collapsed following a brief period at peak levels, but then increased again to a second peak that was smaller than the first. This oscillation in areal biomass appears to be typical of periphyton responses to point source enrichment in the short term, as the same was observed in the 1988 enrichment of the Nechako River (Perrin 1993), in Keogh River fertilization (Perrin et al. 1987), and during Salmon River fertilization (Perrin 1990). In each case, the decline in biomass occurred despite continuous fertilization and was preceded by an obvious senescence of the algal mat.

Causal mechanisms for these dynamics have yet to be investigated, but one hypothesis is related to interactions between the growth of benthic algae and the use of algal-derived dissolved organic carbon (DOC) by bacteria that are part of the periphytic assemblage. Kaplan and Bott (1989) cite observations of changes in bacterial densities in communities following pulses of organic carbon from algae, and Stock and Ward (1989) have observed a close association between algae and bacteria in the development of epilithic communities. Kaplan and Bott (1989) also reported that algal production peaks at lower temperatures than does bacterial production and that bacterial activity is linked to photosynthetic activity (via utilization of DOC) in stream riffles but it is more closely related to temperature variation in depositional areas which are influenced more by algal drift and settlement than by actual growth. In addition, they

found that DOC pulses from algae were uncommon in summer, suggesting that a DOC release from algae and bacterial activity was uncoupled at that time. Thus, at higher temperatures in summer, bacterial activity may be influenced more by temperature, not the availability of DOC produced by algae. If this hypothesis is true, one would expect there to be a close coupling between algae and bacteria at lower temperatures in spring in the Nechako River where bacterial activity may be determined by DOC released from algae. In summer, bacterial activity may be independent of algal production and may impact on algal biomass through decomposition processes regardless of high rates of autotrophic production.

Periphyton accrual at T10, T20, and T50 did not respond as rapidly as at upstream sites, but did produce equally high peak biomass (Figure 4). The small changes in areal biomass in May and early June indicated slower growth rates than at the upstream sites and were consistent with the finding that the added nutrients were assimilated immediately downstream of the fertilizer dispensers, thus maintaining nutrient-deficient growth and biomass accumulation at T10, T20 and T50 at that time. Where, this direct relationship between nutrient concentrations and areal biomass fails is in late June and early July when peak biomass levels equal to those at T1 and T5 were measured despite the apparent complete uptake of the added N and P near the dispensers. To achieve these high biomass levels, there was either an increase in availability of limiting nutrients, since dense algal mats cannot grow or be sustained at the background levels of inorganic N and P in the Nechako River (Perrin 1993), or settlement of biomass sloughed from upstream contributed to high biomass levels, giving a false indication of increased growth of algae at the downstream sites.

Active sloughing of biomass was apparent at all sites beginning in late June, but it was most noticeable at T1 and T5. Further downstream, drifting organic matter was mostly of detrital origin containing little live algal biomass. Settlement must have contributed some of the measured biomass at T10 through T50, but the lack of live sloughed material suggests that it could not have dominated the chlorophyll levels that were measured on the artificial substrata.

Given that the added N and P was assimilated within the first kilometre downstream of the fertilizer dispensers, there are two possibilities as to where added nutrient levels may have originated at downstream sites. One pathway is enrichment from allochthonous sources, including tributary streams and groundwater. Swanson Creek, Targe Creek and Greer Creek which enter the mainstem within 10 km of the dispensers were monitored for nutrient levels at the time of fertilization, but despite active fertilization of agricultural land in those drainages, nutrient levels in the streams were low (Appendix 1) and could not have produced a large algal bloom. Tributaries further downstream and any groundwater supplies were not sampled, so the possibility of any nutrient enrichment from those sources remains unresolved.

A second pathway was mineralization of N and P in algal exudates and the oxidation of organic matter. As algal biomass at the upstream sites collapsed from peak levels in June, the N and P bound into the organic complexes would be made available for uptake downstream as a function of the rate of heterotrophic activity. With high demand for inorganic N and P by periphyton in a nutrient-deficient system like the Nechako River, it is likely that oxidized forms of nutrients would occur only temporarily in solution, if at all, before uptake by the periphyton, and certainly would not be detectable by the routine analyses of grab samples. In this hypothesis, the added nutrients are taken up, released in organic complexes, oxidized, taken up again, and so on in a process known as “spiralling” (Elwood et al. 1983, Newbold et al. 1981, O’Neill et al. 1979a, Webster 1975, Webster and Patten 1979). The net effect of this process is that dissolved inorganic fractions of nutrients occur only as a transient phase that is generally undetectable, in favour of transport in fine particulate fractions. This concept is supported with results in Table 3 which show no evidence of increased dissolved nutrient concentrations at any site despite large increases in periphyton biomass (Table 4, Figure 4).

An uncertainty that remains in this hypothesis is the source of nutrients. Was it the added fertilizer or another unknown, perhaps allochthonous source that spiralled downstream and contributed to the high biomass levels? If fertilizer additions are the only nutrient source, it is not clear whether the fertilizer-derived nutrients could have “spiralled” the 10 to 50 km distances within about 18 days, the period between obvious biomass peaks at the upstream and downstream sites. A rough calculation can be made to determine what the rate of elemental movement downstream would have to be if indeed the fertilizer

did affect reaches down to T20 or T50. The response only at T20 can be considered as an example. The time from peak biomass at T5 to that at T20 was about 18 days (Figure 4). Assuming that most of the added N and P that was initially assimilated at T5 did not significantly affect T20 until about 10 days, prior to peak biomass at T20 (the approximate time required for biomass to increase from low levels to peak levels at exponential growth) the response period at T20 may actually be substantially less than 18 days. To be conservative, however, the 18 day period can be used. For the 15 km reach between T5 and T20, this response time indicates a drift velocity of about 800 $m \cdot d^{-1}$. This estimate means that nutrients from the fertilizer would have to spiral through all ecosystem compartments at an average linear rate of 800 $m \cdot d^{-1}$ if the responses at T20 were due to the fertilizer additions.

A more quantitative approach to determine drift velocity and other indices associated with the recycling of elements is to trace a ^{32}P spike through particle sizes and trophic compartments in its transport downstream, as was done at Walker Branch, Tennessee (Newbold et al. 1983). Walker Branch was a small, first order stream having a complex assemblage of large and small particulate organic matter. Rates of transfer of the P into and out of ecosystem compartments were determined by fitting the ^{32}P data to a partial differential equation model and a non-linear least-squared-error model (O'Neill et al. 1979b). The models were run to steady state to estimate the fluxes of exchangeable phosphorus and to quantify indices of P transport and recycling. The indices included "spiralling length", the distance travelled by a nutrient atom as it completes a cycle through all trophic compartments and "drift velocity", the rate of downstream movement of an atom. At steady state, Newbold et al. (1983) estimated that drift velocity of P in Walker Branch was 10.4 $m \cdot d^{-1}$ and spiralling length was 190 m.

Aside from this one estimate, there are no other data from which to determine if the present drift rate estimate for the Nechako River is reasonable. Since Walker Branch was only a first order stream having completely different particulate structure and likely having very different rates of element processing, the one estimate is difficult to compare to the Nechako River. Intuitively, a drift velocity of 800 $m \cdot d^{-1}$ seems unreasonably high for any site. Although a large river may be less efficient (i.e. longer spiralling length) in utilizing exchangeable nutrients, a two order of magnitude difference between the Walker Branch and Nechako River estimates seems unreasonable and does suggest that factors other than a simple difference in timing of biomass accumulations between sites must be considered in determining the rate of downstream movement of fertilizer-derived nutrients.

Because the source of nutrients responsible for the high biomass levels at T20 and T50 is uncertain, additional monitoring of periphyton biomass without fertilizer additions would be useful to assist in determining the potential importance of allochthonous nutrient sources. It is possible that periphyton accrual at the downstream sites may be similar in a non-treatment year compared to that in 1989 if processes unrelated to fertilization were important. Recent developments in tracing stable isotopes (Ehleringer and Rundel 1989) of nutrients may also be of use in describing the rate of downstream movement of added nutrients. These data would confirm the potential for nutrients from the added fertilizer to affect downstream sites within short periods of time.

Fertilization and Water Quality Criteria

Changes in water quality indices due to the fertilizer additions and increased algal biomass were within national and provincial water quality criteria guidelines (CCREM 1987, Nordin 1985), but the peak levels of algal biomass challenged those guidelines. Dissolved oxygen concentrations in gravels were not affected by the change in periphytic organic matter (Table 9). The higher levels of dissolved organic matter that were produced from increased production of algae did not increase the levels of trihalomethanes, even at chlorine-saturated conditions (Table 8). Also, with the exception of manganese, metals from the fertilizers (Table 2) did not accumulate in periphyton tissue (Table 10). The peak accumulation of algal biomass did exceed provincial standards for water quality (Nordin 1985). Where aesthetics are not a concern, a limit of 100 mg chlorophyll $a \cdot m^{-2}$ is recognized, but aesthetic criteria is set at a level of 50 mg chlorophyll $a \cdot m^{-2}$. The highest level was exceeded for 12 days at T1, 0 days at T5, 2 days at T20, and 4 days at T50 (Figure 4).

Although water quality criteria were established following an exhaustive review of the technical literature, the assignment of critical values remains an arbitrary process (Nordin, B.C. Ministry of Environ-

ment, Victoria; pers. comm.). The level of 100 mg $\cdot m^{-2}$ for chlorophyll *a* is set as a best estimate, but does not consider specific relationships between algal production and trophic dynamics, mainly because there is a general lack of these data that can be used to support water quality standards. For this reason, authors of water quality criteria for algae fully recognize that critical values can not be regarded as definitive and are potentially subject to change at a time when more comprehensive data describing change in trophic indices to algal biomass become available (Nordin; pers. comm.).

Although the existing water quality criteria were temporarily exceeded at some sites in this study, the results do not infer that fertilization reduced water quality for trophic productivity. Fertilization of the Keogh River on Vancouver Island increased algal biomass well in excess of 100 mg chlorophyll $a \cdot m^{-2}$ (Perrin et al. 1987), yet significantly increased the growth and areal biomass of salmonids (Johnston et al. 1990). The same result has been found from fertilization of the Salmon River on Vancouver Island (Perrin 1990). There are no reports that algal biomass in excess of 100 mg chlorophyll $a \cdot m^{-2}$ causes a negative impact on trophic activity, reduces fish abundance, or deteriorates ecosystem function in any way. The only known impact is reduced aesthetic quality due to the appearance of algal mats and increased difficulty in walking over cobble and gravel where periphyton biomass has increased. This change, however, in no way infers that ecosystem function has deteriorated.

The relationship between periphyton production (algae and bacteria) and insect abundance in streams is particularly important to understanding processes that control algal biomass at enriched conditions, and it is fundamental in contributing to water quality criteria for the Nechako River. Aquatic insects are particularly sensitive to change in water quality (e.g. Hart and Fuller 1974) and can provide information on real change in aquatic systems to increased autotrophic production. Although several experiments have been conducted in microcosms to examine relationships between invertebrate activity, grazing pressure, and algal abundance (e.g. Lamberti and Resh 1983, Hershey et al. 1988), mesocosm scale experiments (e.g. Serodes et al. 1984, Mundie et al. In prep.) are more appropriate for an analysis of interactions at the community level. Unfortunately, very few of these have been reported and there are no well established relationships for community-level interactions. Hence, a mesocosm-scale experiment to examine qualitative and quantitative insect responses to increased algae production would be a useful precursor to further fertilization of the Nechako River. In combination with the present algal bioassays, the results could provide precise information in setting treatment levels specific to the production of fish-food organisms and potentially contribute to water quality criteria based on real trophic responses to nutrient manipulation.

Nutrient Limitation and Fertilization Levels

Separate N, P, and N+P additions in the bioassay confirmed earlier evidence (Perrin 1993) that periphyton production in the Nechako River is co-limited by N and P (Figure 5). It also indicated that there were differences in the magnitude of responses between years. An increase in areal biomass from additions of N-alone in 1988 was not observed in 1989 and peak biomass for treatments including N additions were about four times greater in 1988 compared to those in 1989.

One explanation for the differences is that higher levels of P were available in solution in 1988 compared to 1989. Although analyses of SRP in bioassay water samples from 1988 (Perrin 1993) and 1989 (Table 3) indicated that SRP was undetectable in inflow water in both years, there may have been differences in the range less than 1 $\mu g \cdot L^{-1}$, which is undetectable to routine wet chemistry techniques, but can significantly alter cellular growth rates (Bothwell 1985). If there were slightly higher SRP concentrations in 1988, although undetectable, the addition of N would have produced a greater biomass response due to less P deficiency compared to that in 1989.

A second explanation is that NH_4 was a required form of N by the algal community in the Nechako River. Although NH_4 and NO_3 were added in 1988 as NH_4NO_3, the presence of NH_4 in an inorganic N solution can inhibit the uptake of NO_3 and prevent the formation of nitrate reductase (Syrett 1981), the enzyme required for the reduction of nitrate to ammonium before incorporation into organic structures. For this reason, NH_4 is usually taken up in preference to NO_3 if both are available in solution (Syrett 1981). This inhibition is not always complete, however, since both forms have been found to be taken up at equal rates at extreme N-deficiency (Caperon and

Ziemann 1976). Since N-deficiency was found in 1988, both N forms were likely to have been actively assimilated although the possible preference for NH_4 cannot be ruled out, as it may depend on the degree of N-deficiency. In contrast, only NO_3 was available for uptake in the 1989 experiment. This change should not have produced different rates of periphyton growth at similar levels of N-deficiency since maximum growth rates in lab cultures are much the same with either NO_3 or NH_4 as the N source (Syrett 1981). This basic premise suggests that biomass responses, which are based on growth rates (Bothwell 1985), should be comparable between years without confounding by N source.

General similarities between growth rates of cells grown on NO_3 and NH_4 in lab cultures may not be typical in a diatom dominated community in the Nechako River. Some algal taxa are known to grow poorly in NO_3 media (e.g. Antia et al. 1975). *Dunaliella tertiolecta*, for example, grows 10-30% faster on NH_4 than on NO_3 (Paasche 1971). In fact, the reason that many lab cultures do grow well on NO_3 is that they have been isolated from natural populations by selective growth in media containing NO_3 (Antia et al. 1975). This selection, of course, did not take place in the chambers and the natural Nechako River community would likely have included some taxa that did take up NO_3 and some taxa that did not. It is not unreasonable that the community may have had a net requirement for NH_4 under the ambient chemical conditions in order to produce the higher biomass levels that were measured in 1988. Background NH_4 levels, although undetectable, may have been sufficient to partly block nitrate uptake processes and nitrate reductase activity. If this were true, the known N-deficiency may not have been extreme enough to reduce the inhibitory effects of NH_4 on NO_3 uptake, and the uptake of NO_3 may have been limited despite its addition to the ambient solution.

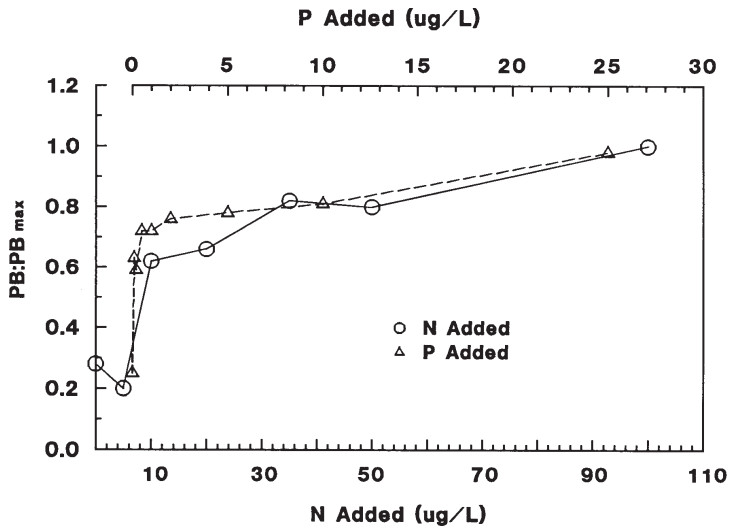
There is one other explanation for differences in responses between years, but it is less convincing than the nutrient supply hypotheses. Aside from the N sources, the only difference in conditions in the bioassay between years was that chamber current velocities in 1989 were almost double those of 1988. Within a range that does not physically remove algae by sloughing, higher current velocities tend to produce greater algal growth rates by reducing chemical diffusion gradients around cells and increasing nutrient uptake and cell respiration rates (Whitford and Schumacher 1964). Clearly, this process would act against the inter-year differences (less response at higher velocities), assuming that the 25 $cm \cdot s^{-1}$ increase in current velocity between 1988 and 1989 could not have reduced the biomass by the four fold difference through higher sloughing rates. Observations of periphyton biomass on the mainstem substrata (Figure 4) that were several fold greater than that in the chambers (Figures 5 and 6), yet in velocities that were 20% higher (average velocity at mainstem sampling sites) suggests that this assumption is reasonable and support the view that current velocity was not a factor in reducing areal biomass in 1989.

Regardless of causal mechanisms responsible for the lower biomass responses in 1989, a curvilinear response to a gradient of N additions at surplus P was apparent (Figure 6). The curve was logarithmic at lowest N concentrations but linear at the higher levels, thus suggesting that two different processes were active in controlling the biomass response. In a similar experiment conducted on the Thompson River in which phosphorus was the limiting nutrient, Bothwell (1989) showed an almost identical curvilinear response and quantitatively separated it into three phases. Phase I was the exponential portion where responses were explained by Monod-Type cellular growth kinetics. The amount of accrued biomass was controlled by the relative saturation of nutrient-limited cellular growth rates. Phase II was linear and could be explained by the kinetics of nutrient diffusion through the periphyton matrix. In Phase III, phosphorus was no longer limiting peak biomass and the periphyton mat was saturated with respect to phosphorus requirements.

The Nechako and Thompson River PB curves can be quantitatively compared by normalizing the data to a maximum PB measured at the highest concentration of the nutrient being tested. To conform to Bothwell's terminology, the normalized value is called relative peak biomass ($PB:PB_{max}$). It corrects for variation unrelated to the nutrient addition and can be a powerful index for comparing changes in areal biomass to a single growth-limiting resource.

Overlaying the two curves from each site shows identical responses for Phases I and II (Figure 7). Phase I of the Nechako River curve occurs at N concentrations $\leq 10\ \mu g \cdot L^{-1}$ and Phase II appears to occur from 10 to not less than 100 $\mu g \cdot L^{-1}$. At both sites, Phase I included a range of $PB:PB_{max}$ from about 0.2 up to 0.6-0.7 and Phase II included the remaining 30% of the PB response. Since the curves in Figure 7

Figure 7
Relative Peak Biomass as a Function of N Concentration at Surplus P (Nechako River) and as a Function of P Concentration at Surplus N (Thompson River)
The Thompson River data are reprinted from Bothwell et al. (1989) with permission.



show responses to levels of different limiting nutrients, their striking similarity suggests that a similar growth curve can be used to describe a diatom community response to additions of a single limiting resource, whether it be N, P, another nutrient, or any other resource. Since the curve is not affected by factors other than the supply of only one limiting nutrient, it can also be used to predict biomass responses to any level of addition of the limiting nutrient at any site where a diatom community exists, assuming that other factors do not limit the growth of the community. Its greatest value for this project is that it may also be used to determine a level of nutrient addition for a given level of peak biomass. It is clear from Figure 7 that 62% of a maximum possible response can be achieved at an N concentration of 10 $\mu g \cdot L^{-1}$ with surplus P. About 75% of the maximum response can be achieved at a P concentration of 1 $\mu g \cdot L^{-1}$ at surplus N. Additions of N greater than 10 $\mu g \cdot L^{-1}$ and additions of P greater than 1 $\mu g \cdot L^{-1}$ result in relatively smaller gains in biomass. To maintain these responses under co-limitation by N and P, the P would have to be added at a concentration greater than 1 $\mu g \cdot L^{-1}$ to maintain surplus P at the N concentration of 10 $\mu g \cdot L^{-1}$. Similarly, N would have to be added at a concentration greater than 10 $\mu g \cdot L^{-1}$ to maintain surplus N if P is added at 1 $\mu g \cdot L^{-1}$.

By examining biomass responses to a gradient of nutrient additions, specific levels of N and P can be selected, given a required peak biomass. These target concentrations can simply be read from the curves in Figures 6 or 7. The curves suggest that the greatest response per microgram of N and P added will be achieved with N and P additions near 10 $\mu g \cdot L^{-1}$ (61% of PB) and 1 $\mu g \cdot L^{-1}$ (75% of PB), respectively. Greater responses require more N or P added per unit of biomass produced and thus are less efficiently attained.

The actual target for fertilization, of course cannot be determined from the periphyton response data as it is not the periphyton response that is of interest for determining the enrichment required for increased growth of chinook. It is the relationship between nutrient loading rates and the potential increase in abundance of fish food organisms and ultimately the chinook that is required to select the level of nutrient addition. Since a factorial experiment designed to examine chinook responses is logistically impractical in the Nechako River (NFCP Technical Committee; pers. comm.), a study that quantifies relationships between fish food organism abundance, periphyton response and nutrient addition would be useful for a more accurate selection of N and P additions. The specific input of N and P can only be quantified from Figure 7 once a target level of periphyton has been determined through this interactive approach.

One consideration in selecting levels of treatment is that concentrations of the added nutrient will decline as the fertilizer travels downstream, due to uptake by periphyton. Unless it can be shown that exchangeable N and P is available to downstream sites through spiralling processes, there may be a need to fertilize at levels higher than those determined as the targets in order to ensure treatment of the entire target reach.

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APPENDIX 1
Chemical Characteristics of Tributary Streams to the Upper Nechako River, 1989

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Chemical Characteristics of Tributary Streams to the Upper Nechako River, 1989

At the time the Nechako River was being fertilized, ammonium nitrate (34-0-0) was also being applied to three areas of grazing land owned and managed by the Big Valley Ranch. A 48 ha block immediately adjacent to Swanson Creek and the Nechako River was fertilized at 80 kg $N \cdot ha^{-1}$ (34-0-0 loading at 235 $kg \cdot ha^{-1}$) for a total loading of 3,840 kg N. An 80 ha block about 1.5 km from Greer Creek was fertilized at the same rate, resulting in a total loading of 6,400 kg N. The third area of 32 ha was 2 km west of the Greer Creek site but not close to any tributary stream or the Nechako River and it was treated at 60 kg $N \cdot ha^{-1}$ (34-0-0 loading at 176 $kg \cdot ha^{-1}$) for a total loading of 1,920 kg N.

The three main tributaries to the upper Nechako River (Swanson Creek, Targe Creek, Greer Creek) were sampled for analysis of dissolved N and P before and during fertilization of the mainstem Nechako River and these ranch lands. The objective was to measure concentrations of inorganic N and P in drainage both from forested areas (Targe Creek) and ranch land areas affected by fertilizer addition (Swanson Creek) and a mixture of forested and ranch lands (Greer Creek). Increased nutrient concentrations in the tributaries after fertilizer additions to the grazing areas would indicate additional loading of N to the Nechako River. Inputs from the tributaries would have to be considered in calculating N loading rates for future fertilization trials in the Nechako River.

Because of the close proximity of field fertilization to Swanson Creek, water samples were collected using a declining frequency approach that is used to measure nutrient concentrations in streams after forest fertilization. Samples were collected once before the field fertilization, then daily for three days and on a declining frequency thereafter for 24 days. In Greer Creek and Targe Creek samples were collected approximately once every ten days. Higher frequency sampling was not required since the field fertilization was not in close proximity to the streams.

Sample collection and analytical procedures were identical to those described in section 2.0.

A very small change in NO_3 levels was found in Swanson Creek immediately after the field fertilization at that site (Table A-1). NH_4 and SRP levels were always undetectable, but for two days following field fertilization, NO_3 concentrations did increase up to 19 $\mu g \cdot L^{-1}$ from background levels <5 $\mu g \cdot L^{-1}$. After declining to pre-treatment levels another increase to the same level was measured three days later. On all other days NO_3 levels were <5 $\mu g \cdot L^{-1}$.

An increase in NO_3 concentrations is expected after field fertilization with NH_4NO_3 since most of the applied NH_4 would be rapidly hydrolysed to NO_3 in the soils and NH_4 is tightly retained at cation exchange sites (Tisdale et al. 1985). Conversely, NO_3 is highly mobile in soils and usually dominates inorganic N losses after fertilization of agricultural fields.

N and P levels in the other streams were also very low. Concentrations of the inorganic forms were generally less than detectable limits. TDP levels ranged from 19 to 50 $\mu g \cdot L^{-1}$ with most concentrations in the 30-40 $\mu g \cdot L^{-1}$ range. These levels were about double those in the Nechako mainstem and did present a possible enrichment source. Higher TDP levels in these streams was expected since agricultural soils generally have a high total phosphorus content. Streams draining forested lands (Targe Creek) have lower levels of total P, reflecting tighter retention in forest soils. The mainstem Nechako had lower levels of TDP than were found in the tributaries mainly because of dilution effects. Although the large dilution ratio of about 52:1 would have reduced TDP concentrations at full mixing to levels <1 $\mu g \cdot L^{-1}$, Swanson Creek was not fully mixed at the confluence. The discharge was likely retained along the south shore immediately downstream of the confluence potentially contributing to enrichment in that localized area. Indeed, in late April and early May, the areal biomass of periphyton along the south shore downstream of the confluence was visually greater than that at other locations across the width profile of the mainstem, thus indicating localized enrichment. As flows in Swanson Creek declined, however, the enrichment effect declined and by mid May, there was no visual effect of enrichment from Swanson Creek.

Table A-1

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Swanson Creek

Date	NO_3+NO_2-N ($\mu g \cdot L^{-1}$)	NH_4-N ($\mu g \cdot L^{-1}$)	SRP ($\mu g \cdot L^{-1}$)	TDP ($\mu g \cdot L^{-1}$)
April 25	< 5	<10		
April 28	19	<10		
April 29	18	<10		
April 30	< 5	<10	<1	38
May 2	< 5	<10	<1	46
May 3	19	<10	<1	46
May 14	< 5	<10	<1	39
May 18	< 5	<10	<1	35
May 24	< 5	<10	<1	30

Greer Creek

Date	NO_3+NO_2-N	NH_4-N	SRP	TDP
April 25	49	<10		
May 3	< 5	<10	<1	50
May 14	< 5	<10	<1	50
May 24	< 5	<10	<1	32

Targe Creek

Date	NO_3+NO_2-N	NH_4-N	SRP	TDP
May 3	40	<10	<1	19
May 14	< 5	<10	<1	27
May 24	< 5	<10	<1	26