Phosphorus limitation of lotic periphyton growth rates: An intersite comparison using continuous-flow troughs (Thompson River system, British Columbia)[1]

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Abstract

   Periphyton growth rates and relative degrees of phosphorus deficiency were compared with on-site, continuous-flow troughs in three parts of the Thompson River system. Soluble reactive phosphorus (SRP) in the lower Thompson, North Thompson, and South Thompson Rivers averaged 3.4, 1.1, and 0.7 μg liter^{-1}. Several physiological and chemical composition parameters ranked the degree of P deficiency in the rivers in the same sequence as did SRP. Among these were alkaline phosphatase activity, V_{max} for $^{32}PO_4^{3-}$ uptake, and cellular N:organic P, Chl *a*:ATP, C:ATP, and C:organic P. Specific growth rates (μ) estimated by biomass accrual and by $^{14}CO_2$ uptake usually, but not always, indicated higher μ with greater availability of P. However, relative specific growth rates (μ:μ_{max}) consistently reflected the influence of P limitation. As assessed from N:organic P and by application of the Droop and Goldman-Carpenter equations, μ:μ_{max} was 0.8–0.9 at the lower Thompson, 0.3–0.6 at the North Thompson, and 0.0–0.3 at the South Thompson sites. Hence, periphyton growth rates in the lower Thompson River were near the maximum set by temperature and light at ambient SRP of only 3–4 μg liter^{-1}. Evidence of P-limited growth rates in the South Thompson and North Thompson Rivers was found at temperatures approaching 0°C.

There have been many studies of the response of lotic periphyton communities to nutrient additions (Wuhrmann and Eichenberger 1975; Traaen 1978; Peterson et al. 1983; Horner et al. 1983), but our ability to extrapolate from these findings and to predict quantitative algal responses to increased loadings of river nutrients is limited. There are several reasons for this situation. The extreme spatial heterogeneity of rivers and the complexity and sometimes overriding importance of physical factors in controlling areal primary productivity has hindered attempts to treat in situ nutrient effects quantitatively. Progress in understanding the mechanisms of lotic eutrophication has been further impeded by the frequent failure to distinguish between the influence of biomass and of specific growth rates on primary productivity. This distinction is particularly critical for attached algae in flowing water where nutrient supply is continuous. Extensive laboratory research on P-limited growth kinetics of unicellular planktonic algae has clearly shown that steady state growth rates saturate at very low levels of ambient dissolved phosphorus (Fuhs 1969; Rhee 1973; Tilman and Kilham 1976; Brown and Button 1979). While the experimental stream work of Wuhrmann and Eichenberger (1975) seems to support this conclusion, many workers have found that much higher levels of phosphorus [>20 μg liter^{-1} soluble reactive phosphorus (SRP)] are required to produce algal bloom problems in streams or rivers (MacKenthun 1968; Wong and Clark 1976; Horner et al. 1983). Discrepancies may arise because of species differences, differing physical factors, the influence of algal mat thickness, and the dynamics of lotic P spiraling. I address here only one aspect of this complex problem. Are the in situ growth rates of native riverine periphyton saturated at ambient P concentrations as low as those reported for many cultured lentic algal species?

   This study was undertaken in response to a perceived eutrophication problem in the Thompson River, British Columbia. Algal biomass downstream of point-source nutrient loadings was found to be an order of magnitude greater than at upstream locations (Federal-Provincial Task Force unpubl. rep.). Although the cultural P loading
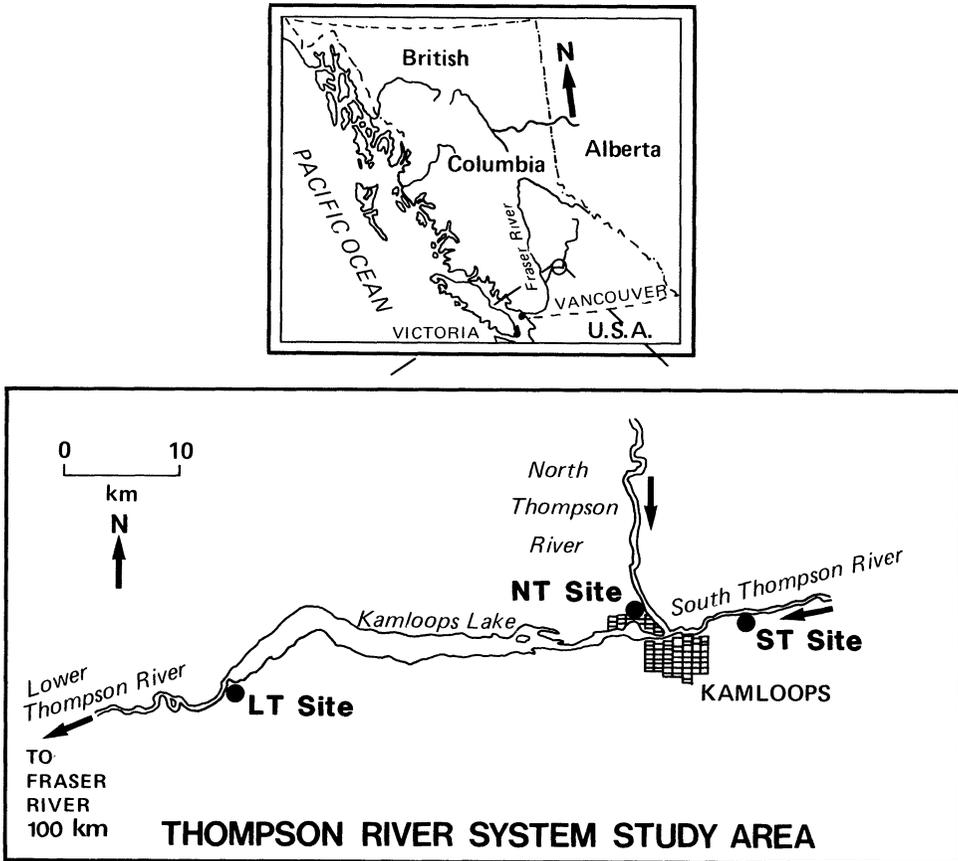
Fig. 1. Location of experimental trough facilities on the South Thompson (ST), North Thompson (NT), and lower Thompson (LT) Rivers.

was substantial in absolute terms, dilution in the river limited the elevation in downstream P concentration to levels (<5 μg liter^{-1} SRP) well below those normally associated with lotic enrichment problems. Attempts to interpret the levels of algal biomass in the Thompson system in terms of P limitation are complicated by systematic differences in the stability of substrata, flow, turbidity, and invertebrate grazer populations upstream and downstream. Under such circumstances, it is only possible to demonstrate a causal link between the elevation in downstream periphyton biomass and increased P loading when phosphorus acts directly to control growth rates; i.e. when algal P nutrition is better and cell division rates faster at downstream sites.

Because of the recognized importance of

physical factors such as current velocity in controlling nutrient availability to attached algal cells (Whitford and Schumacher 1961), the availability of phosphorus to periphyton communities in different river reaches could only be compared by using cells grown under the same physical conditions. This was accomplished by establishing experimental flowing troughs at downstream and upstream locations as part of a long term study of the growth and nutritional physiology of periphyton in this river system (Bothwell 1983; Bothwell and Jasper 1983). These troughs were used in experiments to determine the degree of phosphorus-controlled growth in periphyton algae during low-flow, winter conditions. In this first application of the concept of relative specific growth rates to natural freshwater periphyton com-

munities, direct estimates of cell division rates and physiological indicators of relative P deficiency were compared among the sites and used to determine $\mu{:}\mu_{max}$ at each location.

Field and technical support were provided by R. Mitchell, J. Vande Castle, K. Suzuki, and E. Marles. Algae were counted by M. Bolin. $^{32}PO_4{}^{3-}$-uptake experiments and computations of daily photosynthetic fixation rates were performed by S. Jasper. E. A. Laws, P. J. Harrison, and R. J. Daley provided criticisms on the manuscript.

Site description and background

The Thompson River, in south central British Columbia, is formed by the joining of the North Thompson and South Thompson Rivers with mean annual flows of 430 and 280 m³ s⁻¹ (Fig. 1). Both of these rivers drain mountainous and sparsely populated terrain; however, a few kilometers downstream of their confluence near the City of Kamloops, municipal and industrial nutrient outfalls occur. The river continues westward through Kamloops Lake and then flows another 109 km west-southwest to the Fraser River. The river reach between Kamloops Lake and the Fraser confluence is here called the lower Thompson River and is the section affected by the nutrient inputs originating 30 km upstream.

Two things distinguish the Thompson River situation from other instances of river eutrophication. First, because of the physiography and seasonal hydrograph of the Thompson River, the major impact of phosphorus loading on primary productivity occurs during the colder months when water temperatures range from near 0° to 4°C. Largely fed by snowpack and glacier melt, the Thompson exhibits lowest and most stable flow during winter. This condition is not only conducive to the accumulation of benthic algal biomass but also minimizes the dilution of continuous point-source inputs, with the result that higher phosphorus concentrations occur downstream. Moreover, while the North and South Thompson Rivers above the nutrient outfalls and Kamloops Lake often freeze over during winter, the lake's thermal inertia normally keeps the lower Thompson

River ice-free. Freshet extends from spring through midsummer, when high turbidity and intense turbulence severely restrict areal primary productivity.

Second, the biological impacts from the nutrient discharges are only first visible 30 km downstream at the outlet of the lake. This extraordinary circumstance is explained by the hydrodynamics and seasonal thermal structure of Kamloops Lake. The lake is narrow, deep, and riverine, with a mean residence time of only 60 d (St. John et al. 1976). During summer, phosphorus in the incoming river plume interflows and mixes deep within the lake. However, inverse thermal stratification in winter causes the cold, nutrient-laden river plume to traverse the lake surface rapidly with little dilution and nutrient loss (St. John et al. 1976). These conditions allow the major biological impact to occur in the outlet river, not in the lake.

Materials and methods

Experimental trough installations—Experimental flowing-trough facilities were located on the banks of the lower Thompson River (LT) below Kamloops Lake and, for comparison, on the North Thompson (NT) and South Thompson (ST) Rivers upstream of nutrient outfalls at Kamloops (Fig. 1). Details of the construction and operation of these facilities are given elsewhere (Bothwell 1983). In brief, each installation consisted of a pair of identical, parallel, 2-m-long Plexiglas troughs, one covered with glass and exposed to ambient light, the other enclosed in a light-tight cover. Water was continuously pumped to each trough site. Depth was about 1 cm and velocity about 50 cm s⁻¹. Sheets of styrofoam-DB served as a substratum for periphyton colonization. The unidirectional flow and rapid hydraulic flushing (4-s residence time in the troughs) assured that the troughs continuously reflected water quality in the rivers.

Periphyton accumulation studies—Three experiments on periphyton accumulation rate were done during the low flow, prefreshet conditions of late winter–early spring 1980 (exp. 1, February; exp. 2, March; exp. 3, April). A fourth trial was run in April 1981. Each experiment began with fresh sty-

rofoam sheets and the start of water flow. The time-course of algal biomass accrual was followed with Chl *a*. Quadruplicate cores of the styrofoam were removed with a corkborer from light and dark troughs at each site every 24 h for the first 4–5 d and thereafter at 2–3-d intervals. Samples were taken at about the same time each day (1000–1300 hours). Chlorophyll extracted from the cores with 90% acetone and a Polytron grinder was measured fluorometrically (Holm-Hansen et al. 1965). Experiments lasted 17–24 d and were ended before obvious sloughing began.

Samples for water chemistry taken each sampling day from the headtanks of the trough installations were filtered immediately through prewashed 0.45-μm Sartorius membrane filters. Subsamples for total dissolved phosphorus (TDP), soluble reactive silicate, NH_4^+-N and $NO_3^- + NO_2^-$-N were refrigerated until analysis at the Pacific and Yukon Regional Water Quality Branch Laboratory (North Vancouver) with procedures outlined in the Environment Canada (1979) manual. SRP samples were preserved with chloroform.

LiCor (Lincoln, Nebraska) integrating light meters (LI-550B) with quantum sensors (LI-190SB) recorded continuous light intensity at each experimental site during accumulation trials. River temperatures were measured manually each sampling day.

Periphyton physiology, chemistry, and taxonomy—At least once during each of the accumulation experiments, attached algal material growing in each of the light troughs was removed, suspended in river water, and used to determine the mean cell doubling times and relative degree of phosphorus availability to periphyton communities in the three rivers. After vigorous agitation to disrupt algal clumps, subsamples were withdrawn for determination of alkaline phosphatase activity (APA), $^{32}PO_4^{3-}$ uptake, $^{14}CO_2$ uptake, organic phosphorus (OP), particulate carbon and nitrogen (CHN), Chl *a*, adenosine triphosphate (ATP), and for algal cell counts. Most analyses were in triplicate or quadruplicate. Some of the analytical procedures were slightly modified during the study and some tests were not done in all trials (as noted below).

APA was determined with the fluorometric method of Perry (1972) as elaborated by Healey and Hendzel (1979). However, beginning with experiment 2, 100 μM of 3-O-methylfluorescein phosphate (MFP) was adopted as the routine test substrate concentration (10 μM MFP was used in experiment 1). APA values corrected for soluble activity were normalized to chlorophyll.

In experiments 3 and 4, orthophosphate (P_i) uptake rates as a function of added P_i were determined with carrier-free $^{32}PO_4^{3-}$. Natural levels of P_i (S_n) were augmented with a standard KH_2PO_4 solution to yield final concentrations of S_n plus 1, 2, 5, 10, and 20 μg P liter^{-1}. Incubations were carried out in room light within 1°–2°C of ambient river temperatures. At geometrically increasing time intervals from 1 to 30 min, bottles were swirled and 10-ml subsamples withdrawn and filtered through 0.45-μm Millipore filters. Filter-retained and total activities were counted in Aquasol-2. Checks for abiotic uptake were run on samples fixed with formaldehyde. The uptake rate constants (k) were estimated graphically from the slopes of semilog plots of the percentage of activity remaining in the filtrate vs. time. Initial uptake velocities (V), calculated as the product of k and the total substrate level ($S_n + A$), were plotted against $S_n + A$. For those curves that differed markedly from a normal Michaelis-Menten curve, uptake velocities were recalculated on the assumption of progressively lower values of S_n, as suggested by Rigler (1966); the highest value for S_n that still resulted in curves approximating Michaelis-Menten kinetics was used. V_{max} and the half-saturation constant, K_t, were obtained by plotting V against $V/S_n + A$ (Dowd and Riggs 1965). V_{max} was normalized for algal biomass with Chl *a*.

Periphyton suspensions for Chl *a* determinations were filtered on Whatman GF/F filters, homogenized in 90% acetone, centrifuged, and measured fluorometrically (Holm-Hansen et al. 1965). ATP on 0.45-μm Millipore filters was extracted with boiling 0.02 M Tris, pH 7.75 (Holm-Hansen and Booth 1966) and analyzed by the peak-height method (Karl 1978). Internal standards of ATP were run with all samples. Particulate organic carbon, nitrogen, and

phosphorus were determined in periphyton suspensions filtered through preignited Whatman GF/F filters. Carbon and nitrogen determinations were made with a Hewlett-Packard CHN analyzer (model 185B), and OP by UV digestion (Solórzano and Strickland 1968) with the apparatus manufactured by La Jolla Scientific Co. Particles were kept in suspension during the 2-h irradiation period by continuous bubbling with filtered, compressed air. The acid hydrolysis step was omitted because of the presence of mineralogic apatite-P in many of the samples.

Subsamples fixed with Lugol's solution were used for algal enumeration. The percentage composition by volume of phyla was estimated on 5–7 transects at 200× in Utermöhl chambers. A second, acid-cleaned subsample was used for diatom identification at 1,250×. At least 100 valves of the dominant species were counted and at least 300 valves in total. Beginning with experiment 3 in 1980, the slides for diatom counts were prepared in a reliable quantitative manner by the evaporation dish procedure of Battarbee (1973). The percent viability of each diatom species was estimated on a third subsample by the acid fuchsin procedure of Owen et al. (1979). Live cell numbers of each species were converted to cell volumes from the mean dimensions of 10 specimens and planimetric determination of the valve surface area of each taxon from the drawings of Patrick and Reimer (1966, 1975). Live cell volumes were converted to estimates of living carbon from cell volume: carbon relationships (Mullin et al. 1966).

Estimates of growth rate—Direct estimates of attached algal net growth rates were made from the accrual rates of Chl *a* during each experiment. Following the rationale and procedures of Bothwell and Jasper (1983), I used linear rates of Chl *a* accumulation observed in the dark troughs to correct for passive algal settlement at each site. Mean cell division times during each experiment were determined by iterative solution of the equation

$$y = s \sum_{j=0}^{t-1} (1 + k)^j$$

where *y* is the periphyton biomass (mg Chl *a* m^{-2}) after *t* days of growth, *s* is the daily rate of algal settlement (mg Chl *a* m^{-2} d^{-1}), and *k* is the daily fractional growth rate (div d^{-1}) of the adhering population.

The relationship between irradiance and photosynthesis of the periphyton communities was determined by the $^{14}CO_2$-uptake procedure during all experiments in 1980. Algal suspensions were incubated with $^{14}CO_2$ in 60-ml glass bottles at each of four light intensities (956, 428, 175, and 46 μEinst m^{-2} s^{-1}) in an incubator designed for measurements of phytoplankton primary production (Jasper et al. 1983). Incubations began at 1100–1300 hours and were normally carried out within 1°–2°C of ambient river temperature. At the end of 1–3 h, fixed ^{14}C was measured by the acid-bubbling technique of Schindler et al. (1972) as modified by Jasper et al. (1983). Replicate zero time blanks for each sample were acidified and bubbled immediately after ^{14}C addition. Samples and standards were counted as a gel with Aquasol-2. Total inorganic carbon was measured by gas chromatography (Stainton 1973). Mean daily rates of carbon fixation were computed from photosynthesis-light curves and continuous records of solar irradiation during the accumulation period (Jasper et al. 1983). These rates were converted to the mean number of cell div d^{-1} using estimates of living carbon based on determinations of ATP and, where available, data on live cell volume.

Results

Taxonomy—Periphyton communities in the troughs, as in the rivers, were always dominated by diatoms. Diatoms comprised 95–99% of the total, live-cell volume of algal samples used for physiological and chemical measurements. No direct counts of bacteria were made in any of these experiments. However, in recent trough work on the South Thompson River epifluorescent microscopic examination of periphyton communities (2–3 weeks old) showed that bacteria were <1% of the algal biomass on a carbon basis (R. Daley unpubl. data).

Although many of the same diatom species were often observed at each of the sites, the relative abundance and diversity differed. At the lower Thompson site (LT),
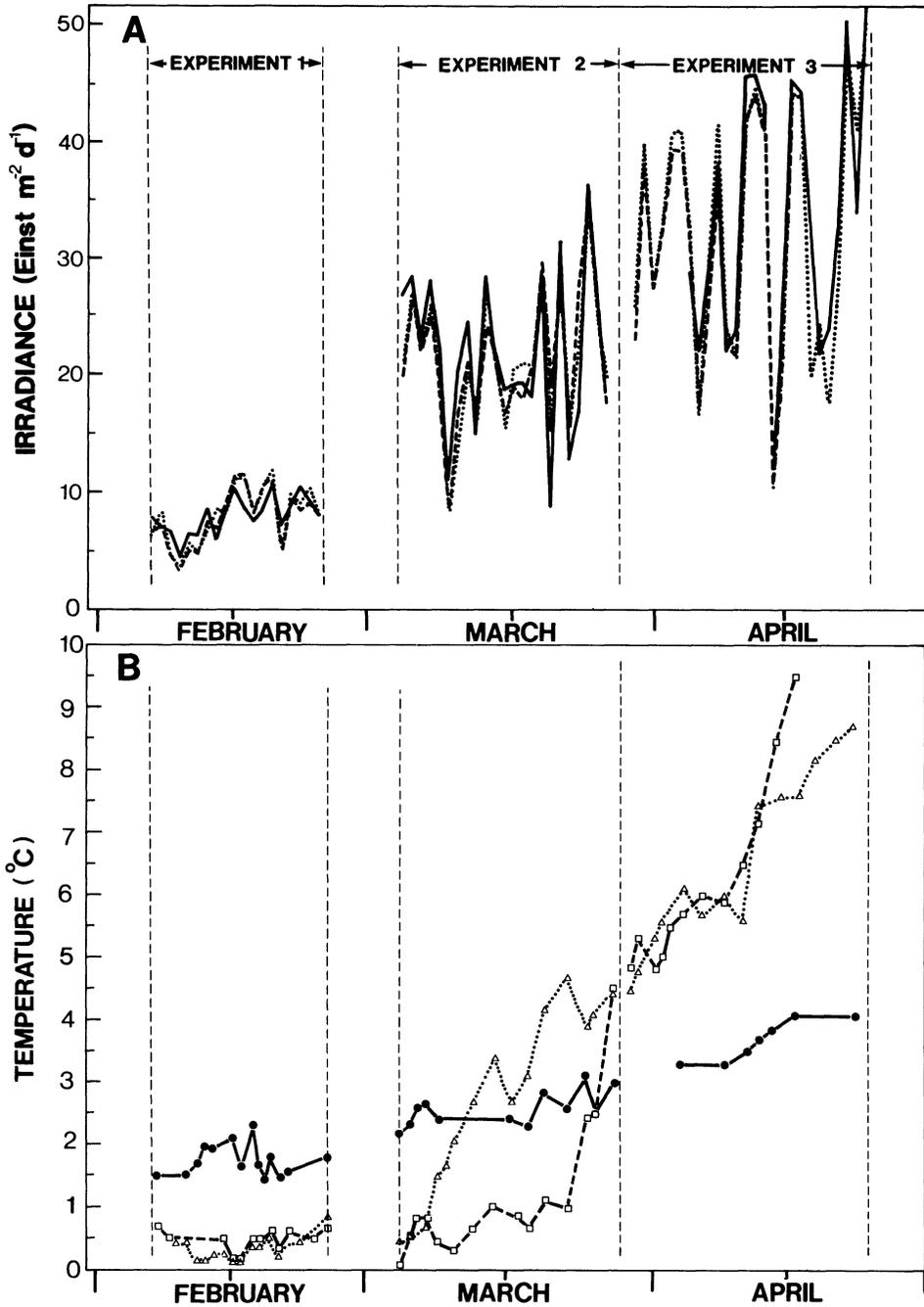
Fig. 2. Solar irradiance and water temperature during three periphyton accumulation experiments in 1980. Solid line—lower Thompson; dashed line—North Thompson; dotted line—South Thompson.

Fragilaria vaucheriae was normally the numerical dominant, although the larger celled species *Hannaea arcus* and *Synedra ulna* were occasionally major contributors to volume. These three species were also present in the North Thompson (NT) and the last two often dominated volumetrically. However, *Diatoma tenue* v. *elongatum* and

Table 1. Summary of nutrient chemistry of Thompson River system during four experiments in 1980–1981. Values are means (SE) of 40–60 samples.

	SRP	TDP	$NO_3^- + NO_2^-$-N	NH_4^+-N	SiO_2	N:P*
		(μg liter⁻¹)			(mg liter⁻¹)	(atom)
South Thompson	0.7(0.11)	2.7(0.27)	62.0(1.4)	11.0(1.2)	6.0(0.05)	231
North Thompson	1.1(0.20)	3.5(0.31)	116.0(1.0)	16.0(1.3)	6.5(0.06)	266
Lower Thompson	3.4(0.44)	6.9(0.47)	145.0(0.90)	8.7(1.3)	5.8(0.04)	100

* Nitrogen values are $NH_4^+ + NO_3^- + NO_2^-$. Phosphorus values are SRP.

Achnanthes minutissima were numerically most abundant at this site. Species diversity at the South Thompson (ST) site was highest and sometimes no species were clearly dominant. *Synedra delicatissima, D. tenue* v. *elongatum, Tabellaria fenestrata, Nitzschia palea, F. vaucheriae, Fragilaria crotonensis,* and *A. minutissima* all contributed ≥ 10% of the cell numbers or cell volume in experiments at ST.

Light and temperature—Progressive seasonal increases in both light and temperature were observed during the three experiments in 1980 (Fig. 2). There were large daily fluctuations in light associated with varying cloud cover, but mean irradiance levels of about 8 Einst m⁻² d⁻¹ in February quadrupled to about 35 at all the sites by April (Fig. 2A). There were no significant differences (*t*-test for paired comparisons, $P > 0.05$) in total irradiance between the three locations in any of the experiments.

However, water temperatures in the rivers upstream and downstream of Kamloops Lake were usually different. Midwinter temperatures in the two upstream rivers were lower (0.1°–0.8°C) than the outlet of the lake

(1.5°–2.4°C), but the upstream tributaries warmed more rapidly in spring so that by the end of April temperatures in the North and South Thompson exceeded those in the lower Thompson by 4° or 5°C (Fig. 2B). This winter-to-spring reversal of temperature regimes in the inflow and outflow rivers is characteristic of temperate lake-river systems and can complicate interpretation of in situ measurements of growth rate in relation to phosphorus availability.

Water chemistry—Small but consistent differences in dissolved phosphorus were found between all three rivers in all experiments (Table 1). Concentrations of TDP in the lower Thompson during the four experiments averaged 6.9 μg liter⁻¹ compared to 3.5 and 2.7 in the North and South Thompson Rivers. This relative ranking of the three rivers was also found for SRP. Mean upstream SRP values of 0.7 and 1.1 μg liter⁻¹ increased to 3.4 downstream. SRP concentrations of about 1 μg liter⁻¹ or lower in the two upstream tributaries place these rivers in the range of potentially P-limited surface waters. This conclusion is reinforced by the relatively high levels of inorganic nitrogen ($NH_4^+ + NO_3^- + NO_2^- \simeq 70–150$ μg N liter⁻¹) and silicate ($SiO_2 \simeq 6$ mg li-

Table 2. Alkaline phosphatase activity (nmol MF μg⁻¹ Chl *a* h⁻¹) in periphyton communities during experiments in 1980–1981. In parentheses—SE.

	River trough site		
Trial	ST	NT	LT
Exp 1 Feb 80	5.8(0.64)	1.4(0.09)	0.56(0.75)
Exp 2* Mar 80	29.0(1.3)	12.0(0.84)	5.3 (0.71)
Exp 3 Apr 80	29.0(1.2)	19.0(0.58)	4.2 (0.41)
Exp 4 Apr 81	130.0(5.7)	86.0(4.0)	16.0 (2.5)

* Mean of quadruplicate analyses on two separate sampling dates during this experiment.

Table 3. Phosphate uptake kinetics in periphyton communities during experiments in April 1980 and 1981. In parentheses—SE.

Trough site	K_t (μg P liter⁻¹)	V_{max} (μg P μg⁻¹ Chl *a* h⁻¹)
	Experiment 3—April 1980	
ST	0.8(0.3)	0.44(0.06)
NT	1.6(0.2)	0.25(0.01)
LT	2.3(0.5)	0.11(0.01)
	Experiment 4—April 1981	
ST	7.2(1.4)	1.10(0.14)
NT	1.2(0.3)	0.57(0.07)
LT	0.5(0.1)	0.15(0.01)

ter^{-1}) in the rivers. The mean atomic ratios of dissolved available N and P were 231–266 for the upstream tributaries and 100 for the lower Thompson River (Table 1). Such high values for supply N:P are known to result in potentially P-limited growth conditions (Goldman et al. 1979).

Alkaline phosphatase—High levels of APA are an indicator of phosphorus deficiency in algae (Healey 1975). APA Chl a^{-1} showed significant (ANOVA, $P < 0.05$) differences between the sites (Table 2) that were consistent with measurements of dissolved phosphorus in the three rivers. During 1980, APA at the LT site varied from near zero to around 5 nmol MF μg^{-1} Chl a h^{-1}, i.e. in the range indicative of negligible to slight phosphorus deficiency (Healey and Hendzel 1979). In contrast, APA at the ST site was 5–10 times higher and often in the range indicative of severe phosphorus deficiency (Healey and Hendzel 1979; Smith and Kalff 1982). Values at the NT site always fell between these two extremes. APA at all sites was higher in April 1981 but the relative differences persisted.

Phosphate uptake kinetics—The relative ranking of the three rivers in terms of P deficiency was also observed with P_i-uptake kinetics of the periphyton communities. P_i-uptake rates of unicellular algae are inversely related to their internal P content (Rhee 1973, 1974; Brown and Harris 1978). The maximum uptake rate, V_{max}, of P_i increases in response to P limitation (Fuhs et al. 1972; Gotham and Rhee 1981; Rivkin and Swift 1982). V_{max} in some species can increase by an order of magnitude during extreme P deficiency over that in P replete conditions (Perry 1976; Nalewajko and Lean 1978). In experiments 3 and 4, V_{max} (normalized to Chl a) was about 4–7 times greater at ST than at LT. Such a large difference again indicates more P deficient conditions upstream of Kamloops than at the downstream site (Table 3). As with APA, the V_{max} values for P_i uptake at NT were between these two extremes.

The half-saturation constants for P uptake, K_t, varied from 0.5 to 7.2 μg P liter^{-1} with most values in the lower end of this range (Table 3). These values are among the lowest ever reported for natural algal populations (*see* Nalewajko and Lean 1980; Cembella et al. 1984) and suggest periphyton adapted to low levels of P. There is some support in the literature for long term, genetically fixed adaptation in K_t of phytoplankton communities (Perry and Eppley 1981). However, K_t is not generally believed to be a kinetic parameter readily altered with short term, transient changes in the P status of cells. With some exceptions, where K_t has been noted to increase with V_{max} (Nalewajko and Lean 1978) or decrease with increasing V_{max} during P limitation (Brown et al. 1978), most workers have found no short term alteration in K_t with changes in P sufficiency (Rhee 1973; Perry 1976; Burmaster and Chisholm 1979; Rivkin and Swift 1982). Such observations are consistent with my failure to find a repeatable pattern among the sites in the two April experiments. The large standard errors associated with K_t resulted in failure to reject the null hypothesis ($P > 0.05$) for most of the intersite comparisons. More extensive ^{32}P-uptake work done later at ST and LT (data not shown) showed no significant correlation between K_t and V_{max} ($r = -0.141$, $n = 27$) with a mean K_t of 1.8 μg P liter^{-1}.

Periphyton chemical composition ratios—The atomic N:OP ratios of the periphyton communities supported the other parameters in the ranking of the three rivers in terms of P sufficiency (Table 4). Cellular N:P ratios are a well established indicator of phosphorus nutrition in algal cultures and reflect relative growth conditions (Perry 1976; Sakshaug and Holm-Hansen 1977; Rhee 1978; Goldman et al. 1979). Rhee (1974, 1978) has demonstrated the absence of dual nutrient limitation in algal cells. The optimum N:P, defined as the ratio at which one nutrient limitation switches to the other, is species-specific (Rhee and Gotham 1980). For eight algal species investigated by Rhee and Gotham (1980) the mean optimum N:P was 17, a value similar to atomic N:P ratios from cultured algae grown under nutrient replete conditions (Healey 1975; Goldman et al. 1979; Healey and Hendzel 1980). My values of N:OP at LT averaged 16, remarkably close to this optimum and to the Redfield ratio (Table 4). Since diatoms generally appear to have a slightly low-

er optimum N:P than green algae (Rhee and Gotham 1980) this ratio in a diatom-dominated system such as the Thompson River may still reflect some slight degree of P deficiency, although the N:OP ratios reported here do not include polyphosphate. In contrast, the mean N:OP values at NT and ST of 58 and 107 (Table 4) indicate moderate to extreme P deficiency.

Chl a:ATP ratios are also useful in identifying P as well as N limitation in algae. The ratio increases during P deficiency and decreases during N limitation (Perry 1976; Sakshaug and Holm-Hansen 1977). These changes seem to result from the greater sensitivity of ATP than of Chl a under P stress, with the opposite under N deficiency. In the present experiments Chl a:ATP ratios were lower at LT than at ST or NT (Table 4). These data also indicate greater P deficiency at the two upstream sites.

Another suite of cellular composition ratios that have proven useful in establishing relative P and N sufficiency are those in which carbon is normalized to other cell constituents, such as N, OP, ATP, and chlorophyll. Culture studies have shown that all four of these ratios increase with increasing P deficiency (i.e. decreasing growth rate) (Perry 1976; Sakshaug and Holm-Hansen 1977; Goldman 1979; Hunter and Laws 1981). Two of these ratios, C:OP and C:ATP, are more sensitive to P than to N limitation (Perry 1976; Goldman 1979; Hunter and Laws 1981). Computation of these carbon-based ratios in this study was complicated by problems in measuring live cell carbon. Particulate carbon values from CHN analysis of trough samples may overestimate live cell carbon by the inclusion of detritus. This problem, common to all natural environmental studies, is potentially exacerbated in some periphyton communities by the presence of autotrophic species with copious amounts of extracellular mucopolysaccharides in the form of capsules and attachment stalks. I also noted in later 1984 work that the CHN samples may have been unavoidably contaminated with very small flakes of the styrofoam (polystyrene) substratum. C:N ratios from CHN analyses at LT ranged from 12 to 16 and were in fact high for algal cells shown by other indicators to be nearly

Table 4. Trends in N:OP and Chl a:ATP ratios of periphyton from the Thompson River system during experiments in 1980–1981. Values shown are means; observed range in parentheses.

Ratio	Trough site		
	ST	NT	LT
N:OP	107	58	16
(atomic)	(94–120)	(45–82)	(13–21)
Chl a:ATP	7.3	6.6	3.1
(w/w)	(5.0–9.5)	(3.1–9.3)	(2.5–3.6)

P replete. Because of these uncertainties with chemically measured carbon, cell carbon was estimated indirectly from live cell volume measurements using cell carbon : volume relationships obtained from nutrient-sufficient cultures (Mullin et al. 1966). Although this procedure should be adequate for field samples thought to be nutrient replete (LT), it may well underestimate the carbon content of P-deficient cells since carbon : cell volume ratios can vary inversely with μ (Caperon and Meyer 1972). Hence, while the absolute values of cellular constituent ratios with carbon (determined by cell volume) in the numerator may be low for nutrient-deficient samples (NT and ST) such ratios are nonetheless valuable in the present comparative study because they provide a conservative estimate of the relative differences between the sites.

Ratios of C:ATP are correlated negatively with cellular growth rate under P-limited conditions (Perry 1976; Hunter and Laws 1981). This relationship is complex and nonlinear in some species (Hunter and Laws 1981). The mean C:ATP values for these three rivers were ST, 1,140; NT, 420; and LT, 235 (Table 5). These C:ATP ratios confirm the relative degrees of P deficiency found with estimates of dissolved phosphorus in the rivers as well as with the physiological and compositional indicators APA, $^{32}P_i\text{-}V_{max}$, N:OP, and Chl a:ATP. The value of 235 at LT is close to the factor of 250 frequently used to convert ATP to carbon biomass in nutrient replete cells (Holm-Hansen 1973). The values at NT and ST are underestimates if C:volume varies with growth rate. Data of Caperon and Meyer (1972) with N-limited phytoplankton cultures indicate that the potential magnitude

Table 5. Trends in chemical composition ratios of periphyton using carbon estimates from cell volume determinations. (Carbon computed from cell volume estimates using the relationship of Mullin et al. 1966. Since C:μm^3 may increase under P deficiency, ratios at ST and NT are probably underestimates. Explanation given in results.) Values shown are the means during experiments 3 and 4 in April 1980 and April 1981.

	River trough site		
Ratio	ST	NT	LT
C:ATP (w/w)	1,140	420	235
C:OP (atomic)	750	370	115
C:Chl *a* (w/w)	150	60	81
C:N (atomic)	7.9	5.4	7.7

of this error is twofold to threefold. The present range of C:ATP values (235 to 1,140) is, in fact, smaller than often reported for P-limited growth conditions in algal cultures and natural phytoplankton populations (Perry 1976; Cavari 1976; Sakshaug and Holm-Hansen 1977; Hunter and Laws 1981).

It is possible, for the sake of corroboration, to compute C:ATP ratios in a second way. If one assumes, for the moment, that the C:N ratio of 7.7 at LT (Table 5) is approximately correct for P replete periphyton and that the C:N ratio does not increase with increasing P deficiency, C:ATP ratios can be calculated from ATP and N (from CHN) data. To the extent that C:N does increase with P limitation, these estimates of C:ATP will place conservative limits on the spread of values between the sites. C:ATP ± C.V. computed in this manner was ST, 1,010 ± 24; NT, 529 ± 35; and LT, 232 ± 22. The agreement with C:ATP ratios computed from cell volume data (Table 5) is remarkable and suggests one of two possibilities. Either C:N and C:μm^3 cell volume did not change with growth rate under P deficiency or, more likely, they both changed in roughly the same manner.

C:OP ratios computed from cell volume: carbon data also ranked the three rivers in the same relative sequence of P deficiency (Table 5). Although the mean C:OP value at LT (115) may be slightly high for non-P-limited algae (Perry 1976; Sakshaug and Holm-Hansen 1977), the value at ST of 750 is certainly indicative of low P-limited growth rates. C:OP at NT was intermediate, 370.

The C:Chl *a* ratio is also a useful indicator of nutrient-limited algal growth. Many workers have demonstrated the close negative correlation between C:Chl *a* and cell division rates under both N- and P-limited conditions (Caperon and Meyer 1972; Laws and Bannister 1980; Goldman 1980; Hunter and Laws 1981). However, I did not see the same ranking of the rivers here (Table 5), although high values at ST indicate low relative growth rates. The apparent insensitivity of C:Chl *a* ratios may be due, in part, to the fact that increases in C:μm^3 cell volume with increasing nutrient deficiency were not accounted for. The mean C:Chl *a* value at LT (81) is higher than many ratios reported for nutrient-saturated algal cultures (Perry 1976; Goldman 1980). However, the C:Chl *a* vs. μ curve is influenced by the level of irradiance (Bannister and Laws 1980; Cullen 1982), and periphyton algae grown under high surface-light conditions might be expected to have greater C:Chl *a* ratios at high relative growth rates than laboratory-grown cultures.

The C:N ratios (C from cell volume; N from CHN) also did not show a trend across the three sites consistent with the previously discussed estimates of relative P sufficiency (Table 5). This indicator of nutrient sufficiency also suffers here from the lack of accurate estimates of cell carbon under nutrient-deficient conditions. Furthermore, some studies have shown that C:N responses can be substantially lower under P than under N limitation and may even be species-specific (Panikov and Pirt 1978; Goldman 1979).

Cellular growth rates—Direct estimates of specific growth rates (μ) determined by biomass accrual generally agreed with the relative assessments of P limitation in three of the four experiments. In these trials (exp. 1, 2, and 4), μ at LT was 2–4-fold that at ST, with values at NT either intermediate or aberrantly low (Table 6). In experiment 3 there was no significant difference between accrual estimates of μ at ST and LT, but $^{14}CO_2$-uptake rate estimates of μ did show differences. Furthermore, during this experiment (and exp. 4) water temperatures at ST and NT were about 3°–5°C warmer than at LT. Such a temperature differential would likely elevate the maximum growth

rate (μ_{max}) at ST and NT. Hence the relative specific growth rate ($\mu:\mu_{max}$) at LT was probably higher than at ST even though accrual estimates of μ were not. The reverse was probably also true of experiment 1, but the temperature differential between the sites was smaller (1°–2°C). Since growth rates of unicellular algae have a Q_{10} of about 1.9–2.2 (Eppley 1972; Goldman and Carpenter 1974), this thermal difference would translate into a shift in μ_{max} too small to counterbalance the observed 4-fold difference in μ between LT and ST. Hence, both μ and $\mu:\mu_{max}$ were higher at LT in February. The extremely low μ at NT in experiment 4 was likely due to attrition by sloughing.

Specific growth rates computed from mean daily photosynthetic rates usually, but not always, corroborated the differences in μ between the sites; best agreement was found at ST and LT. Slowest cell doubling times were always observed at ST. With the exception of the very high μ calculated for NT during experiment 2, the fastest doubling times were observed at LT. Specific growth rates at LT were 2–4-fold those at ST—the same relative magnitudes found with the biomass accrual technique.

Discussion

The strength of the overall conclusion regarding the relative degree of P deficiency among the three rivers examined here derives from the convergence of multiple lines of evidence. Chemical measurements of SRP and TDP in the water were corroborated by levels of APA and V_{max} for P_i uptake as well as cellular ratios of N:OP and Chl a:ATP. Estimates of C:ATP and C:OP also highlighted differences in P nutrition between the sites. Two other carbon-based ratios (C: Chl a and C:N) appeared to be more sensitive to errors associated with the lack of an accurate estimate of living carbon under P-limited conditions and consequently did not always rank the rivers in the same sequence of P deficiency as the other parameters.

Since the primary reason for comparing P deficiency in these experiments was to determine its influence on specific growth rate (μ), direct comparison of μ might seem more appropriate. The growth rates determined by biomass accrual and $^{14}CO_2$ uptake

Table 6. Comparison of the mean number of cell divisions d^{-1} (SE) estimated from biomass accrual rates and determinations of $^{14}CO_2$ photosynthesis. Data not available—NA.

Trough site	Biomass accrual* (div d^{-1})	^{14}C uptake† (mg C fixed mg^{-1} C d^{-1})
Experiment 1—February 1980		
ST	0.05 (0.02)	0.04(0.01)
NT	0.17 (0.02)	0.06(0.02)
LT	0.21 (0.02)	0.19(0.05)
Experiment 2—March 1980		
ST	0.10 (0.01)	0.06(0.02)
NT	0.17 (0.01)	0.32(0.06)
LT	0.22 (0.01)	0.20(0.06)
Experiment 3—April 1980		
ST	0.19 (0.02)	0.14(0.05)‡
NT	0.11 (0.01)	0.24(0.07)
LT	0.18 (0.02)	0.32(0.10)
Experiment 4—April 1981		
ST	0.13 (0.01)	NA
NT	0.006(0.002)	NA
LT	0.29 (0.03)	NA

* Details of calculation procedures given in methods section.
† Based on living carbon estimates from ATP and conversion factors in Table 5.
‡ In experiment 3 values are the means of computations based on carbon from ATP and carbon from cell volume data. Since C:μm^3 may increase under P deficiency, estimates of div d^{-1} at NT and ST may be high.

usually confirmed the ranking of the rivers revealed by the various physiological indicators of P deficiency. However, they did not always agree. In addition, there are fundamental uncertainties about the accuracy of both these measures of growth rate. Biomass accrual estimates of primary production have been the hallmark of periphyton research (Newcombe 1949; Cooke 1956; Sládeček and Sládečková 1964). As often practiced under uncontrolled in situ conditions with infrequent sampling, this method is of questionable value. Losses due to grazing and sloughing are usually not considered. As a result errors are produced that tend to systematically underestimate actual net primary production. By contrast, the accrual procedure used here included frequent sampling (24–48-h intervals), rigorous control of physical conditions, control of grazing pressure (for details *see* Bothwell 1983), use of a rough-textured substratum to reduce sloughing, and correction for passive settlement by use of dark controls. These refinements increase both the accuracy and reproducibility of the method (Bothwell 1983). Nevertheless, the possibility of low
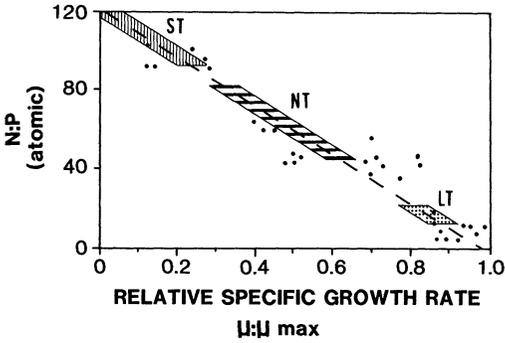
Fig. 3. Cellular N:P ratios vs. relative specific growth rate for P-limited *Pavlova lutheri*. Data from Goldman (1979). Line drawn by inspection. Hatched and stippled areas indicate range of ratios observed at ST, NT, and LT.

level attrition by physical scouring action is always present and cell division rates may still be underestimated, even during experiments of short duration. The use of dark controls to correct for passive settlement makes the possibility of overestimating net growth rate unlikely. However, as I reported earlier, passive settlement in the dark does not always fully correct for settlement occurring in the light on a 24-h basis (Bothwell and Jasper 1983). Consequently, the biomass accrual procedure, even corrected for settlement in the dark, may still in certain cases overestimate specific growth rates. Likewise, the $^{14}CO_2$-uptake procedure can either over- or underestimate specific growth rates of algae in different circumstances (*see* Peterson 1980; Carpenter and Lively 1980).

Even with accurate measures of μ, the use of these values alone in comparing algal communities in terms of nutrient limitation is clearly restricted. Since temperature, light, and species composition all influence the maximum growth rate, communities with similar μ, but grown under varying physical conditions, may have different degrees of nutrient-controlled growth. An example of this complexity was evident in experiment 3 where similar biomass accrual estimates of μ at ST and LT were likely caused by higher temperature at ST. One way of dealing with this problem is to normalize μ to μ_{max}.

Goldman (1980) has elaborated on the use of $\mu:\mu_{max}$ in estimating degrees of nu-

trient limitation in algae from the ratios of the cellular macronutrients C, N, and P. With phosphorus as the limiting nutrient, the most useful elemental ratios for assessing steady state cell-division rates are C:P and N:P (Fuhs et al. 1972; Goldman 1979; Goldman et al. 1979). Expressing μ as a fraction of μ_{max} helps reduce the influence of physical factors. For example, under different temperature and light conditions C:N correlates much better with $\mu:\mu_{max}$ than with μ alone in N-limited cultures (Donaghay et al. 1978). The inability to estimate μ with this approach is, for many purposes, a major drawback. However, it is actually advantageous in studies like the present one where the desire is to compare P-limited growth rates among algal communities under varying physical conditions.

To assess the value of this approach for periphyton, I compared the Thompson River N:OP ratios graphically with the data of Goldman (1979) for P-limited growth of *Pavlova lutheri* (Fig. 3). Algae at ST, NT, and LT had relative specific growth rates of about 0.0–0.3, 0.3–0.6, and 0.8–0.9. This analysis indicates that specific growth rates at LT were near the maximum set by temperature and light in all experiments.

The ranking of $\mu:\mu_{max}$ among the three river sites based on N:OP ratios is corroborated by the cellular OP μm^{-3} determined in experiment 3, where a complete set of cell volume and OP data was available. The Droop equation for internal nutrient control is

$$\mu = \bar{\mu}\left(1 - \frac{k_q}{Q}\right)$$

where μ is the specific growth rate (d^{-1}), $\bar{\mu}$ is the specific growth rate (d^{-1}) when the cell quota, Q, is infinite, and k_q is the subsistence cell quota. This equation can be used to compute $\mu:\mu_{max}$ because, for phosphorus, $\bar{\mu} \simeq \mu_{max}$ (Goldman and McCarthy 1978). The mean value of k_q for phosphorus in eucaryotic algae is $\simeq 0.4$ fg μm^{-3} (calculated from the literature compilation of Shuter 1978 with five aberrant values omitted). Using this k_q and the values for cellular OP μm^{-3} in experiment 3 (ST, 0.48; NT, 0.81; LT, 3.2 fg P μm^{-3}), I found $\mu:\mu_{max}$ at ST, NT,

and LT to be 0.17, 0.51, and 0.88 (Table 7). These relative specific growth rates are in the range indicated by the N:OP ratios. However, the usefulness of the Droop equation is known to decrease at temperatures other than the optimum for growth because both k_q and the maximum cell quota are temperature-dependent (Goldman 1979). Although the temperature optima of the species in the present experiments are unknown, they are likely higher than the observed river temperatures.

A third independent assessment of $\mu:\mu_{max}$ is possible with the observed μ values in experiment 3 and theoretical μ_{max} values calculated from the equation of Goldman and Carpenter (1974):

$$\mu_{max} = (1.8 \times 10^{10}) \times \exp(-6,842/T)$$

where T is the temperature (K°). For this purpose μ was the average of cell division rates calculated from ^{14}C-uptake rates based on carbon both from ATP and cell volume data (Table 6). These data from incubations at defined temperatures were used to avoid the complication of interpreting biomass accrual rates during experiments when temperatures were changing rapidly. Computed in this fashion, I found $\mu:\mu_{max}$ to be 0.27, 0.46, and 0.93 for ST, NT, and LT. These values agree surprisingly well with those calculated from the Droop equation (Table 7).

In spite of the acknowledged uncertainties in applying the Droop and the Goldman-Carpenter equations to mixed natural diatom populations, both approaches yielded results comparable with each other and with the analysis of elemental ratios. This three-way confirmation of $\mu:\mu_{max}$ ranking of the sites provides conclusive evidence that algal growth rates in these rivers are controlled by the availability of phosphorus. This study is the first successful application of the concept of $\mu:\mu_{max}$ to natural, freshwater algal communities and indicates the value of this approach in assessing and comparing nutrient limitation. Instead of simply determining the degree of nutrient limitation, $\mu:\mu_{max}$ gauges it in terms of specific growth rate under the existing physical conditions. Relative growth rate estimates coupled with ambient SRP data would allow a rough evaluation of potential growth rate

Table 7. Comparison of relative specific growth rates ($\mu:\mu_{max}$) at the three sites during experiment 3, 1980. Values computed using the Droop and Goldman-Carpenter equations.

	$\mu:\mu_{max}$	
	Droop*	Goldman-Carpenter†
South Thompson	0.17	0.27(9°C)
North Thompson	0.51	0.46(9°C)
Lower Thompson	0.88	0.93(4°C)

* $\mu = \mu_{max}[1 - (k_q/Q)]$.
† μ estimates from ^{14}C-uptake experiments; theoretical $\mu_{max} = (1.8 \times 10^{10}) \times \exp(-6,842/T)$.

responses to changes in P concentration. Such an approach may prove of value for environmental assessment.

Algal growth rates in the lower Thompson River (SRP $\simeq$ 3–4 μg liter^{-1}) were nearly saturated with respect to phosphorus. This finding confirms for natural river periphyton the long-standing observation from chemostat work that growth rates of unicellular algae saturate at very low ambient phosphorus concentrations (Fuhs 1969; Rhee 1973; Brown et al. 1978). Because SRP at ST and NT was 2–3 μg liter^{-1} lower than at LT, it was not surprising to find convincing evidence of greater P limitation at these two sites. However, differences in SRP at ST and NT were normally <1 μg liter^{-1} and on occasion were analytically indistinguishable. Nevertheless, certain physiological differences in the periphyton between ST and NT were discernible. The ability of algae to exhibit a wide range of growth rates under steady state, P-limited conditions with very small (analytically undetectable) changes in SRP is well known. The experiments reported here provide a clear example of this effect in nature. An important implication of this finding for lotic systems is the demonstration that even periphyton in rivers whose P levels apparently differ insignificantly may still have greatly different relative specific growth rates. In the present instance, $\mu:\mu_{max}$ in the NT was about double that in the ST. This conclusion re-emphasizes the need for direct measurements of cellular chemistry, physiological indicators, and growth rates in making judgments about the nutritional status of periphyton in rivers.

Notwithstanding the above argument, it is significant that in all experiments agree-

ment was good between the mean dissolved phosphorus concentration of grab samples and most of the physiological indicators of P nutrition. Hence, while SRP is known to overestimate absolute orthophosphate in natural waters (Rigler 1966, 1968), it is a good relative measure of the phosphorus available for immediate uptake in these rivers. The correlation between SRP concentration and algal P nutrition also suggests that the periphyton was growing in an equilibrium condition with respect to the ambient medium and that nutrient pulsing was unimportant during these periods of low, stable flow.

Some of the determinations of P-limited algal growth rates in the South and North Thompson Rivers were made at low temperatures, <1°C. Nutrient limitation of growth rate at very low temperatures, while possible, is thought to be unusual in nature (Eppley 1972). Normally nutrient concentrations and temperature vary inversely, spatially and temporally, in both lakes and oceans. My experiments present the first strong documentation of in situ, P-limited growth rates at temperatures approaching 0°C. The concurrence of low temperature and low nutrients may be more common in lotic than in lentic environments; in the latter, nutrient regeneration and water-mass mixing processes in winter usually elevate euphotic levels of dissolved N and P.

Another noteworthy aspect of lotic P limitation is revealed by the comparison of cellular and ambient medium N:P ratios. At the lower Thompson site, where $\mu:\mu_{max} \simeq$ 0.8–0.9, cellular N:P averaged about 16 while extracellular N:P was close to 100. This situation is analogous to a P-limited chemostat run at high dilution rate. In both cases, ambient (supply) N:P determines which of the two nutrients would potentially limit growth rate, if in short enough supply. Actual $\mu:\mu_{max}$ under steady state is related to the extracellular concentration of the critical nutrient. Soluble N:P ratios are often used to determine whether lakes are N or P limited (Chiaudani and Vighi 1974). In lentic environments the distinction between potential and actual nutrient limitation, based on ambient N:P, is not critical because during the growing season phyto-

plankton activities normally drive nutrient concentrations to very low levels. However, in lotic systems with large water-discharge: biomass ratios, periphyton is less likely to alter the ambient concentrations of N or P. In these environments the distinction between potential and actual P limitation based on bulk-water N:P is crucial. High external N:P in rivers only means that they are potentially P limited. Whether growth rates are actually controlled by P depends on the concentration. Data from the Thompson River at temperatures below 10°C indicate that P limitation begins at about 3–4 μg liter^{-1} SRP.

My conclusion that periphyton growth rates are saturated at very low levels of SRP differs from that of Horner et al. (1983) who found that the periphytic green alga, *Mougeotia* sp., continued to show increases in growth rate with additions of PO_4^{3-} up to 25 μg P liter^{-1}. This disparity may not be surprising, considering that their experiments were run at higher temperatures (>15°C) and with algal mat densities 10–20 times greater than the biomass levels examined here (ca. 10–30 mg Chl *a* m^{-2}). Intuitively, thicker algal mats would seem to require higher ambient P concentrations to saturate growth of the mat as a whole. However, in cases where algal mat thickness is controlled by either physical conditions or grazing pressure, it is clear from my results that periphyton growth rates can be saturated with respect to phosphorus at much lower concentrations than previously reported in the river-stream literature.

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