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Spatial and temporal variability of nutrient limitation in 6 North Shore tributaries to Lake Superior

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Abstract. Nutrient availability varies both spatially and temporally in temperate systems because of timing of seasonal and hydrological events (e.g., spring snowmelt). Most studies have found either N or P to be primarily limiting. A nutrient-diffusing bioassay was used to determine if N, P, neither nutrient, or both nutrients were limiting to periphyton growth (measured by chlorophyll *a*) in 6 tributaries to Lake Superior during the ice-free season of 1994. Molar ratios of dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) were also calculated to predict potential limitation conditions and determine agreement with bioassay results. Co-limitation predominated (N + P > all other treatments). No limitation was also common during the late portion of the ice-free season. DIN:SRP ratios were not useful in predicting nutrient-limitation conditions. Results showed that nutrient limitation of periphyton biomass varied over space and time on a relatively small regional scale. This result is significant because many studies extrapolate results from a single stream or time period to a much larger spatial or temporal scale.

Key words: nutrient limitation, DIN:SRP, spatial and temporal variability, co-limitation, bioassays, Lake Superior tributaries.

Nutrient limitation determines algal production in many stream ecosystems, and is an important aspect of stream ecosystem dynamics. Elwood et al. (1981) demonstrated the importance of nutrient limitation in a woodland stream where the addition of P increased rates of detrital processing. Periphyton accrual rate has also been enhanced when limiting nutrients are provided (Grimm and Fisher 1986, Winterbourn 1990, Rosemond et al. 1993).

Many studies have shown nutrient limitation in stream ecosystems. In the southwestern US, streams are primarily N limited (Coleman and Dahm 1990, Grimm and Fisher 1986). Primary N limitation is also common in streams of southern Australia (Chessman et al. 1992). Phosphorus limitation has been detected in a coastal rain forest stream (Stockner and Shortreed 1978), a Michigan trout stream (Hart and Robinson 1990), and a tundra stream (Peterson et al. 1983). Co-limitation of both N and P occurs in streams of Australia (Winterbourn 1990), Walker Branch, Tennessee (Rosemond 1993), and Kings Creek, Kansas (Tate 1990). Periphyton accumulation rates were experimentally en-

hanced by both N and P addition in a British Columbia river (Perrin et al. 1987). Studies such as these might suggest regional patterns of nutrient limitation, but not all adequately consider spatial and temporal variability within a region. In most cases results were restricted to 1 stream or watershed and in some cases results were restricted to a short time frame.

Nutrient limitation could vary seasonally because there is a strong seasonality of the hydrologic cycle, organic matter inputs, and biotic activity in streams of temperate areas. Snow melt entering northern streams during spring contains NO_3^- from acid precipitation (Aber et al. 1989). In summer, streams on the Minnesota North Shore of Lake Superior are characterized by increasing biomass and low but consistent nutrient concentrations, except when affected by high discharge events. In the fall, leaf litter inputs to these streams can be quite significant, and have the potential to alter nutrient concentrations as a result of increased nutrient leaching from leaves. In a previous study on the French River on the North Shore, nutrient limitation shifted from P in May to N in June and August, to no nutrient limitation in October (Allen and Hershey 1996).

Nutrient limitation also has the potential to vary spatially across watersheds. This variability

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ty may come from differences in topography of the watersheds, spring meltwater runoff patterns, riparian vegetation, presence of wetlands, and non-homogenous summer precipitation events (Meyer et al. 1988, Tate 1990, Paul et al. 1991). Spatial variability of minor floods may also impact nutrient cycling (Mosisch and Bunn 1997). North Shore streams have very similar physical characteristics, which suggests that generalizing among watersheds is appropriate. However, general understanding of spatial variability of nutrient limitation in streams is poor.

Various methods have been used to determine nutrient limitation, including whole-stream manipulations, flow-through channels, and nutrient-diffusing substrata. Nutrient-diffusing substrata are useful for bioassays because they 1) stimulate significant growth (Pringle 1987), 2) reduce sampling variability because of uniform size, 3) are economical in terms of cost and effort, 4) allow simultaneous manipulation of >1 nutrient in the same stream reach at the same time, 5) are less disruptive to a stream than is whole-stream addition of fertilizer, and 6) permit assessment of temporally changing conditions.

Ratios of nutrient concentrations in the water column have often been used to infer a limiting nutrient. Molar N:P ratios of <10 may indicate a N-limiting state, whereas ratios >20 could mean P limitation (Redfield 1958, Schanz and Juon 1983, Lohman et al. 1991, Axler et al. 1994). Dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) ratios and DIN to total phosphorus (DIN:TP) ratios both have been used as tools to discern limitation because nutrients in these forms are or can become available to stream periphyton (Axler et al. 1994). Total nitrogen to TP (TN:TP) ratios may overestimate available nutrients to stream epilithic algae because not all N forms are readily available to algae (Axler et al. 1994).

We hypothesized that nutrient limitation would vary spatially across different watersheds and seasonally within a watershed, given the potential for variability in these river systems. We used 2 approaches to evaluate this variability in 6 Minnesota tributaries to Lake Superior: 1) nutrient concentration and molar DIN:SRP ratio analysis, and 2) nutrient-diffusing bioassay experiments to determine nutrient limitation.

Study Sites

The 6 Minnesota tributaries to Lake Superior we examined were the Knife, Stewart, Encamp-

ment, East Split Rock, and West Split Rock rivers and Silver Creek (Fig. 1). These streams are all relatively high-gradient and lie within small watersheds (Waters 1977). All study reaches were 2nd order and ~200 m in length. Three sites were established at each reach; all sites were at least 100 m upstream from road access points. Substratum at all sites was similar, consisting of cobble and boulder (60%), with some sand and gravel (30%), and silt in depositional areas (10%). Discharge and temperature for these streams are shown in Figs. 2 and 3.

Methods

Samples for analysis of ambient nutrient concentrations were collected using 250-mL acid-washed polyethylene bottles at each of the 3 sites in each river throughout the ice-free season. The samples were filtered through Gelman glass fiber filters and the filtrate was analyzed for NO_3^- -N, NH_4^+ -N, and SRP by using standard methods (APHA 1989). Detection limits were 5 $\mu\text{g/L}$ for NO_3^- -N and NH_4^+ -N, and 2 $\mu\text{g/L}$ for SRP. The concentrations ($\mu\text{g/L}$), were converted to molar concentrations of N and P to determine molar DIN:SRP ratios. The DIN:SRP ratios were calculated to ascertain potential nutrient limitation in these streams.

Discharge was determined each time a site was visited by measuring mean velocity at $0.6\times$ depth at 5 points along a transect across the stream and multiplying the mean velocity by the cross-sectional area of the cell. These cell discharges were summed to obtain total stream discharge (Gore 1996). Temperature also was recorded at each sampling time.

Nutrient-diffusing bioassay experiments

Nutrient-diffusing bioassay experiments were conducted at all sites in the 6 study rivers to determine spatial and temporal variability in nutrient limitation of algal biomass. Nutrient-diffusing bioassay tubes capped with porous silica disks were used to diffuse either N, P, or N + P from an agar medium. The 4 treatments consisted of N (0.5 M NaNO_3), P (0.05 M Na_2HPO_4), N + P (0.5 M NaNO_3 + 0.05 M Na_2HPO_4), and a control treatment of untreated bacto agar (after Allen and Hershey 1996). The broth of deionized water, nutrient, and agar was

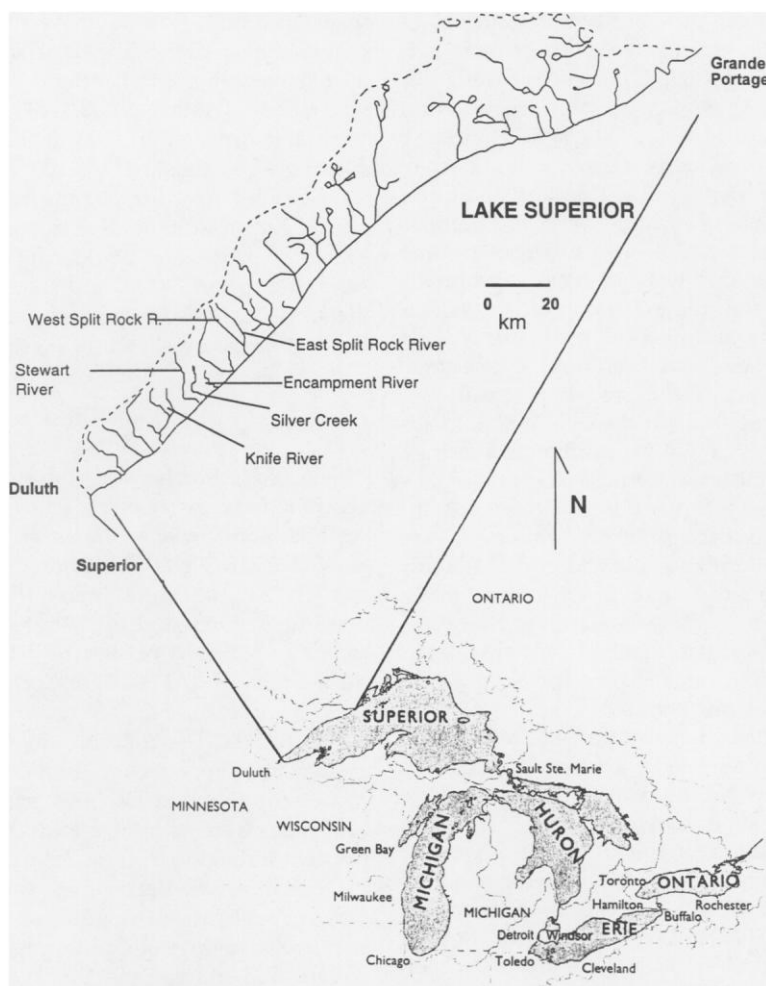


FIGURE 1. Minnesota tributaries to Lake Superior showing the 6 rivers used in this study. Map adapted from Environment Canada and US Environmental Protection Agency (1995) and Waters (1997).

autoclaved and poured into 10-dram (~40 mL) plastic vials. Vials were capped with acid-washed 26-mm-diameter fused silica disks (Gibeau and Miller 1989). All tubes were secured in plastic test tube racks and anchored to the river bottom using rebar. Racks containing 2 replicates of each nutrient treatment were placed at 3 sites in each of the 6 rivers. All sites were located at the heads of riffles to minimize potentially confounding spatial downwelling and upwelling effects associated with runs, riffles, and pools (Valett et al. 1994).

Bioassay experiments were run for 3-wk periods, beginning 11 May 1994. When tubes were removed after the 3-wk incubation period, they

were replaced with fresh sets of treatments. We overlapped the study periods during the first 2 mo and the last 2 mo of the study (i.e., new tubes were placed in rivers 1 or 2 wk prior to removing an earlier set). The final sets of tubes were removed from all rivers on 17 and 18 November 1994. Each time the tubes were removed from the test tube racks, the porous silica disks were separated from the plastic vials, and the disks were placed in glass vials for laboratory analysis.

Chlorophyll *a* concentrations on the disks, corrected for phaeopigments, were determined following extraction with 90% acetone for 24 h at 4°C (APHA 1989). Concentration of chloro-

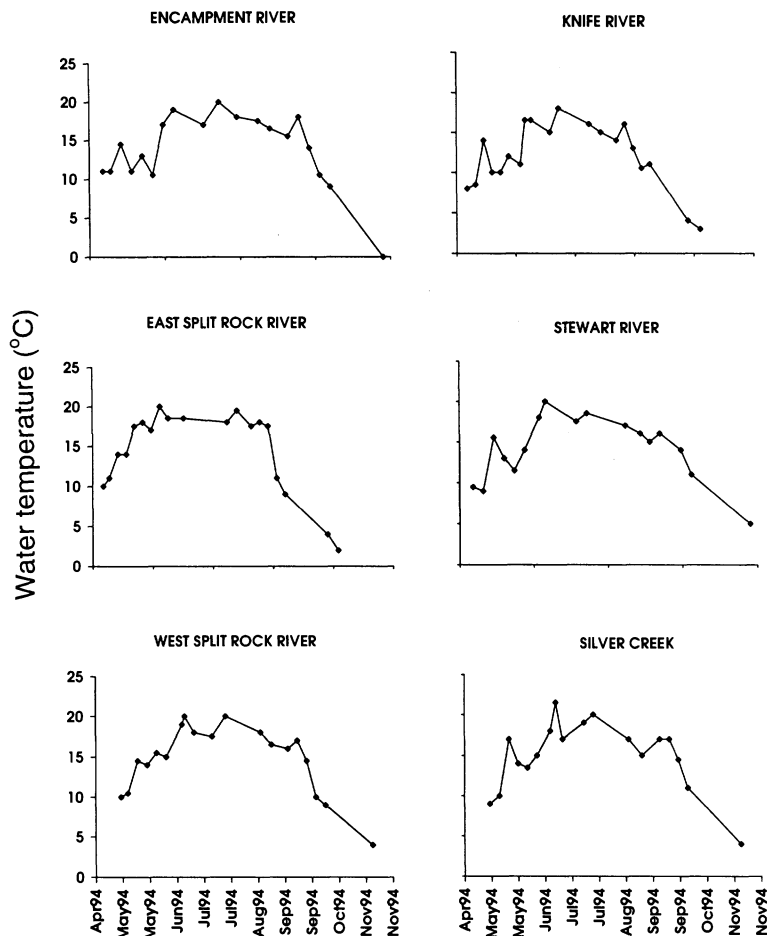


FIGURE 2. Daytime water temperature for the 6 study streams during the ice-free period of 1994.

phyll *a* was determined spectrophotometrically with a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer. Concentrations were converted to mg chlorophyll *a*/m².

Seven different nutrient-limiting conditions were defined (level of significance $p < 0.05$ for all): 1) **no limitation**, where no treatment differed significantly from other treatments; 2) **P-limitation**, where all treatments containing P (including N + P) were significantly greater than the control; 3) **N-limitation**, where all treatments containing N (including N + P) were significantly higher than the control; 4) **co-limitation**, where the N + P treatment was significantly higher than the control but neither the N nor P treatments alone were significantly greater than the control; 5) **both N and P limited**, where all nutrient treatments were significantly

greater than the control; 6) **P primarily limiting with N secondarily limiting**, where the P treatment was significantly greater than the control, and the N + P treatment was greater than the P treatment; and 7) **N primarily limiting with P secondarily limiting**, where the N treatment was significantly greater than the control, and the N + P treatment was greater than the N treatment.

Statistical design and analysis

Bioassay chlorophyll *a* results were analyzed following a randomized complete block design where sites served as blocks and each block contained 2 replicates of each nutrient treatment. The randomized block design was used to min-

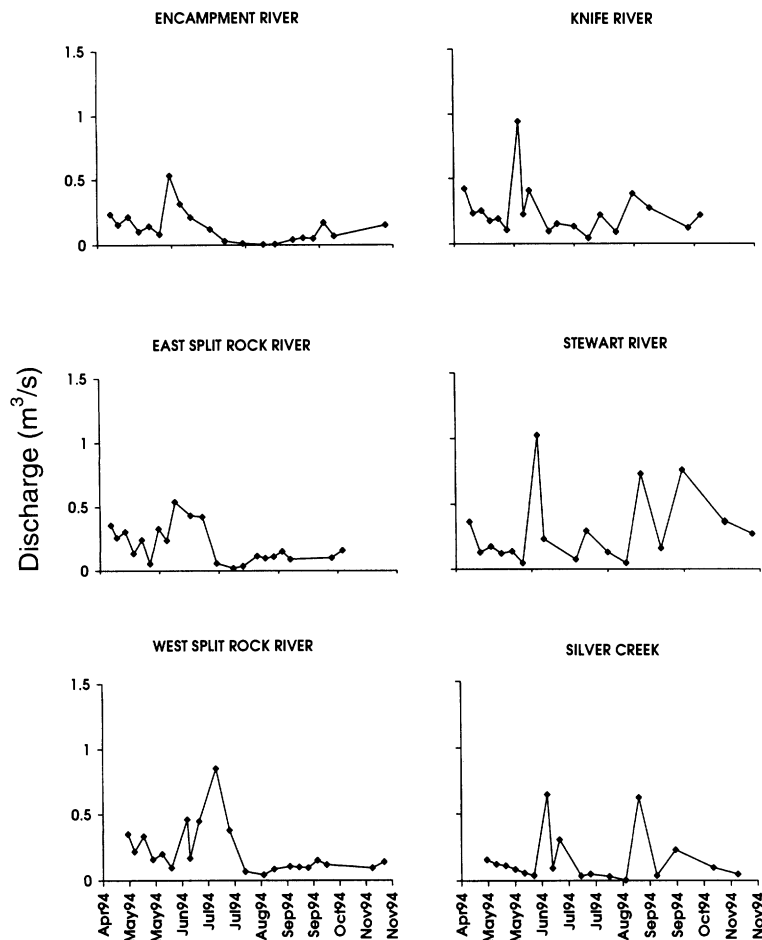


FIGURE 3. Instantaneous discharge for the 6 study streams during the ice-free season of 1994.

imize the influence of spatial patchiness within streams. Nutrient limitation conditions were determined for each date and river using the general linear models procedure (proc GLM) in SAS (6.03 edition, SAS Institute, Inc., Cary, North Carolina). The response variable was *ln* chlorophyll *a*. Significant differences were determined using Dunnett's method of comparing all treatments to the control. Significance level was $p < 0.05$ for all statistical analyses.

Results

Water chemistry

Inorganic nutrient concentrations were very low for all 6 rivers throughout the ice-free season. NO_3^- -N ranged from below detection to 25 $\mu\text{g/L}$, NH_4^+ -N ranged from below detection to

17 $\mu\text{g/L}$, and SRP ranged from below detection to 14 $\mu\text{g/L}$ (Fig. 4). NO_3^- -N concentration was typically highest in the spring and the fall. Ammonium and SRP concentrations were generally lower than NO_3^- -N in all 6 streams and showed no discernible seasonal pattern. Nutrient concentrations were generally similar among all rivers, although the Knife and Stewart rivers had slightly lower and less variable NO_3^- -N concentrations than the others. Molar DIN:SRP ratios indicated potential N limitation (DIN:SRP ratios < 10 indicate N limitation and > 20 indicate P limitation) most of the time (Fig. 5). Molar DIN:SRP ratios were quite variable and most of the > 10 values occurred early in the open-water season. All of the rivers also exhibited an increase in the DIN:SRP ratio at the end of the ice-free season.

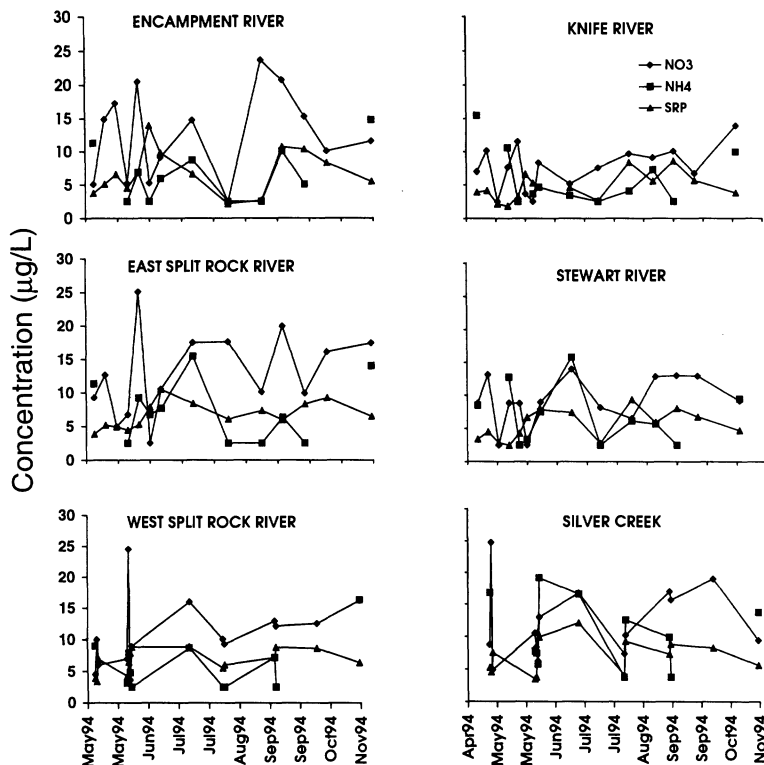


FIGURE 4. Nutrient concentrations in the study streams for the ice-free season of 1994. SRP = soluble reactive phosphorus.

Nutrient-diffusing substrata

Of the 72 nutrient bioassay experiments conducted, 36 showed co-limitation (Table 1). No limitation was the 2nd most common result, occurring in 19 assays. P-limitation was observed in 7 of the experiments, and N-limitation was found in 3 experiments. Phosphorus was the primary limiting nutrient, with N secondarily limiting in 4 of the experiments. Both N and P were limiting in 3 of the assays. Nitrogen primarily with P secondarily limiting did not occur in any of the experiments.

Bioassay results showed that nutrient limitation fluctuated throughout the ice-free season (Table 1). In the East Split Rock River, algal biomass appeared to be sequentially co-limited, limited by both N and P, N-limited, and P primarily, N secondarily limited during the early and middle portions of the season, but by the end of the ice-free season, no limitation predominated (Table 1). The Encampment River exhibited a similar pattern to the East Split Rock River

but was also limited by P on 1 occasion during the summer. The Knife River was co-limited during much of the early part of the year with 2 cases of P-limitation, but showed no limitation by the end of the season. Silver Creek exhibited co-limitation, both, N, and no limitation, although co-limitation dominated. However, Silver Creek did not exhibit an end-of-season trend toward no limitation as observed in the East Split Rock, Encampment, and Knife rivers. The Stewart River was co-limited for most of the year, but also tended toward no limitation at the end of the season, except for the last date when P-limitation was the result. The West Split Rock River showed co-limitation and no limitation in almost alternate weeks with only 2 other results. Therefore, no seasonal trend was apparent. Overall, in 4 of the 6 rivers studied, there appeared to be a seasonal trend of some form of nutrient limitation during most of the season followed by no limitation at the end of the season (Table 1). Nutrient limitation was commonly

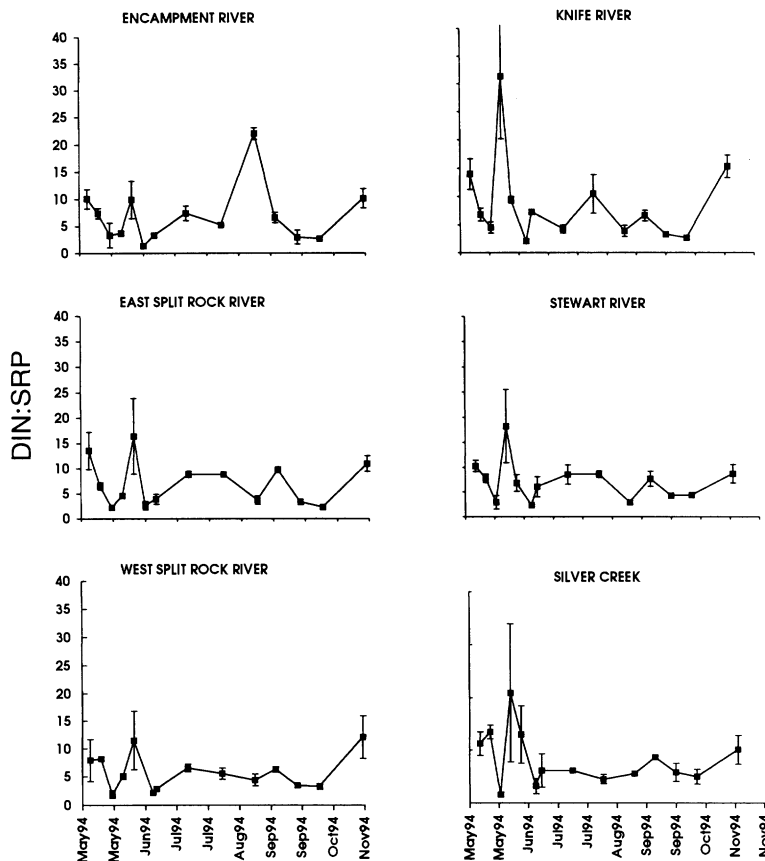


FIGURE 5. Molar dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) ratios (± 1 SE) in the 6 study streams during the ice-free season of 1994. DIN:SRP < 10 may indicate N limitation and DIN:SRP > 20 may indicate P limitation.

observed in the other 2 rivers, but we did not detect a seasonal pattern (Table 1).

Bioassay experiments for the 1st date in 1994 corresponded to the widest range of DIN:SRP ratios found during the ice-free season. DIN:SRP ratios were < 20 on most occasions and 86% of all experiments were ≤ 10 , indicating N-limitation. Even with the low molar ratios, co-limitation and no limitation predominated and N-limitation was uncommon (Fig. 6).

Discussion

We found a generalized pattern from early summer to late fall in 4 rivers, where early season assays indicated some form of nutrient limitation by N and/or P, but most late season assays indicated no nutrient limitation. This result is similar to that of Allen and Hershey (1996)

where no limitation was observed at the end of the field season in the nearby French River. However, there were specific differences among rivers throughout the season, especially during the early and middle portions of the season; no single nutrient-limitation pattern described all rivers.

Nutrient limitation

Variable nutrient concentrations and corresponding DIN:SRP in all 6 rivers of this study indicated the potential for seasonally variable nutrient-limitation conditions. DIN:SRP was relatively high (> 10) in the early part of the season and generally low (< 10) for the rest of the ice-free season in 5 of the 6 study streams, the exception being Encampment River. This pattern is similar to that found in the French River of

TABLE 1. Mean \pm 1 SE chlorophyll *a* (mg/m², *n* = 6) on bioassay disks for each nutrient treatment. Week = the week the assay was completed. Asterisk = significant difference from control (Dunnett's method of multiple comparisons, *p* < 0.05). Conclusion abbreviations are defined as: LIM = limitation, PRIM = primary, SEC = secondary, BOTH N + P = both nutrients limiting.

River	Week	C	N	N + P	P	Conclusion
East Split Rock	1 Jun 94	2.61 \pm 0.12	2.85 \pm 0.13	4.02 \pm 0.15*	2.58 \pm 0.10	CO-LIM
	8 Jun 94	2.33 \pm 0.08	2.79 \pm 0.07*	2.99 \pm 0.1*	2.32 \pm 0.11	N-LIM
	15 Jun 94	3.32 \pm 0.09	3.16 \pm 0.08	4.08 \pm 0.12*	3.63 \pm 0.05*	P-PRIM, N-SEC
	22 Jun 94	3.88 \pm 0.10	4.03 \pm 0.03	4.30 \pm 0.05*	3.83 \pm 0.06	CO-LIM
	12 Jul 94	3.58 \pm 0.17	3.94 \pm 0.07*	4.54 \pm 0.03*	4.10 \pm 0.09*	BOTH N + P
	3 Aug 94	3.48 \pm 0.16	3.97 \pm 0.05*	4.63 \pm 0.10*	3.93 \pm 0.09*	BOTH N + P
	24 Aug 94	3.11 \pm 0.11	3.3 \pm 0.13	4.51 \pm 0.14*	3.5 \pm 0.09	CO-LIM
	13 Sep 94	2.93 \pm 0.12	2.88 \pm 0.08	4.01 \pm 0.06*	3.02 \pm 0.07	CO-LIM
	27 Sep 94	3.07 \pm 0.13	2.95 \pm 0.18	3.57 \pm 0.42	3.63 \pm 0.08	NO LIM
	11 Oct 94	2.58 \pm 0.17	2.66 \pm 0.12	3.38 \pm 0.23*	3.07 \pm 0.26	CO-LIM
	25 Oct 94	1.84 \pm 0.08	2.24 \pm 0.29	2.49 \pm 0.26	2.55 \pm 0.19	NO LIM
	17 Nov 94	2.36 \pm 0.32	2.63 \pm 0.16	2.96 \pm 0.22	3.16 \pm 0.17	NO LIM
Encampment	1 Jun 94	2.58 \pm 0.20	3.01 \pm 0.26	4.24 \pm 0.19*	2.28 \pm 0.10	CO-LIM
	8 Jun 94	2.12 \pm 0.19	2.6 \pm 0.15	3.82 \pm 0.17*	2.33 \pm 0.13	CO-LIM
	15 Jun 94	3.14 \pm 0.09	2.76 \pm 0.09*	4.01 \pm 0.06*	3.60 \pm 0.14*	P-PRIM, N-SEC
	22 Jun 94	3.45 \pm 0.10	3.43 \pm 0.10	3.69 \pm 0.42	3.65 \pm 0.07	NO LIM
	12 Jul 94	3.88 \pm 0.06	4.19 \pm 0.08*	4.63 \pm 0.06*	4.03 \pm 0.06	N-LIM
	3 Aug 94	3.51 \pm 0.13	3.79 \pm 0.09	5.00 \pm 0.08*	3.50 \pm 0.18	CO-LIM
	24 Aug 94	2.84 \pm 0.08	3.01 \pm 0.20	4.43 \pm 0.09*	3.46 \pm 0.07*	P-LIM
	13 Sep 94	2.83 \pm 0.11	2.9 \pm 0.09	3.81 \pm 0.07*	2.57 \pm 0.05	CO-LIM
	27 Sep 94	2.8 \pm 0.11	2.68 \pm 0.15	3.7 \pm 0.26	2.25 \pm 0.06*	NO LIM
	11 Oct 94	2.47 \pm 0.08	2.2 \pm 0.14	3.31 \pm 0.17*	2.22 \pm 0.15	CO-LIM
	25 Oct 94	1.52 \pm 0.22	1.34 \pm 0.18	1.83 \pm 0.15	1 \pm 0.1	NO LIM
	17 Nov 94	2.72 \pm 0.19	2.78 \pm 0.16	3.38 \pm 0.15	3.17 \pm 0.28	NO LIM
Knife	1 Jun 94	2.76 \pm 0.18	3.20 \pm 0.15	4.21 \pm 0.37*	3.35 \pm 0.28	CO-LIM
	8 Jun 94	2.68 \pm 0.06	2.98 \pm 0.10	3.70 \pm 0.12*	3.07 \pm 0.11	CO-LIM
	15 Jun 94	3.46 \pm 0.08	3.20 \pm 0.09	3.98 \pm 0.11*	3.62 \pm 0.07	CO-LIM
	22 Jun 94	3.52 \pm 0.04	3.48 \pm 0.09	3.98 \pm 0.08*	3.63 \pm 0.10	CO-LIM
	12 Jul 94	3.39 \pm 0.12	3.61 \pm 0.05	4.32 \pm 0.05*	3.71 \pm 0.02*	P-LIM
	3 Aug 94	3.4 \pm 0.12	3.39 \pm 0.11	4.42 \pm 0.07*	3.59 \pm 0.11	CO-LIM
	24 Aug 94	3.19 \pm 0.06	3.35 \pm 0.08	4.31 \pm 0.05*	3.61 \pm 0.09*	P-LIM
	13 Sep 94	2.83 \pm 0.17	3.03 \pm 0.11	3.53 \pm 0.19*	2.61 \pm 0.16	CO-LIM
	27 Sep 94	2.94 \pm 0.29	3.03 \pm 0.1	3.82 \pm 0.24*	3.07 \pm 0.21	CO-LIM
	11 Oct 94	3.09 \pm 0.23	2.59 \pm 0.1	4.46 \pm 0.23*	3.1 \pm 0.14	CO-LIM
	25 Oct 94	1.76 \pm 0.24	2.38 \pm 0.21	2.45 \pm 0.30	2.45 \pm 0.32	NO LIM
	17 Nov 94	2.85 \pm 0.29	2.9 \pm 0.17	3.38 \pm 0.25	3.31 \pm 0.23	NO LIM
Silver	1 Jun 94	1.89 \pm 0.21	2.27 \pm 0.09	3.68 \pm 0.11*	2.33 \pm 0.08	CO-LIM
	8 Jun 94	2.53 \pm 0.09	3.04 \pm 0.7*	3.72 \pm 0.13*	3.04 \pm 0.08*	BOTH N \pm P
	15 Jun 94	3.91 \pm 0.06	3.80 \pm 0.04	4.29 \pm 0.06*	3.93 \pm 0.13	CO-LIM
	22 Jun 94	3.64 \pm 0.11	3.58 \pm 0.05	4.10 \pm 0.08*	3.62 \pm 0.08	CO-LIM
	12 Jul 94	3.39 \pm 0.04	3.62 \pm 0.04*	3.98 \pm 0.07*	3.47 \pm 0.11	N-LIM
	3 Aug 94	3.82 \pm 0.15	3.86 \pm 0.07	4.16 \pm 0.12	3.57 \pm 0.13	NO LIM
	24 Aug 94	2.99 \pm 0.21	3.18 \pm 0.21	4.2 \pm 0.2*	3.5 \pm 0.21	CO-LIM
	13 Sep 94	2.6 \pm 0.32	3.07 \pm 0.08	3.4 \pm 0.19	3 \pm 0.25	NO LIM
	27 Sep 94	3.14 \pm 0.16	3.11 \pm 0.12	3.82 \pm 0.19*	3.3 \pm 0.16	CO-LIM
	11 Oct 94	2.57 \pm 0.16	2.73 \pm 0.1	3.63 \pm 0.18*	3.08 \pm 0.15	CO-LIM
	25 Oct 94	1.95 \pm 0.31	2.1 \pm 0.18	2.55 \pm 0.18	2.38 \pm 0.2	NO LIM
	17 Nov 94	2.4 \pm 0.21	2.55 \pm 0.07	2.99 \pm 0.21*	2.67 \pm 0.13	CO-LIM

(continued on page 10)

TABLE 1. Continued.

River	Week	C	N	N + P	P	Conclusion
Stewart	1 Jun 94	1.91 ± 0.11	2.38 ± 0.25	3.21 ± 0.13*	2.22 ± 0.26	CO-LIM
	8 Jun 94	2.23 ± 0.16	2.71 ± 0.08*	3.56 ± 0.15*	2.93 ± 0.08*	P-PRIM, N-SEC
	15 Jun 94	3.66 ± 0.10	3.68 ± 0.08	4.21 ± 0.07*	3.76 ± 0.09	CO-LIM
	22 Jun 94	3.66 ± 0.11	3.59 ± 0.07	4.18 ± 0.13*	3.56 ± 0.06	CO-LIM
	12 Jul 94	3.58 ± 0.08	3.75 ± 0.17	4.53 ± 0.11*	3.83 ± 0.10	CO-LIM
	3 Aug 94	3.67 ± 0.2	3.85 ± 0.1	4.73 ± 0.1*	4.04 ± 0.14	CO-LIM
	24 Aug 94	3.29 ± 0.22	3.67 ± 0.23	4.45 ± 0.09*	4.08 ± 0.18*	P-LIM
	13 Sep 94	2.97 ± 0.003	2.73 ± 0.12	4.01 ± 0.11*	3.51 ± 0.08*	P-LIM
	27 Sep 94	2.28 ± 0.32	2.9 ± 0.04	3.51 ± 0.01*	3.04 ± 0.22	CO-LIM
	11 Oct 94	2.09 ± 0.41	2.44 ± 0.17	2.83 ± 0.21	2.67 ± 0.28	NO LIM
	25 Oct 94	1.4 ± 0.04	1.53 ± 0.32	2.13 ± 0.51	1.64 ± 0.11	NO LIM
	17 Nov 94	2.2 ± 0.07	2.3 ± 0.05	2.9 ± 0.22	3.03 ± 0.38*	P-LIM
West Split Rock	1 Jun 94	2.59 ± 0.13	2.87 ± 0.16	3.67 ± 0.16*	2.96 ± 0.07	CO-LIM
	8 Jun 94	2.86 ± 0.18	2.92 ± 0.06	3.67 ± 0.06*	3.32 ± 0.15*	P-PRIM, N-SEC
	15 Jun 94	4.07 ± 0.07	3.61 ± 0.19*	4.30 ± 0.05	4.16 ± 0.10	NO LIM
	22 Jun 94	3.93 ± 0.10	3.90 ± 0.09	4.03 ± 0.15	3.90 ± 0.16	NO LIM
	12 Jul 94	3.70 ± 0.07	4.06 ± 0.07*	4.43 ± 0.06*	4.11 ± 0.05*	CO-LIM
	3 Aug 94	4.17 ± 0.24	4.22 ± 0.18	4.73 ± 0.16	4.04 ± 0.23	NO LIM
	24 Aug 94	3.44 ± 0.11	3.81 ± 0.17	4.56 ± 0.13*	3.8 ± 0.08	CO-LIM
	13 Sep 94	3.03 ± 0.19	3.11 ± 0.11	4.11 ± 0.1*	2.72 ± 0.09	CO-LIM
	27 Sep 94	3.25 ± 0.14	3.08 ± 0.19	3.69 ± 0.14	3.2 ± 0.14	NO LIM
	11 Oct 94	2.61 ± 0.16	2.57 ± 0.15	3.64 ± 0.18*	2.49 ± 0.33	CO-LIM
	25 Oct 94	1.7 ± 0.2	1.9 ± 0.4	2.46 ± 0.43	1.75 ± 0.24	NO LIM
	17 Nov 94	2.37 ± 0.14	2.94 ± 0.1	3.37 ± 0.21*	3.38 ± 0.19*	P-LIM

northeastern Minnesota by Allen and Hershey (1996). The DIN:SRP ratios were indicative of N-limitation (<10) for most of the season, but the bioassay experiments indicated co-limitation. The DIN:SRP ratios in these systems were useful in indicating that potential nutrient-limitation conditions did exist, but were not useful in predicting the limiting nutrient(s). Other systems have had similar nutrient concentrations throughout their growing seasons and experienced limitation by only 1 nutrient (Peterson et al. 1983, Grimm and Fisher 1986, Coleman and Dahm 1990). N:P ratios sometimes are not indicative of nutrient-limitation conditions because of factors such as N fixation (Mulholland et al. 1991). Production of alkaline phosphatase can make organic P available to algae (Axler et al. 1994) and therefore limit the usefulness of SRP as a true measure of bioavailable P. Although alkaline phosphatase activity and total P were not measured, we conclude that DIN:SRP ratios were not reliable predictors of nutrient-limitation status.

Bioassay results confirm that during the early

and middle portions of the growing season, periphytic algal abundance was limited by N + P. Co-limitation may be caused by many factors including low nutrient concentrations (Tate 1990), increased nutrient cycling within the periphytic mat (Paul et al. 1991, Mulholland et al. 1994, 1995, Steinman et al. 1995), and N fixation in the periphyton (Mulholland et al. 1991). We did not study nutrient cycling rates, but N fixation likely was not a factor in these North Shore streams because blue-green algae were never observed at the study sites or on the disks.

Results of concurrent bioassays and molar N:P ratios from selected studies are summarized in Table 2. DIN:SRP ratios and bioassay results agreed in some studies that indicated N or P limitation (Grimm and Fisher 1986, Chessman et al. 1992). According to DIN:SRP in our study, N limitation should be the dominant result of the bioassay, but co-limitation was the most common condition. Other study streams where co-limitation was observed had low concentrations of inorganic nutrients similar to streams on the North Shore of Lake Superior (Tate 1990,

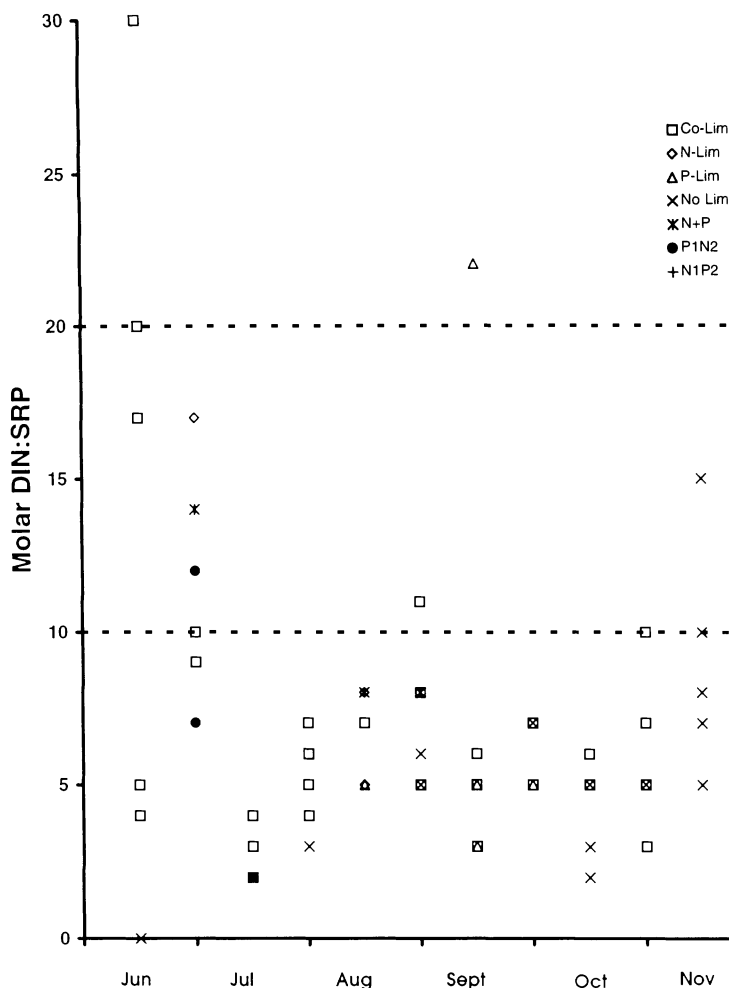


FIGURE 6. Bioassay result corresponding to measured dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) versus month for all study rivers and experiments in 1994. Lim = limitation, P1N2 = P primary and N secondary limitation, N1P2 = N primary and P secondary limitation. DIN:SRP <10 may indicate N limitation and DIN:SRP >20 may indicate P limitation. (Dashed lines show these limitations.)

Rosemond et al. 1993). Nutrient limitation was not observed under higher nutrient concentrations in the Blue Earth River, Minnesota, although N:P ratios indicated P limitation (Kutka and Richards 1997). Thus, N:P ratios also did not appear to be useful predictors of nutrient-limitation status in the Blue Earth River.

Previous studies on the North Shore indicated either N or P as the single limiting nutrient to periphyton growth in 4 North Shore streams (Allen and Hershey 1996, J. A. Schuldt, University of Minnesota, unpublished data). Therefore, co-limitation could be the result of a shift in limitation during the assay from N to P or vice versa.

One would expect N or P to be significantly higher than the control if either factor was primarily limiting for a portion of the assay, but in most cases N and/or P was not significantly different ($p > 0.05$) from the control. The co-limitation result is more plausibly explained by an additive effect of N and P amendment (Tate 1990). The additive effects model suggests that when N and P concentrations are low, addition of the primary limiting nutrient depletes the secondary limiting nutrient at a fast rate so that both nutrients limit growth. The North Shore rivers were an example of this model of co-limitation in 1994. Other studies involving the ef-

TABLE 2. Indication of nutrient-limitation status from N:P ratios and bioassays from selected studies. Secondly limiting nutrients are in parentheses.

River	Water N:P	Bioassay	Reference
Blue Earth	P	no limitation	Kutka and Richards 1997
Sycamore Creek	N	N	Grimm and Fisher 1986
Kings Creek	N	co-limitation	Tate 1990
Darebin Creek	N	N	Chessman et al. 1992
Dandenong	N	no limitation	Chessman et al. 1992
Shady Creek	P	N?	Chessman et al. 1992
Merriman	N	N(P)	Chessman et al. 1992
Shaw Creek	N	N(P?)	Chessman et al. 1992
Watchbed	N	N(P?)	Chessman et al. 1992
Brodribb	P	P	Chessman et al. 1992
Carp Creek	P	P	Pringle 1987
Walker Branch	P	co-limitation	Rosemond et al. 1993
Walker Branch	N	co-limitation	Rosemond et al. 1993
6 North Shore rivers	N	co-limitation	This study

fects of decomposing salmon on North Shore streams have shown significant increases in periphytic algae downstream of the carcasses that contributed both N and P to stream water (Schuldt and Hershey 1995, Fisher Wold 1995). Salmon-derived N has been detected in algae downstream of salmon carcasses (Schuldt and Hershey 1995, Fisher Wold 1995), but salmon are also an important source of P in North Shore streams (Schuldt and Hershey 1995).

Variability of nutrient limitation

The hypothesis that nutrient limitation varies spatially across watersheds and seasonally within watersheds was supported for the ice-free season of 1994. Co-limitation of periphyton by both N and P during the bulk of the ice-free season was not observed by Allen and Hershey (1996) in the French River. Unlike the French River study, we did not observe a consistent pattern of P-limitation early in the season. The French River study was conducted a few weeks earlier in the season, however, when snow melt was still entering the stream and DIN concentrations were quite high (>90 µg/L, Allen and Hershey 1996).

There were noticeable differences in patterns of nutrient limitation within a relatively small geographic area. Some studies have demonstrated nutrient-limitation regimes in a single watershed and suggested a similar regional pattern (Stockner and Shortreed 1978, Grimm and Fisher 1986). Similar patterns of nutrient limitation

in North Shore streams might be expected given the similarities in geology, vegetation, and location, but patterns differed. The observed spatial variability in nutrient limitation suggests that regional-scale extrapolation cannot be done without 1st assessing spatial variability, regardless of how similar streams may appear. It is also exceedingly important that nutrient-limitation conditions are not only determined by DIN:SRP ratios in these systems, but in combination with a reliable bioassay.

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