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APPLIED ISSUES

Development and application of a nutrient-diffusing bioassay for large rivers

GARRY J. SCRIMGEOUR*
Sustainable Forest Management Network of Centres of Excellence, G-208, Biological Sciences Building, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

PATRICIA A. CHAMBERS
National Hydrology Research Institute, 11 Innovation Boulevard, Saskatoon, Saskatchewan S7N 3H5, Canada

**Author to whom correspondence should be sent.*

SUMMARY

1. Laboratory and field experiments were performed to develop and then apply a nutrient-diffusing substratum (NDS) design suitable for use in large, fast-flowing rivers.
2. Initial laboratory experiments quantified diffusion of PO_4 and NO_3 from new and previously used clay pots, which were soaked in deionized distilled water. Mean release rates initially exceeded 2.4 and 725 μmol l^{-1} day^{-1} P and 0.22 and 18 μmol l^{-1} day^{-1} N from new and used pots, respectively, but declined rapidly with increasing time spent in deionized distilled water and were below detectable levels after about 18 and 29 days, respectively.
3. A phosphorus (P) dose–response experiment in a P-limited reach of the Athabasca River, Alberta, Canada showed that epilithic biomass and macroinvertebrate density on NDS increased with increasing concentrations of KH_2PO_4 up to about 0.5 M. Beyond this threshold, biomasses and densities were unaffected by initial KH_2PO_4 concentration. Coefficients of variation of epilithic biomass estimates declined with increasing KH_2PO_4 whereas invertebrate density appeared to be unaffected by KH_2PO_4 levels.
4. Release rates of both P and N from NDS filled with 0.5 M KH_2PO_4 or 0.5 M $NaNO_3$ declined at a log-negative rate from about 5000 μmol $N\text{-}NO_3$ l^{-1} day^{-1} and 3500 μmol $P\text{-}PO_4$ l^{-1} day^{-1} on day 2, to 200 μmol l^{-1} day^{-1} for both N and P on day 32.
5. After development, we used the diffusing substrata to identify spatial patterns in nutrient limitation at seven sites along a 120 km reach in the Athabasca River, that receives two known point-source nutrient inputs. NDS consisting of N, P, N + P and unenriched controls were attached to the river bottom for 22–23 days and then retrieved and sampled for epilithic chlorophyll *a*. Physicochemical parameters and epilithic biomasses on upper stone surfaces were also quantified when NDS were deployed and retrieved from each site.
6. Sites located immediately downstream of the two point source inputs had higher water column concentrations of PO_4 and epilithic biomasses than the site immediately upstream; epilithic biomass was positively related to PO_4 in the late autumn ($r^2 = 0.58$) but not in early autumn. Sites located immediately below nutrient inputs were not nutrient-limited, whereas upstream reference sites were P-limited.

Introduction

Nutrient enrichment is one of the most widespread anthropogenic stressors of aquatic ecosystems and can affect aquatic ecosystem structure and function by altering abundance, biomass and species diversity of epilithic, macroinvertebrate and fish communities (Lowe, Golladay & Webster, 1986; Pringle, 1987; Bothwell, 1988; Biggs, 1989; Johnston *et al.*, 1990; Winterbourn, Hildrew & Orton, 1992; Peterson *et al.*, 1993). Our ability to predict the effects of nutrient inputs to river systems is generally poor because of the dependency of the magnitude of enrichment effects upon interactions between a suite of abiotic and biotic factors.

Nutrient diffusing substrata (NDS) have been widely used to investigate spatial and temporal patterns in nutrient limitation in lakes (Fairchild & Lowe, 1984; Pringle & Bowers, 1984; Fairchild, Lowe & Richardson, 1985; Barnese & Schelske, 1994), small streams (Lowe *et al.*, 1986; Winterbourn & Fegley, 1989; Winterbourn, 1990) and larger, low-gradient rivers (Corkum, 1996). However, our preliminary field trials to identify spatial patterns in nutrient limitation in the Athabasca River, Alberta, Canada showed that plastic Petri dish designs (Corkum, 1996) and conventionally used clay designs (Fairchild & Lowe, 1984) with high vertical profiles were unsuitable for use in the fast-flowing Athabasca River and were either damaged or scoured from the substratum over 30-day incubations (Scrimgeour *et al.*, 1995; G. J. Scrimgeour unpublished data). Further, attachment of other designs (Pringle & Bowers, 1984) is difficult because dramatic declines in river stage of the Athabasca during the autumn, when nutrient enrichment is a concern (Scrimgeour *et al.*, 1995; Chambers, 1996), requires that NDS be attached to the river bottom in areas where water depths range between 55 and 95 cm. The areas of these sites are located in the upper reaches of the Athabasca River, where gradient is relatively high, so current velocities typically range from 70 to 110 cm s^{-1}.

In this study we describe laboratory and field experiments, initially to develop, and then apply, a nutrient diffusing bioassay for use in large, fast-flowing rivers. To accomplish this we: (i) quantified nitrogen (N) and phosphorus (P) release rates from new and previously used NDS to identify the length of time that NDS should be soaked in deionized-distilled water before field deployment; (ii) determined the minimum molar concentration of P as KH_2PO_4 that should be mixed with agar in the P-enriched treatment; and (iii) completed field experiments to determine whether our design could identify spatial patterns in nutrient limitation along a 120-km reach of a large river system in Alberta, Canada.

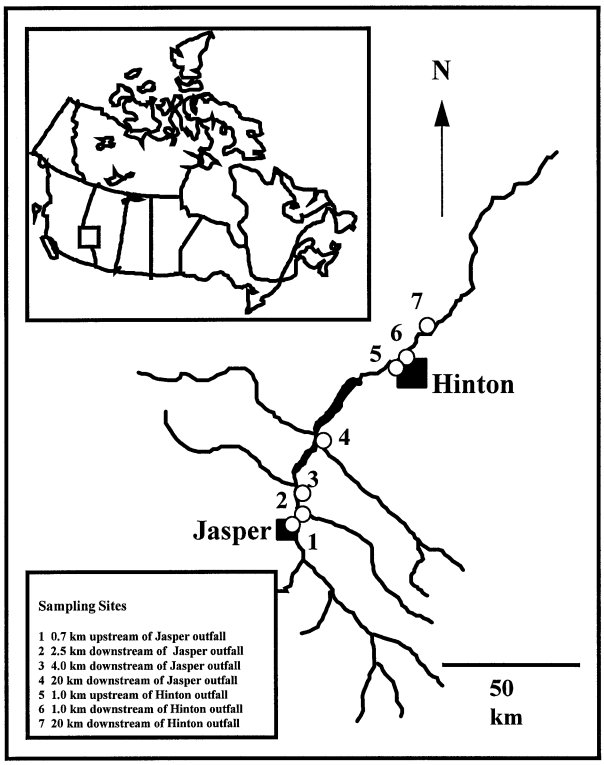

Fig. 1 Location of study sites in the upper reaches of the Athabasca River, Alberta, Canada.

Study area

The glacial-fed Athabasca River originates in the Rocky Mountains of west-central Alberta, Canada and flows north-east across Alberta to Lake Athabasca where it joins with the Peace River to form the Slave River. The Athabasca River is unregulated and mean daily flows at Hinton (Fig. 1) average 407 m^{-3} s^{-1} (1980–93) with peak flows occurring in June after mountain snow-pack melt (1016 m^{-3} s^{-1}, 1980–93) and lowest flows in February (62 m^{-3} s^{-1}, 1980–93) (Environment Canada, 1994).

Fieldwork was conducted at seven sites within a 120-km reach of the upper Athabasca River, Alberta,

Canada (Fig. 1). This reach receives municipal sewage from the Town of Jasper and a combined municipal-industrial effluent consisting of sewage from the Town of Hinton and a 1050 air-dry tonnes day^{-1} bleached kraft pulp mill (Anderson, 1991; Tones, 1994; Chambers, 1996). Jasper, with a resident population of about 3600 (summer tourist population = 10 000), discharges 3948 ± 272 m^{-3} day^{-1} (mean ± SE, $n = 13$, 1988–93) effluent with total P (TP) and total N (TN) concentrations of 4.3 ± 0.1 and 19.9 ± 1.9 mg l^{-1}, respectively, from aerated stabilization basins. The Hinton combined effluent is treated by aerated stabilization basins and discharged at $106\,613 \pm 415$ m^{-3} day^{-1} ($n = 1450$) with 0.7 ± 0.3 mg l^{-1} TP ($n = 204$) and 4.9 ± 0.1 TN ($n = 138$) (Chambers, 1996).

Immediately upstream of the Hinton effluent discharge, total dissolved phosphorus and total dissolved nitrogen to total dissolved phosphorus ratios (TDN : TDP) average 2 ± 1 g l^{-1} and $110 \pm 64 : 1$ (± SD, $n = 7$), respectively, during winter low flows (1988–92). Daily inputs of N and P from the mill's aerated stabilization basin averaged 535 ± 115 kg TN (± SD, $n = 138$, 1990–93) and 79 ± 3 kg TP (± SD, $n = 203$, 1990–93) (Chambers, 1996). Nutrient enrichment effects (high epilithic biomasses) are evident within this reach downstream of the effluent outfall and are most pronounced in spring and autumn when discharge is low and water clarity is high (immediately preceding and following the large spring freshette) (Anderson, 1991; Scrimgeour *et al*., 1995; Scrimgeour & Chambers, 1996).

Materials and methods

Experiment 1. Selection of a clay-diffusing substrate design and quantification of N and P diffusion rates from new and previously used pots

A commercially available ribbed, clay flower pot (outer diameter = 11 cm, height = 6 cm, internal volume = 325 ml) was tested for use as a candidate for a nutrient-diffusing bioassay (Fig. 2). This design was chosen because it is has a relatively low vertical profile and is more robust than either the typical cone-shaped design (Fairchild & Lowe, 1984) or plastic Petri dishes (Corkum, 1996), which were unable to withstand physical stresses of the Athabasca River (Scrimgeour *et al*., 1995; G. J. Scrimgeour unpublished data).

The NDS consists of a pot with a 12-cm-diameter polypropylene base (thickness = 4 mm) and two 20-cm-long attachment pegs. One hole was drilled on each side of the pot and a strand of nylon cord (2-mm diameter) was passed through each side to produce two attachment loops. The strand was secured within the pot using an electrical cable tie. During deployment, the plastic pegs are passed through the attachment loops and driven into the river bottom (Fig. 2).

Prior to deployment, NDS are typically soaked in deionized and/or distilled water to ensure that N and P released from the clay does not contaminate enrichment and control treatments. While a 2- to 3-day period is commonly used for clay-diffusing substrata (Fairchild *et al*., 1985; Ghosh & Gaur, 1994), there is no experimental evidence that this length of time is sufficient. To quantify releases of N and P from new and previously deployed NDS, we placed six pots (three new and three previously deployed) in individual 2-l acid-washed beakers which contained 1 l of distilled-deionized water. Previously deployed NDS had been filled with either 0.5 M KH_2PO_4 or 0.5 M $NaNO_3$ and incubated for 32 days in riffles of the Athabasca River after which the agar-nutrient mixture was discarded and the pot washed thoroughly in distilled-deionized water. Beakers were maintained at 18 °C and agitated eight to ten times per day. Water from each of three replicate beakers was replaced daily and concentrations of NO_3 and PO_4 measured at 1- to 5-day intervals for 32 days. Sampling of water from individual beakers consisted of mixing the water in the beakers thoroughly and then pouring a 250-ml sample into a polyethylene bottle for analysis for PO_4 and NO_3. The beaker was then rinsed thoroughly with deionized-distilled water before the pot was placed back into the beaker and refilled. Concentrations of P-PO_4 and N-NO_3 were measured on a Technicon II autoanalyser (Stainton, Capel & Armstrong, 1977) using the molybdenum blue and the cadmium reduction techniques, respectively, (APHA, 1975). These techniques result in detection limits of 0.1 μg l^{-1} PO_4 and 0.5 μg l^{-1} NO_3.

Experiment 2. Phosphorus dose–response experiments

Experiments identifying nutrient limitation with NDS typically rely on four treatments consisting of nitrogen (0.33–1.5 M $NaNO_3$), phosphorus (0.02–0.5 M KH_2PO_4, K_2HPO_4, or NaH_2PO_4), N + P and unamended controls (Fairchild & Lowe, 1984; Winterbourn, 1990;

Winterbourn *et al*., 1992; Barnese & Schelske, 1994; Ghosh & Gaur, 1994; Corkum, 1996). Nutrient concentrations should affect algal accrual and production costs, therefore we completed an initial field trial to determine the minimum amount of P, as KH_2PO_4, that must be added to an agar solution to saturate algal growth. We selected P enrichment levels of 0.01, 0.02, 0.05, 0.1, 0.5, 1.0, 2.0 M KH_2PO_4 to span likely ranges that would saturate growth requirements, along with nutrient-absent controls. Ten replicates of each treatment were deployed at site 6 where the epilithic community was known to be P limited (Scrimgeour *et al*., 1995).

To prepare the NDS, the new clay pots were soaked in deionized-distilled water for 15 days and then allowed to air dry before being filled with hot agar solutions. Agar solutions were prepared in 4-l beakers by mixing agar and autoclaved, deionized-distilled water (20 g agar l^{-1}) on continually stirring hot plates. Solutions were heated to 90 °C to ensure that the agar was fully dissolved and then stirred for an additional 20 min without heating to allow solutions to cool to 70 °C. In nutrient-enriched treatments, N and/or P were added gradually over 5 min and stirred for an additional 10 min to allow nutrients to dissolve fully. The agar solutions were then poured into NDS and the entire pot attached to a polypropylene base with aquarium-safe silicone (silicone that does not contain an active fungicide). The agar solution is partially absorbed into the clay wall, so 350 ml was typically added to each 325-ml NDS. NDS were stored in the dark at 5 °C before deployment in the field.

During these trials, NDS were attached to the river bottom, retrieved after 22 days and sampled for epilithic chlorophyll *a* and macroinvertebrates. NDS were attached to the river bottom in riffles where current velocities and depths ranged from 0.65–1.1 m s^{-1} and 55–95 cm, respectively. To reduce interference of nutrients leached from upstream treatments, NDS were separated by a minimum of 1.5 m along the direction of flow. Under high water velocities, accrual of epilithic biomass is considerably higher on the downstream-facing surface compared to the upstream-facing surface. Thus to standardize sampling position, epilithic biomass was removed from the downstream-facing surface of each NDS with a stout brush from within 9.6 cm^{-2} as delimited by a plastic template. The sample was placed into a scintillation vial, frozen immediately and stored in the dark until analysed. Epilithic biomass, measured as chlorophyll *a*, was determined following ethanol extraction on a Turner designs model 10 series fluorometer.

Invertebrate fauna associated with NDS were sampled with a U net sampler (mesh size = 0.25 mm) (Scrimgeour, Culp & Glozier, 1993). Substrata were approached from downstream to minimize disturbance of macroinvertebrates, encompassed with the U net sampler and then the attachment pegs were removed from the substratum and the NDS placed within the sampler. The NDS fauna included individuals present on the outer wall as well as those found in a thin layer of sediment immediately beneath the NDS. These animals may use the upper substratum surfaces during the hours of darkness (Culp & Scrimgeour, 1993) and we therefore considered them a valid part of the NDS macroinvertebrate fauna. This microhabitat immediately below the NDS was sampled by agitating sediments to a depth of 5 mm. The net, and the enclosed NDS, were then lifted out of the river and placed into a plastic dish. Total invertebrate density was determined by combining invertebrates collected in the U net with those attached to the NDS. Invertebrates were preserved in 70% ethanol and later sorted and counted in the laboratory.

The effects of nutrient treatment on epilithic chlorophyll *a* and macroinvertebrate abundances were tested with a single factor ANOVA using SAS statistical software (SAS Inc., 1987). When ANOVA indicated a significant treatment effect, treatment means were compared with Least Significant Difference (LSD) criteria (Neter, Wasserman & Kutner, 1990) using an alpha of 0.05. Where appropriate, data were transformed to meet assumptions of ANOVA. Linear regression was used to determine whether there was any functional relationship between macroinvertebrate density and epilithic biomass on NDS, assuming that colonizing macroinvertebrates are responsive to spatial variation in epilithic biomass and that epilithic chlorophyll *a* measured on the downstream surface is related to the total algal food resource on each NDS in some manner.

Experiment 3. Quantifying nutrient release coefficients

Laboratory experiments were performed to quantify release rates from NDS that were filled with an agar solution containing 0.5 M KH_2PO_4 or 0.5M $NaNO_3$. Release rates of N-NO_3 and P-PO_4 were estimated over 32 days for individual NDS placed in 2-l glass

beakers filled with 1 l of deionized-distilled water. Water from each beaker was changed daily and the removed water sampled for NO_3 and PO_4 at 1- to 5-day intervals as described for experiment 1. A thin microbial biofilm formed on the inside of each beaker during the experiment and to minimize uptake of NO_3 and PO_4 by this community, beakers were replaced with acid-washed beakers every 4 days.

Release rates could be underestimated if diffusion of nutrients from the NDS resulted in similar nutrient concentrations in the surrounding water and the NDS (the lack of a concentration gradient). To ensure release rates were constant over the 24-h period before water within each beaker was changed, a 5-ml sample was removed with an autopipette from each beaker at 1- to 3-h intervals on day 15. Samples were placed in acid-washed 50-ml test tubes, stored in the laboratory for 1 day and analysed for NO_3 and PO_4 as stated previously. Linear regression was used to determine whether release rates of NO_3 and PO_4 were constant over 24 h.

Experiment 4. Field test of the nutrient bioassay

After development, we field tested the NDS design at seven sites along a 120-km reach of the Athabasca River, Alberta between September and October 1994. Ten replicates of each nutrient-enriched treatment (N, P and N + P amended) and unamended controls were randomly dispersed throughout a 150-m riffle-reach at each site. To reduce contamination among treatments, we ensured that NDS were separated by a minimum of 1.5 m along the direction of flow. River stage declines dramatically in the late autumn when input from the Athabasca Glacier is reduced, and so NDS were attached to the river bottom in deep (range = 55–90 cm) and, thus, fast-flowing water (0.7–1.2 ms^{-1}). While ten replicates of each treatment were prepared, one of each treatment was often lost due to scouring or breakage during deployment. NDS were deployed on 26–28 September and retrieved from the river bottom after either 22 days (sites 1–3, 5 and 6) or 23 days (sites 4 and 7) and sampled for epilithic chlorophyll *a* as described previously.

Water chemistry and estimates of epilithic biomass on upper stone surfaces were sampled at each site upon placement and retrieval of NDS. Instantaneous estimates of pH and water temperature (°C) were recorded with a Fisher Scientific Accumet 1000 series hand-held meter; samples for dissolved oxygen were collected in 500-ml dissolved oxygen bottles and determined according to Carpenter's (1965) modified Winkler technique. Samples for dissolved inorganic nitrogen (DIN; NO_2 + NO_3, NH_4^+) were collected in 50-ml polystyrene bottles, whereas samples for PO_4 were collected in 500-ml Nalgene polyethylene bottles. All samples were stored on ice in the field and refrigerated at 4 °C in the laboratory before analysis. Nitrite + nitrate samples were filtered through pre-washed 0.45-m HAWP millipore membrane filters; nitrite + nitrate and NH_4^+ concentrations were determined with a Technicon autoanalyser (Stainton *et al*., 1977). Samples for PO_4 were analysed as stated previously. Estimates of epilithic biomass on upper surfaces of stones from riffles where NDS were deployed were determined using a 9.6 cm^{-2} template as previously stated for epilithic chlorophyll *a* on NDS.

The effects of nutrient treatment on epilithic chlorophyll *a* were tested for each site with a single factor ANOVA on $\log_{10}$ transformed data to remedy inequality of variances, and LSD tests were subsequently employed to compare treatment means when the ANOVA was significant. When the ANOVA tests were not significant we estimated the power of each test as described in Zar (1984).

Results

Experiment 1. Selection of a clay-diffusing substrate design and quantification of N and P diffusion rates from new and previously used pots

Daily release rates of PO_4 and NO_3 from new and previously deployed NDS declined rapidly over time in distilled-deionized water (Table 1). For new pots, P-PO_4 and N-NO_3 declined below analytical detection levels (0.1 µg l^{-1} PO_4, 0.5 µg l^{-1} NO_3) after 18 and 15 days, respectively. Release rates of PO_4 and NO_3 from previously used pots were considerably higher than from new pots but also declined below detection limits by 29 days (Table 1). That N release rates were below detection by 15 days, and P were less than 0.003 µmol l^{-1} day^{-1} on day 15, suggests that 15 days is a suitable minimum length of time that new pots should be soaked prior to field deployment. In contrast, previously used pots should be soaked for at least 29 days prior to field deployment.

Table 1 Mean (± 1 SE) daily release rates (μmol l^{-1} day^{-1}) of PO_4 and NO_3 from (a) new and (b) previously used clay diffusing substrata. < DL = below detection limits of 0.1 μg PO_4 l^{-1} and 0.5 μg NO_3 l^{-1})

(a) New substrata			(b) Previously used substrata		
Day	PO_4	NO_3	Day	PO_4	NO_3
1	2.48 ± 0.01	0.22 ± 0.08	1	725.95 ± 21.07	18.89 ± 0.55
4	0.95 ± 0.05	1.88 ± 1.58	3	262.47 ± 26.15	6.83 ± 0.68
8	0.68 ± 0.10	0.78 ± 0.67	4	121.57 ± 7.38	3.16 ± 0.19
12	0.81 ± 0.03	0.09 ± 0.04	8	65.86 ± 3.66	1.71 ± 0.09
15	0.003 ± 0.003	< DL	13	38.92 ± 3.18	1.01 ± 0.09
18	< DL	< DL	19	31.05 ± 5.79	0.81 ± 0.15
21	< DL	< DL	25	17.13 ± 3.28	0.44 ± 0.09
			29	< DL	< DL
			32	< DL	< DL

Experiment 2. Phosphorus dose–response experiments

Epilithic biomass and density of macroinvertebrates on NDS were both significantly affected by P treatment (ANOVA: epilithon: $F_{(6,77)} = 60.1$, $P < 0.0001$, macroinvertebrates: $F_{(6,77)} = 83.4$, $P < 0.0001$) and increased with increasing molar concentration of KH_2PO_4 (Fig. 3). Asymptotic accrual and macroinvertebrate densities were reached when substrata contained about 0.5 M KH_2PO_4 (LSD tests, $P < 0.05$). Moreover, macroinvertebrate density, comprising predominantly mayfly and chironomid larvae, was significantly related to epilithic biomass on NDS ($\log_{10}$ density = 1.85 + 0.06 chlorophyll *a*, $P < 0.0001$, $r^2 = 0.50$). For algal communities, sample variation differed markedly with KH_2PO_4 concentration and decreased fivefold with increasing KH_2PO_4 molarity (Fig. 3). In contrast, coefficient of variation was relatively low and stable for estimates of macroinvertebrate density. These data suggest that for the phosphorus treatments, mixing agar with a 0.5 M KH_2PO_4 maximizes epilithic accrual and invertebrate colonization, and minimizes chemical costs and sample variation.

Experiment 3. Quantifying nutrient release coefficients

Release rates of NO_3 and PO_4 on day 1 were lower than on day 2 probably because N and P are initially absorbed into the clay wall. When data from day 1 are excluded from the analyses, release rates of NO_3 and PO_4 decreased at a log-linear rate over the remaining 31-day period ($\log_{10} NO_3 = 3.40–0.036$ days, $F_{(1,52)} = 33.44$, $P < 0.001$, $r^2 = 0.69$; $\log_{10} PO_4 = 3.39–0.032$ days, $F_{(1,52)} = 108.9$, $P < 0.001$, $r^2 = 0.75$) (Fig. 4). Thus, N and P release rates declined from about 5000 μmol NO_3 l^{-1} day^{-1} and 3500 μmol PO_4 l^{-1} day^{-1} on day 2 to about 200 μmol l^{-1} day^{-1} for both NO_3 and PO_4 on day 32. Moreover, concentrations of N and P in beaker water over a 24-h period on day 15 increased in a linear fashion (NO_3: $r^2 = 0.89$; PO_4: $r^2 = 0.92$) suggesting that concentration gradient did not decline appreciably throughout the 24-h period during which water was not replaced.

Experiment 4. Field test of the nutrient bioassay

While there were minor differences in pH, DO and DIN concentrations among sites, PO_4 concentrations and N : P ratios were 2–4 times higher and 2–3 times lower at downstream sites (Table 2). Epilithic biomass on upper stone surfaces differed significantly between sites ($P < 0.0001$) and was significantly higher downstream than upstream of nutrient inputs (LSD tests, Table 2). Linear regressions showed that epilithic biomass was significantly related to PO_4 in the late autumn when NDS were retrieved from the river (epilithic biomass = 2.54 + 3.27 PO_4, $F_{(1,5)} = 8.0$, $P < 0.001$, adjusted $r^2 = 0.58$), but not earlier in the season when NDS were deployed ($P > 0.05$).

Results from NDS showed that the effects of the nutrient enrichment treatments differed among sites (Fig. 5). Epilithic biomass was significantly higher on P and N + P-enriched compared with N-enriched and control substrata 1 km upstream and 20 km downstream of Jasper, and 1 km upstream of Hinton, indicating that these sites were primarily P limited. In contrast, ANOVAs were not significant 2.5 and 4 km downstream of Jasper or 1 and 20 km downstream of Hinton suggesting that these sites were not nutrient

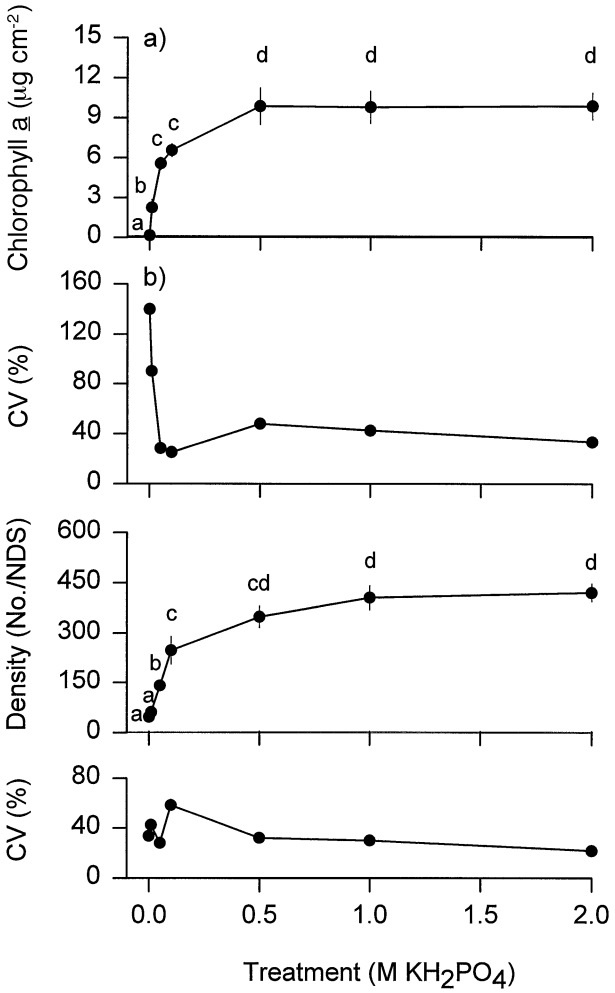

Fig. 3 Mean ($\bar{x} \pm 1$ SE) and coefficient of variation of epilithic chlorophyll *a* (g cm^{-2}) (a and b) and macroinvertebrate densities (c and d) on nutrient-diffusing substrata (NDS) enriched with 0–2 M KH_2PO_4. Standard errors for chlorophyll *a* and macroinvertebrate density estimates may be contained within symbols. Means sharing the same letter are not significantly different (Least Significant Difference tests, $P > 0.05$).

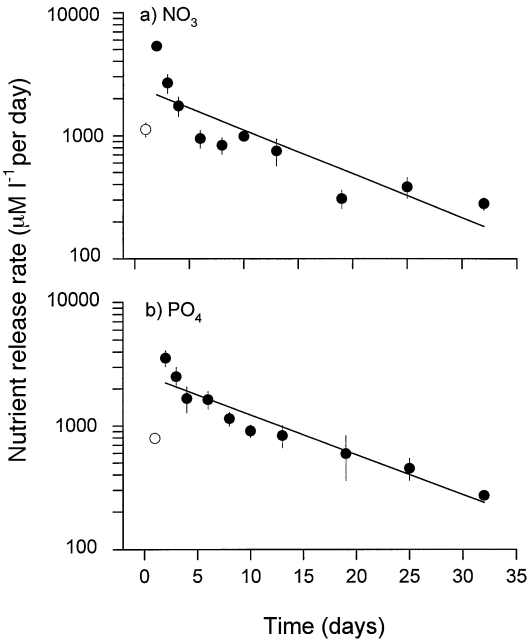

Fig. 4 Mean ($\bar{x} \pm 1$ SE) diffusion rates (µMol l^{-1} day^{-1}) of PO_4 (a) and NO_3 (b) released from NDS containing 0.5 M KH_2PO_4 or 0.5 M $NaNO_3$ over 32 days. Open symbols are release rates on day 1 which were excluded from analyses. Standard errors may be contained within symbols.

limited (Fig. 5). However, the lack of significant treatment differences at these sites should be viewed cautiously as our estimates of statistical power for these analyses were low (power < 35%).

Discussion

Large volume (325 ml) clay NDS are a useful design to identify spatial patterns in nutrient limitation in a large river system where dramatic declines in water stage necessitate placement of NDS in deep, fast-flowing areas. Our ability to identify P-limited reaches of the Athabasca River reaches was enhanced by adopting a rigorous experimental approach to validate methodology, which was: (i) P and N release rates from new and used substrata; (ii) nutrient release rates from P- and N-amended NDS; and (iii) the effect of KH_2PO_4 concentration on mean and sample variance of algal biomass accrual and macroinvertebrate colonization.

Diffusion of nutrients from NDS should be strongly affected by the material comprising the diffusion membrane. In general, NDS can be divided into three types based on whether the nutrients in an agar or agar-sand mixture diffuse through: (i) clay (Fairchild & Lowe, 1984; Fairchild *et al.*, 1985; Barnese & Schelske, 1994; Ghosh & Gaur, 1994); (ii) an agar-sand layer (Pringle & Bowers, 1984); or (iii) fine-mesh (Winterbourn, 1990; Corkum, 1996). Release of nutrients should be lowest through clay, and considerably higher where nutrients diffuse through an agar-sand mixture or fine mesh. The quantity of nutrients released is probably determined by concentrations of N and P, both in the agar and surrounding water, current velocity, the nature of the diffusing membrane, and the presence of other materials within the agar matrix (for example, sand, Pringle & Bowers, 1984).

Table 2 Longitudinal patterns in physicochemical characteristics and epilithic chlorophyll *a* at seven study sites in the Athabasca River, Alberta, 1994. DIN = dissolved inorganic nitrogen, N : P ratios calculated as molar ratios of DIN : PO_4. Values are means of samples taken in early (September) and late (October) autumn, u/s = upstream, d/s = downstream, – data not included in analyses

Site	°C	pH	DO ($g\ l^{-1}$)	DIN ($\mu g\ l^{-1}$)	PO_4 ($\mu g\ l^{-1}$)	N : P ratio	Chlorophyll *a* ($\mu g\ cm^{-2}$)
Jasper							
1 km u/s sewage	6.8	7.7	13.3	85.7	1.7	122	2.5 ± 0.4
2.5 km d/s sewage	6.8	7.9	13.3	66.1	3.4	43	22.7 ± 2.7
4 km d/s sewage	5.8	8.2	13.9	78.4	3.7	47	31.7 ± 3.9
20 km d/s sewage	6.0	8.0	13.4	86.5	–	–	5.9 ± 1.0
Hinton							
1 km u/s effluent*	7.3	8.1	14.3	56.7	2.2	58	7.9 ± 1.3
1 km d/s effluent*	7.5	8.2	14.2	58.2	7.2	18	21.8 ± 2.4
20 km d/s effluent*	7.3	7.9	15.3	40.4	4.1	22	18.9 ± 2.1

*Effluent discharged into the Athabasca River at Hinton comprises a discharge from a bleached kraft pulp mill that is combined with municipal sewage.

Our data showed that diffusion rates of N and P from NDS containing 0.5 M KH_2PO_4 or 0.5 M $NaNO_3$ declined at a log-negative rate through time. While our diffusion rates are qualitatively similar to those found for another clay-diffusing design (Fairchild *et al.*, 1985) and a Petri dish design filled with an agar-sand mixture (Pringle & Bowers, 1984), they are intermediate in magnitude between the clay and Petri dish designs. For instance, Fairchild *et al.* (1985) found that release rates of N and P from clay NDS containing 0.5 M K_2HPO_4 and 0.5 M $NaNO_3$ declined from about 10 000 µmol N l^{-1} day^{-1} and 5000 µmol P l^{-1} day^{-1}, respectively, to about 2000 µmol for both N and P after 23 days. Loss of nutrients from Petri dishes filled with 0.5 M KH_2PO_4 and 0.5 $NaNO_3$ within an agar-sand mixture declined from about 200 µmol NO_3 l^{-1} day^{-1} and 100 µmol PO_4 l^{-1} day^{-1} to < 25 µMol l^{-1} day^{-1} for both N and P after only 6 days. Our results showed that release rates of N and P from NDS filled with 0.5 M KH_2PO_4 or 0.5 M $NaNO_3$ declined from about 3500 and 5000 µmol, respectively, on day 2 to 200 µmol l^{-1} day^{-1} for both N and P on day 32.

Losses of nutrients from NDS are typically quantified by placing substrata in beakers containing distilled and/or deionized water (Pringle & Bowers, 1984; Fairchild *et al.*, 1985). Water is changed daily and N and P concentrations are typically measured at 1- to 5-day intervals. Release rates of N and P would be underestimated if diffusion from the NDS results in similar nutrient concentrations in the NDS and the surrounding water (no diffusion gradient) prior to water replacement (< 24 h). In this situation, concentrations of N and P in the surrounding water would increase at a decelerating rate. Our data showed that N and P losses from NDS over a 24-h period increased at a linear rate with time and there was no evidence of a decelerating function. While these data suggest that nutrient losses from our design can be reasonably estimated in standing water microcosms providing that the water is replaced at 24 h, additional nutrient loss experiments, using a range of water velocities, are required before the effect of water velocity on N and P losses from substrata are fully understood.

We are not suggesting that previous NDS studies which used other than 0.05 M KH_2PO_4 employed nutrient enrichment levels that were above or below those required. The fact that Pringle & Bowers (1984), Fairchild *et al.*, (1985), Winterbourn (1990), Winterbourn *et al.*, (1992) and Corkum (1996) identified significant differences among N, P or N + P treatments compared with nutrient-absent controls indicates that N and P enrichment levels were sufficient for their NDS design methodology. Rather, we suggest that researchers need better to understand diffusion rates from their chosen designs, especially when results fail to identify a limiting nutrient.

Enrichment of NDS with nutrients is typically achieved by adding N as $NaNO_3$ and P as K_2HPO_4, KH_2PO_4 or NaH_2PO_4 (Pringle & Bowers, 1984; Fairchild *et al.*, 1985; Winterbourn & Fegley, 1989; Winterbourn, 1990; Winterbourn *et al.*, 1992; Barnese

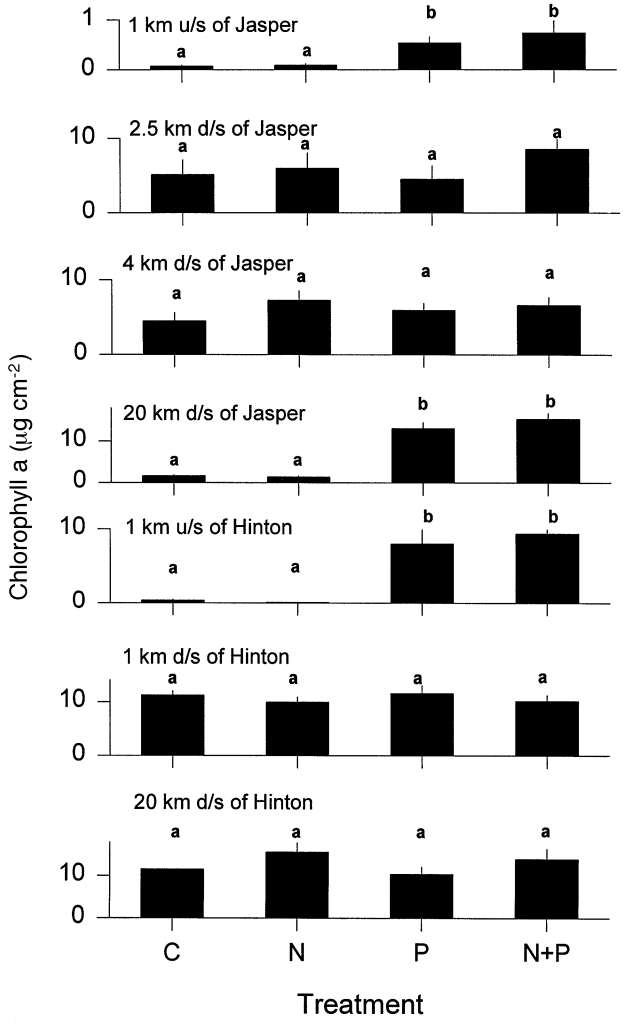

Fig. 5 Mean ($\bar{x} \pm 1$ SE) chlorophyll *a* concentration (g cm^{-2}) on control and nutrient-enriched diffusing substrata in the upper Athabasca River, Alberta, Canada. Means sharing the same letter are not significantly different (Least Significant Difference tests, $P > 0.05$). Note differences in *y*-axis scales.

& Schelske, 1994; Corkum, 1996). Molar concentrations of these chemicals are highly variable, both among and within studies, depending on whether the NDS contain solely N, P or other nutrients. In the majority of cases, the rationale for using a particular level is not provided or is based on laboratory studies which compare diffusion rates of two different molarities. This approach precludes identifying nutrient enrichment levels that maximize algal accrual or the numbers of colonizing macroinvertebrates, or minimize sample variation or chemical costs. Our P dose–response experiment showed that P molarity significantly affected means and variances of algal biomass and total invertebrate density estimates. Mean algal biomass increased with increasing P concentration up to about 0.5 M KH_2PO_4, whereas sample variance decreased with increasing P levels up to 0.05 M KH_2PO_4, and was relatively low and stable beyond these levels. These data suggest that for our design, selecting an appropriate KH_2PO_4 molarity represented a balance between maximizing algal biomass accrual and minimizing sample variance. Finally, while different nutrient molarities would almost certainly affect algal species composition, and thus estimates of epilithic chlorophyll *a*, these effects remain to be quantified.

Field tests of the NDS

Field tests using our NDS design showed that upper regions of the Athabasca River comprise both P-limited sites and sites that were presumably nutrient unlimited. Nutrient-unlimited sites were located 1–4 km downstream of known nutrient inputs, whereas P-limited sites were located immediately upstream of nutrient inputs or at greater distances downstream. The lack of a significant difference among N-, P-, N + P-enriched and control substrata results either when: (i) nutrient concentrations are sufficiently high to saturate epilithic requirements; (ii) algal growth is limited by other factors, such as light availability; or (iii) as an artefact of low statistical power.

While we cannot ignore our low estimates of statistical power following ANOVA tests (35%), we present three lines of evidence which suggest that the absence of significant differences among the four nutrient treatments at sites downstream of nutrient inputs (sites 2, 3, 6 and 7) results from nutrient saturation rather than low statistical power. First, that concentrations of PO_4 were about twofold higher downstream of known nutrient inputs (overall mean of sites 2, 3, 6 and 7 = 4.6 μg l^{-1}) than at upstream sites (sites 1 and 5 = 2.0 μg l^{-1}), is consistent with the notion of P limitation. This line of argument is strengthened further because flow-through mesocosm studies completed 1 km upstream of the combined Hinton effluent (site 5) show that these levels of P enrichment at downstream sites are sufficient to saturate epilithic mat requirements (Chambers, 1996). Second, algal biomass on all four NDS treatments downstream of the nutrient inputs were substantially higher than that on control and N-enriched substrata but relatively similar to P- and N + P-enriched substrata upstream where NDS showed P limitation. Third, there is little

evidence of marked differences in either water temperatures or light attenuation coefficients at any of the study sites. Instantaneous water temperatures at all study sites ranged from 5.8 to 7.5 °C and did not differ in a predictable fashion between upstream P-limited and downstream nutrient-limited sites. Finally, the river bottom at all sites received $> 1\%$ surface light (Scrimgeour & Chambers, 1996).

NDS as a management tool

Historically, NDS have been used to address predominantly theoretical questions related to algal succession (Fairchild & Lowe, 1984), nutrient limitation (Lowe *et al*., 1986), or trophic interactions between nutrients, algae and invertebrates (Winterbourn & Fegley, 1989; Winterbourn, 1990; Winterbourn *et al*., 1992). More recently, NDS have been used to address a wider array of questions including applied, management issues (Barnese & Schelske, 1994; Scrimgeour *et al*., 1995; Corkum, 1996; Dube, Culp & Scrimgeour, 1997). For example, Corkum (1996) used NDS to identify the effects of different land use types on spatial patterns in nutrient limitation in six Ontario streams. Similarly, NDS have been used successfully to identify spatial and temporal patterns in nutrient limitation in the Athabasca (Scrimgeour *et al*., 1995) and Thompson (Dube *et al*., 1997) rivers resulting from enrichment from bleached kraft pulp mill effluents.

If NDS are to be used as a nutrient bioassay, the technique will need to include more rigorous experimental designs and levels of replication that increase statistical power. The effects of replication on statistical power will probably be affected by a complex suite of biotic and abiotic factors which determine algal accrual rates, and will probably be specific to the type of NDS that is deployed. Our field trials showed that using 6–10 (overall mean = 9.2) replicates per treatment was sufficient to identify three of the seven sites on the Athabasca River as being P limited, even where mean epilithic accrual on P-enriched substrata was < 1 g chlorophyll *a* cm^{-1} and the difference between P-enriched and control substrata was < 0.7 g chlorophyll *a* cm^{-1} as at site 1.

Finally, the utility of NDS as a management tool will be enhanced if, in addition to identifying river reaches which are susceptible to enrichment, NDS can be used to make quantitative predictions of the responses of epilithon to enrichment. For example, in P-limited reaches, can the difference in epilithic biomass on P-enriched vs. control substrata be used to quantify the magnitude of the response of the natural epilithic assemblage? Our data showed that in late autumn, epilithic biomass on upper surfaces of stones was about sixfold higher at nutrient-unlimited sites (combined mean of sites 2, 3, 6 and 7) than nutrient-limited sites (combined mean of sites 1, 4 and 5). In contrast, algal biomass on P-enriched substrata at sites 1, 4 and 5 was about twofold higher than on nutrient-absent controls. Thus, the difference in algal accrual on control vs. P-enriched NDS was about three times lower than the difference in epilithic biomass between sites upstream vs. downstream of nutrient inputs. Developing rules of thumb such as this combined with a rigorous scientific approach will enhance the utility of NDS as a management tool.

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Fig. 2 Ribbed clay nutrient-diffusing substratum attached to the substratum of the Athabasca River. Attachment pegs located on either side pass through a 2 mm diameter nylon cord that extends through the clay pot. Clay pot diameter = 11 cm.