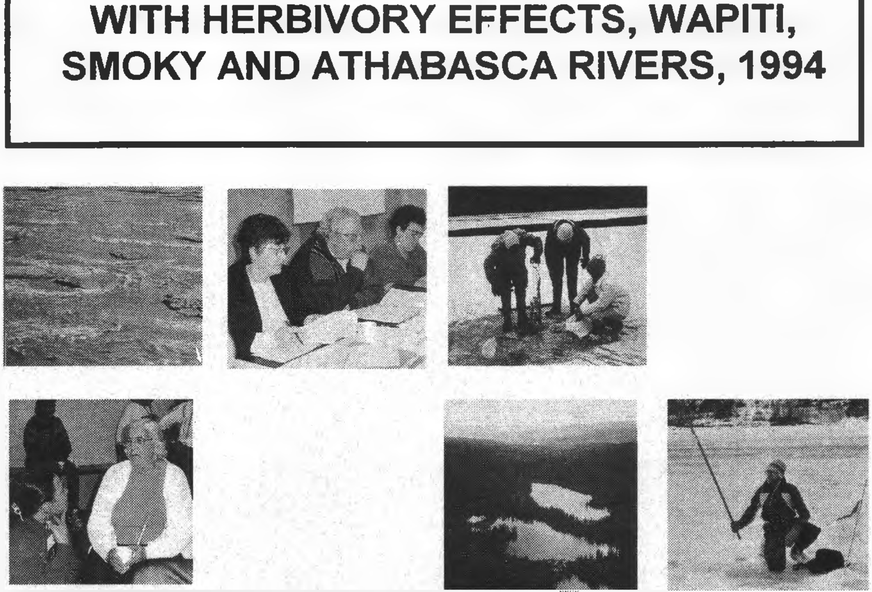
Canada Alberta Northwest Territories

# Northern River Basins Study

NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 96

# IDENTIFICATION OF SPATIAL AND TEMPORAL PATTERNS IN NUTRIENT LIMITATION

## WITH HERBIVORY EFFECTS, WAPITI, SMOKY AND ATHABASCA RIVERS, 1994



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Prepared for the
Northern River Basins Study
under Projects 2614-D1 and 2614-E1

by

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**PREFACE:**

The Northern River Basins Study was initiated through the "Canada-Alberta-Northwest Territories Agreement Respecting the Peace-Athabasca-Slave River Basin Study, Phase II - Technical Studies" which was signed September 27, 1991. The purpose of the Study is to understand and characterize the cumulative effects of development on the water and aquatic environment of the Study Area by coordinating with existing programs and undertaking appropriate new technical studies.

This publication reports the method and findings of particular work conducted as part of the Northern River Basins Study. As such, the work was governed by a specific terms of reference and is expected to contribute information about the Study Area within the context of the overall study as described by the Study Final Report. This report has been reviewed by the Study Science Advisory Committee in regards to scientific content and has been approved by the Study Board of Directors for public release.

It is explicit in the objectives of the Study to report the results of technical work regularly to the public. This objective is served by distributing project reports to an extensive network of libraries, agencies, organizations and interested individuals and by granting universal permission to reproduce the material.

# NORTHERN RIVER BASINS STUDY
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Whereas the above publication is the result of a project conducted under the Northern River Basins Study and the terms of reference for that project are deemed to be fulfilled,
**IT IS THEREFORE REQUESTED BY THE STUDY OFFICE THAT;**
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F. J. Wrona
(Dr. Fred J. Wrona, Science Director)

14 Feb 96
(Date)

Whereas it is an explicit term of reference of the Science Advisory Committee "to review, for scientific content, material for publication by the Board",
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**SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No**

P. Larkin
(Dr. P. A. Larkin, Ph.D., Chair)

14 Feb 1996
(Date)

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**IT IS HERE APPROVED BY THE BOARD OF DIRECTORS THAT;**
this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

Lucille Partington
(Lucille Partington, Co-chair)

23 Feb./96
(Date)

Robert McLeod
(Robert McLeod, Co-chair)

23 Feb/96
(Date)

# IDENTIFICATION OF SPATIAL PATTERNS IN NUTRIENT LIMITATION WITH HERBIVORY EFFECTS, WAPITI, SMOKY AND ATHABASCA RIVERS, 1994

## STUDY PERSPECTIVE

A particular area of concern related to water quality in the northern river basins is the effect of nutrients, primarily nitrogen (N) and phosphorus (P), on the aquatic environment. Nutrients enter a river from municipal and industrial effluents, agricultural and timber-harvesting runoff, natural runoff, ground water sources and tributary inflow. Added nutrients can cause changes in abundance and production of algae, benthic biota and fish. Nutrients may also affect dissolved oxygen concentrations as a result of enhanced plant growth, which in turn, is decomposed by bacteria that consume oxygen. The increased productivity of biological communities resulting from the addition of nutrients and their subsequent effect on the chemical and physical components of the aquatic ecosystem is referred to scientifically as eutrophication. Understanding the effects of nutrients on the aquatic environment will be critical for managing industrial and municipal effluent discharges to the Wapiti, Smoky and Athabasca rivers.

### *Related Study Questions*

| | |
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| *2)* | *What is the current state of water quality in the Peace, Athabasca and Slave River basins, including the Peace-Athabasca Delta?* |
| *5)* | *Are the substances added to the river by natural and man-made discharges likely to cause deterioration of the water quality?* |
| *13b)* | *What are the cumulative effects of man-made discharges on the water and aquatic environment?* |

One of the objectives of this project was to identify spatial patterns of nutrient limitation in the Athabasca and Wapiti-Smoky rivers. Two experiments were conducted to accomplish this goal. The first experiment used nutrient diffusing substrata (NDS) downstream of the Hinton combined effluent to identify the interactive effects of both nutrient enrichment and herbivory (grazing by aquatic insects) on algal biomass. The second experiment used NDS to quantify large scale patterns in algal biomass and nutrient limitation, with four levels of nutrient enrichment, upstream and downstream of the major effluent and tributary input sources along the Athabasca and Wapiti-Smoky rivers.

Results using NDS placed downstream of the Hinton effluent indicated that nutrient treatment (control, N, or P enriched) had no effect on algal biomass, but algal growth on malathion (an insecticide to control grazing by aquatic insects) enriched NDS was about twice that on NDS without malathion. NDS placed at 33 sites along the Athabasca and Wapiti-Smoky rivers aided in the identification of river reaches having no nutrient limitation (14 sites), and those having some form of nutrient limitation (19 sites). Algal communities were typically nutrient unlimited at sites located immediately downstream of point-source inputs, but nutrient limited at sites located immediately upstream or at considerable distances downstream of these inputs. Input sources associated with higher nutrient loading in this study were downstream of Grande Prairie, Hinton, Whitecourt, Athabasca and Fort McMurray.

Results from this study indicate that spatial patterns of nutrient limitation in the Athabasca and Wapiti-Smoky rivers are strongly affected by the location of point-source nutrient inputs. In addition, these effects were found to be relatively complex and likely influenced by other environmental factors, such as herbivory. Similar to the previous NDS project, these experiments further support the hypothesis that prolonged nutrient additions can result in localized increases in primary and secondary production in northern rivers. Management of eutrophication in these rivers from point source inputs should be viewed in terms of the availability of both nitrogen and phosphorus, rather than solely by the abundance of one nutrient type.

# REPORT SUMMARY

Nutrient enrichment is one of the most common anthropogenic stressors of aquatic ecosystems and can alter the abundance, biomass and species diversity of epilithic, macroinvertebrate and fish communities. The Wapiti-Smoky and Athabasca River systems, Alberta, receive a diversity of point and non-point effluent discharges including municipal sewage, agricultural runoff and effluent from oil sands and pulp mills. To evaluate the effects of nutrient enrichment in the wapiti-Smoky and Athabasca rivers, we documented large scale patterns in epilithic biomass and nutrient limitation in these systems between 1993-1994 to determine whether nutrients added to the rivers by natural and man-made discharges cause deterioration of water quality. In February-March 1994 the interactive effects of herbivory and nutrient enrichment on epilithic biomass were investigated using nutrient diffusing substrata at Hinton in the Athabasca River. A two factorial multivariate analysis of variance (MANOVA) design was used in Experiment 1 to investigate the roles of nutrient availability and herbivory on epilithic biomass at a site located immediately downstream of the combined Hinton effluent discharge. In this experiment, we tested the hypothesis that algal biomass, measured as chlorophyll *a* and ash free dry mass was significantly affected by nutrient enrichment (no nutrients, nitrogen enriched, phosphorus enriched), herbivory (presence, absence of a grazer-inhibitor compound, malathion) and the interaction of these factors.

Results from this experiment showed that downstream of Hinton epilithic biomass was unaffected by nutrient treatment but strongly affected by herbivory. Epilithic biomass on nutrient diffusing substrata was two fold higher when nutrients contained a herbivore-inhibiting compound compared to the treatment without a herbivore deterrent. The absence of a nutrient response downstream of Hinton suggests that nutrient released from the mills aerated stabilization basins results in nutrient unlimited conditions 1 km downstream of the outfall but that grazing by invertebrate herbivores reduces this biomass from the maximum achievable.

A second experiment quantified spatial patterns in nutrient limitation and epilithic biomass throughout the Wapiti-Smoky and Athabasca rivers for sites located immediately upstream and downstream of point-source nutrient inputs and tributary mouths. Nutrient diffusing substrata (NDS) containing either nitrogen, phosphorus, nitrogen+phosphorus or nutrient-absent controls were placed on the river bottom at 5 sites in the Wapiti-Smoky and 28 sites in the Athabasca basins and retrieved after 14-31 days for epilithic chlorophyll *a* determination. Epilithic biomass on natural stone surfaces in the river was also quantified upon placement and retrieval of nutrient diffusing substrata.

Data from nutrient diffusing substrata experiments indicated that 19 of the 33 sites were nutrient limited (i.e., epilithic chlorophyll *a* was greater on substrata that contained nitrogen (N), phosphorus (P), or N+P than on nutrient absent controls). The remaining 14 sites were nutrient unlimited. Of the 19 nutrient limited sites 10 were nitrogen limited, 5 were phosphorus limited and four were limited by the availability of both nitrogen and phosphorus. The majority of nutrient unlimited sites were located downstream of known point-source nutrient inputs. This suggests that point-source discharges largely determine the number and extent of nutrient unlimited reaches in these river systems. In addition, epilithic biomass on stones was found to differ significantly among sites in both early and

late fall. Epilithic biomass was up to 50 times higher immediately downstream of point source inputs compared to sites upstream and at sites located at further downstream of nutrient inputs. Predictive models of epilithic chlorophyll *a* using multiple regression showed that epilithic biomass was significantly and positively related with concentrations of bioavailable phosphorus and nitrogen. These two variables explained 40 - 60% of the observed variation in epilithic biomass in these river systems in early and late fall, respectively.

Multiple discriminant function analysis further identified the combined concentrations of bioavailable phosphorus and nitrogen as a significant discriminator between nutrient limited and nutrient unlimited sites with combined concentrations of bioavailable phosphorus and nitrogen being significantly higher at nutrient unlimited than nutrient limited sites. Overall, the model had a high classification success and correctly identified 70% of all sites as either nutrient limited or nutrient unlimited based on their known nutrient status from results of the diffusing substrata experiments. The model was more successful at identifying nutrient limited than unlimited sites.

In conclusion, our results showed that nutrient diffusing substrata are a valuable technique for assessing spatial patterns in nutrient limitation in aquatic systems. In general, sites located immediately downstream of known point-source nutrient inputs were nutrient unlimited and characterized by elevated epilithic biomasses compared to upstream locales which were typically nutrient limited and characterized by lower biomass epilithic mats. Taken together, our results indicate that spatial patterns in nutrient limitation in the Wapiti-Smoky and Athabasca rivers represent a complex mosaic of nutrient unlimited sites interspersed with nitrogen, phosphorus and nitrogen+phosphorus limited sites. Thus, management of epilithic biomass in these rivers must be viewed in terms of the availability of both nitrogen and phosphorus rather than solely by the abundance of one nutrient type. Further studies are required to establish the impact of nutrient loading from anthropogenic sources and tributaries and the seasonality of these impacts on epilithic biomass and higher trophic level effects and to relate nutrient enrichment to other ecological concerns (e.g., dissolved oxygen concentrations) in the river systems.

# ACKNOWLEDGEMENTS

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## 1.0 INTRODUCTION

Nutrient enrichment is one of the most common 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 (e.g., Lowe *et al.* 1986, Bothwell 1988, Biggs 1989, Scrimgeour 1989, Winterbourn *et al.* 1992, Peterson *et al.* 1993). Our ability to predict the effects of nutrient inputs in river systems is generally poor because the magnitude of enrichment effects are affected by a suite of biotic (e.g., competition, predation, herbivory) and abiotic factors (e.g., light intensity and duration, flow regime, water temperature) (Bothwell 1988, Hill and Knight 1988, DeNicola *et al.* 1990, Bothwell *et al.* 1992).

The Wapiti-Smoky and Athabasca River systems, Alberta, currently receive a diversity of point and non-point effluent discharges including municipal sewage, agricultural runoff and effluent from oil sands and pulp mills (Anderson 1989, Terrestrial and Aquatic Environmental Managers 1990, Anderson 1991, Swanson *et al.* 1992, Tones 1994). Recent expansions and the addition of new pulp mills in the region have raised concerns as to whether the additional effluent loads will adversely affect the quality of these aquatic resources. The purpose of the Northern River Basins Study, a joint Federal, Provincial and Territorial study, is to gather comprehensive information on water quality; fish and fish habitat; riparian vegetation and wildlife; hydrology and hydraulics; and the use of aquatic resources. This information will form a database that will be used to develop a capability to predict and assess the cumulative effects of development on the water and aquatic environment of the Peace, Athabasca and Slave rivers within Alberta and the Northwest Territories.

This report presents results from Contract 2614-D1 of the Northern River Basins Study to identify spatial patterns in nutrient limitation in the Wapiti-Smoky and Athabasca Rivers. Our primary objectives were to: (1) identify the interactive effects of nutrient enrichment and herbivory on epilithic biomass (Experiment 1) and (2) quantify larger scale patterns in epilithic biomass and nutrient limitation in the Wapiti-Smoky and Athabasca rivers (Experiment 2). To test the effects of nutrient enrichment and herbivory on epilithic biomass, epilithic chlorophyll *a* was determined from samples recovered from nutrient diffusing substrata (NDS) amended with nitrogen (N), phosphorus (P), malathion (herbivore-deterrent) and unamended controls at a site immediately downstream of the combined effluent from Hinton and Weldwood of Canada Ltd. bleached kraft mill. To assess large scale patterns in nutrient limitation, the response of epilithic biomass to N, P and N+P addition (as determined from NDS) was determined for 33 sites in the Athabasca and Wapiti-Smoky rivers. These experiments allowed us to: (1) determine whether herbivory reduced the epilithic algal response to enrichment in the Athabasca River downstream of Hinton, and assess nutrient enrichment responses throughout the Athabasca and Wapiti-Smoky systems.

## 2.0 STUDY AREA

### 2.1 EXPERIMENT 1 - THE EFFECTS OF NUTRIENT ENRICHMENT AND HERBIVORY ON EPILITHIC BIOMASS.

Experiment 1 was conducted approximately 1 km downstream (53° 25' 47" N, 112° 55' 26" W) of the combined effluent discharge from the Weldwood of Canada Ltd. Hinton Division bleached kraft mill and Town of Hinton between February-March, 1994. While much of the river is ice-covered during this period, the experiment was performed in the open-water area immediately downstream of the Hinton combined effluent discharge. The combined Hinton-Weldwood effluent is a known source of nutrient loading to the Athabasca River (Anderson 1989, 1991). Upstream of the Hinton effluent discharge, total dissolved phosphorus (TDP) concentrations and total dissolved nitrogen (TDN) to total dissolved phosphorus ratios average 2±1 $\mu$g/L and 110:1 (SD = 64, $N = 7$), respectively during winter low flows (1988 - 1992) (Tones 1994). Daily inputs of nitrogen (N) and phosphorus (P) from the mill's aerated stabilization basin averaged 572±29 kg total N ($\bar{x}$ ± SE, $N = 68$; 1990-1993) and 79±3 kg total P ($\bar{x}$ ± SE, $N = 203$; 1990-1993) (Chambers 1996).

### 2.2 EXPERIMENT 2 - SPATIAL PATTERNS IN NUTRIENT LIMITATION IN THE WAPITI-SMOKY AND ATHABASCA RIVERS.

Large scale patterns in epilithic biomass and nutrient limitation were investigated at 33 sites in the Wapiti-Smoky and Athabasca rivers, Alberta between September - November, 1994 (Fig. 1; Table 1). These sites represent upstream and downstream locations of several of the known nutrient point-source inputs into the basin (i.e., upstream and downstream Jasper, Hinton, Whitecourt, Fort McMurray and Grande Prairie). The sites were divided into five convenient groups: (1) Wapiti-Smoky (5 sites); (2) Athabasca River upper reaches (7 sites); (3) Athabasca River middle-upper reaches (9 sites); (4) Athabasca River middle-lower reaches (6 sites); (5) Athabasca River lower reaches (6 sites) (Table 2).

The initial Terms of Reference identify 14 sites in the Wapiti-Smoky and Athabasca Rivers for epilithic and macroinvertebrate studies. The number and data collection requirements for these sites, however was changed prior to field studies. Subsequent discussions indicated that the Terms of Reference be changed to include: 1) the Herbivory experiment at Hinton (i.e., Experiment 1) and, 2) increased numbers of study sites (i.e., 33 compared to the initial 14). These changes were accompanied with removal of the macroinvertebrate data set. Changes to the Project were agreed upon by Drs Scrimgeour, Chambers and the NRBS.

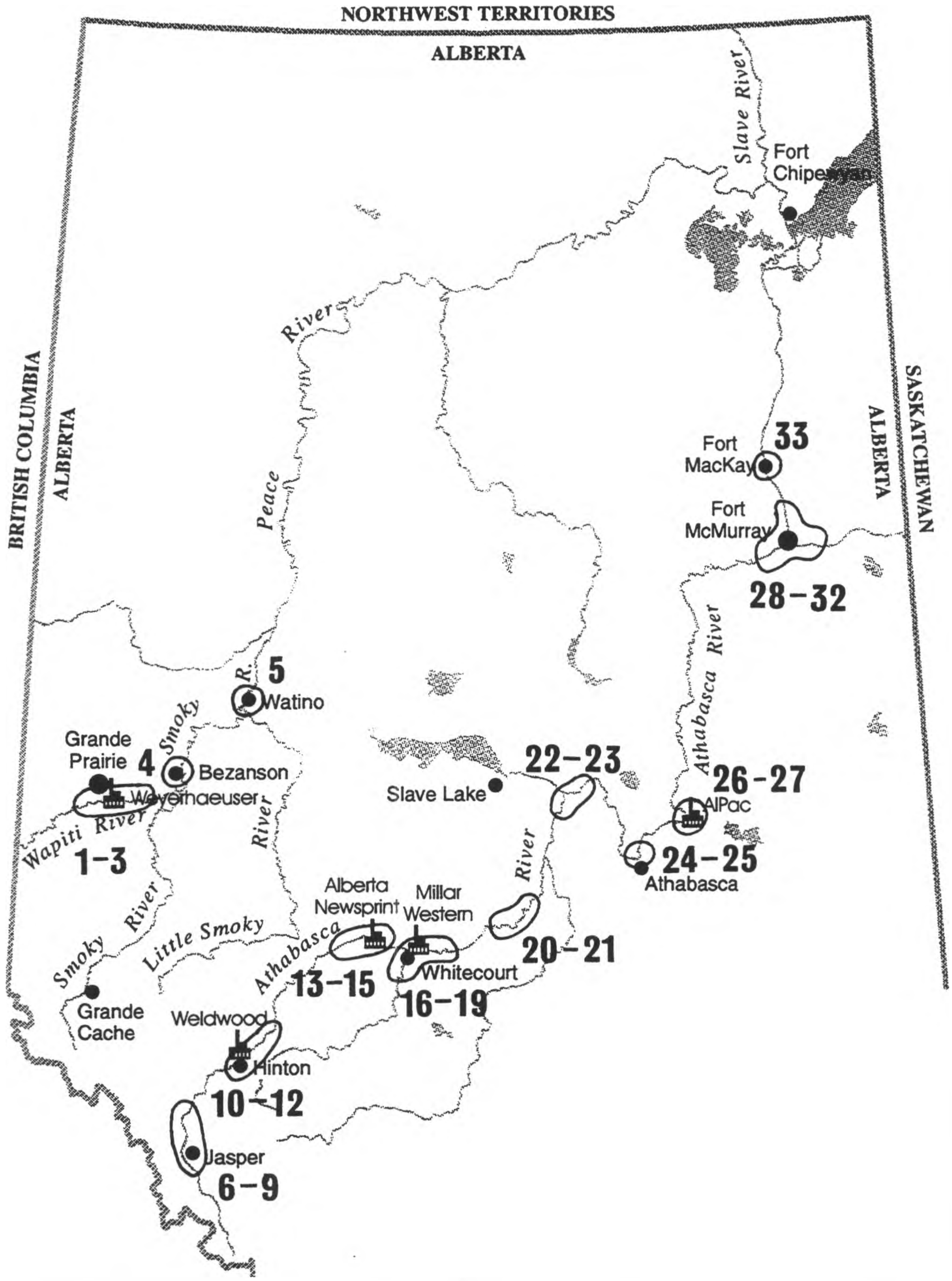

Figure 1. Location of study sites used to quantify spatial patterns in epilithic biomass and nutrient limitation in the Wapiti-Smoky and Athabasca rivers, fall 1994. Circumscribed areas indicate the longitudinal extent of study sites in each river reach.

Table 1. Location of study sites in the Wapiti-Smoky and Athabasca rivers, Alberta, fall 1994. Note that NDS were placed at two additional sites downstream of the town of Watino and immediately upstream of Whitecourt on the Athabasca River but were lost due to scouring and reduced flow, respectively. STP = sewage treatment plant, WEY = Weyerhaeuser Grande Prairie Ltd., ANC = Alberta Newsprint Company, MWPL = Millar Western Pulp Ltd., AlPac = Alberta Pacific Forest Industries Inc., conf = confluence, WH = Whitecourt, STP = sewage treatment plant, Fort McM = Fort McMurray. River distances in the Athabasca River and the Wapiti-Smoky are expressed as distance downstream of the uppermost sites (i.e., Site 1 in the Wapiti-Smoky River and Site 6 in the Athabasca River).

| Site | Start Date | Duration (days) | Location | River distance (km) |
|---|---|---|---|---|
| Wapiti-Smoky rivers | | | | |
| 1 | 29/9 | 31 | 3 km u/s Grande Prairie STP | 0 |
| 2 | 30/9 | 31 | 4.5 km d/s Grande Prairie STP, | 7.5 |
| 3 | 30/9 | 31 | 11.5 km d/s Grande Prairie STP, 2 km d/s WEY | 14.5 |
| 4 | 29/9 | 30 | 40 km d/s Weyerhaeuser, 12 km d/s Wapiti-Smoky confl | 43 |
| 5 | 29/9 | 31 | 135 km d/s Weyerhaeuser, 94 km d/s Wapiti-Smoky confl | 138 |
| Athabasca River - Upper reaches | | | | |
| 6 | 28/9 | 22 | 0.7 km u/s Jasper STP | 0 |
| 7 | 28/9 | 22 | 2.5 km d/s Jasper STP | 3.2 |
| 8 | 28/9 | 22 | 4 km d/s Jasper STP | 4.7 |
| 9 | 27/9 | 23 | 12 km d/s Jasper STP | 12.7 |
| 10 | 27/9 | 22 | 1 km u/s Hinton | 81.5 |
| 11 | 27/9 | 22 | 1 km d/s Hinton | 83.5 |
| 12 | 26/9 | 23 | 20 km d/s Hinton | 102.5 |
| Athabasca River - Middle-Upper reaches | | | | |
| 13 | 15/10 | 18 | 66 km u/s ANC, 120 km d/s Hinton | 219.3 |
| 14 | 15/10 | 18 | 30 km u/s ANC | 255.3 |
| 15 | 15/10 | 18 | 1.5 km d/s ANC, 8.5 km u/s MWPL, 12.5 km u/s WH STP | 286.8 |
| 16 | 5/10 | 24 | McLeod River 2.5 km u/s confl with Athabasca River | - |
| 17 | 16/10 | 19 | 12 km d/s ANC, 2 km d/s MWPL, 2 km u/s WH STP | 293.5 |
| 18 | 16/10 | 19 | 9 km d/s WH STP | 304.5 |
| 19 | 16/10 | 16 | 20 km d/s WH STP | 315.5 |
| 20 | 16/10 | 16 | 1 km d/s Fort Assiniboine STP, 50 km d/s Site 19 | 387.7 |
| 21 | 16/10 | 16 | 25 km d/s Fort Assiniboine STP | 411.7 |

Table 1 - continued. Location of study sites in the Wapiti-Smoky and Athabasca rivers, Alberta, fall 1994.

| Site | Start Date | Duration (days) | Location | River distance (km) |
|---|---|---|---|---|
| Athabasca River - Middle-Lower reaches | | | | |
| 22 | 7/10 | 17 | 10 km u/s of confl with Lesser Slave River | 519.7 |
| 23 | 8/10 | 16 | 3 km d/s from Lesser Slave River | 532.7 |
| 24 | 7/10 | 16 | 1 km u/s Athabasca STP | 636.7 |
| 25 | 7/10 | 16 | 1 km d/s Athabasca STP | 638.7 |
| 26 | 8/10 | 15 | 3 km d/s Athabasca STP, u/s AlPac | 640.7 |
| 27 | 8/10 | 14 | 4 km d/s AlPac | 656.7 |
| Athabasca River - Lower reaches | | | | |
| 28 | 9/10 | 18 | 4 km u/s Fort McMurray STP | 1027.7 |
| 29 | 9/10 | 18 | Clearwater River, 7 km u/s confl with Athabasca River | - |
| 30 | 9/10 | 17 | 1 km u/s Fort McMurray STP, 1 km d/s Clearwater River confl | 1030.7 |
| 31 | 9/10 | 17 | 1 km d/s Fort McMurray STP | 1032.7 |
| 32 | /10 | 17 | 3 km d/s Fort McMurray STP | 1034.7 |
| 33 | 10/10 | 17 | 1 km d/s Fort McKay STP, 60 km d/s Fort McMurray STP | 1080.7 |

# 3.0 MATERIALS AND METHODS

## 3.1 DIFFUSING SUBSTRATA

Diffusing substrata have been widely used to investigate spatial and temporal patterns in nutrient limitation in lotic and lentic ecosystems (Fairchild and Lowe 1984, Pringle and Bowers 1984, Fairchild *et al.* 1985, Lowe *et al.* 1986, Pringle *et al.* 1986, Rushforth *et al.* 1986, Pringle 1987, Hill and Knight 1988, Steinman and Lamberti 1988, Gibeau and Miller 1989, Winterbourn and Fegley 1989, Winterbourn 1990, Winterbourn *et al.* 1992, Corkum 1996). Spatial patterns in nutrient limitation in the Wapiti-Smoky and Athabasca river systems were investigated using clay nutrient diffusing substrata (NDS) that had been previously designed for use in this river system (Scrimgeour *et al.* 1995). Each NDS consists of a porous clay pot (height = 6 cm, width = 11 cm, volume = 325 ml) filled with a test compound and sealed with a 4 mm polypropylene base (diameter = 12 cm) using aquarium safe silicone sealant.

To prepare the NDS, the clay pots were soaked in deionized-distilled water for one week and then dried and filled with a hot agar solution containing agar (12 g/L agar in autoclaved deionized-distilled water) mixed with the test compound (i.e., nutrients and/or a herbivore-inhibiting compound). Agar-water solutions were heated to about 90°C, stirred continuously for about 20 minutes and subsequently left to cool to about 60°C before test compounds were added. The agar solution was then poured into the clay pots and allowed to cool before attachment of the polypropylene base. Because the agar solution is partially absorbed into the clay NDS walls, 350 ml was typically poured into each NDS. NDS were attached to the river bottom with plastic pegs for 2-4 weeks. During this period the test compounds diffused through the porous clay pot and were released into the boundary layer surrounding the outer surface of the substrata. NDS are colonized by bacteria, fungi and algae; biomass accrual at any one time depends upon the test compounds present within the pot. Epilithic biomass on NDS was determined by removing the material with a stout brush from within a 9.6 $cm^2$ area on each NDS. The sample was then placed into a scintillation vial, frozen immediately and later analyzed for chlorophyll *a* (Chl*a*) and/or ash free dry mass (AFDM) (Experiment 1 = Chl*a* and AFDM; Experiment 2 = Chl*a* only). When the sample was analyzed for both Chl*a* and AFDM, the sample was split into two equal portions in the laboratory. Chlorophyll *a* samples were extracted in 90% ethanol at 80°C for 6 minutes then cooled for 0.5 h and the concentration determined fluoremetrically on a Turner designs model 10 series fluorometer (Nusch 1980). AFDM was determined as the change in mass after ignition at 550°C for 1h. Epilithic samples on natural substrata at each site were collected upon placement and retrieval of NDS by scraping a 9.6 $cm^2$ area from the top of 10 stones. Stone scrapings were immediately frozen and epilithic biomass, expressed as Chl*a*, determined in the laboratory following the methods stated previously.

Water quality measurements were taken at each site upon placement and retrieval of NDS. Instantaneous estimates of pH and water temperature were taken with a Fisher Scientific Accumet 1000 series handheld meter. Samples for dissolved oxygen were collected in 500 ml dissolved oxygen bottles and analyzed according to Carpenter's (1965) modified Winkler technique. Samples for water-column Chl*a*, total phosphorus (TP), TDP, and soluble reactive phosphorus (SRP) were collected in 500 ml Nalgene polyethylene bottles, stored on ice in the field and then refrigerated at 4°C in the laboratory until analyzed. Water column Chl*a* was determined following the ethanol extraction technique (Bierhuizen and Prepas 1985) after M. Ostrovsky (Biology Department, Alleghany College, Meadville, P.A. unpubl.). Samples for TDP were filtered through prewashed 0.45 um HAWP millipore membrane filters. TP and TDP were digested and analyzed by Menzel and Corwin's (1965) potassium persulfate method and SRP following the molybdenum blue technique (APHA 1975). Samples for $NO_2$+$NO_3$ and $NH_4^+$ were collected in 50 ml polystyrene bottles, stored on ice in the field and then refrigerated at 4°C in the laboratory. Nitrite+nitrate samples were filtered through prewashed 0.45 um HAWP millipore membrane filters. Nitrite+nitrate and $NH_4$ concentrations were analyzed on Technicon autoanalyzer (Stainton *et al.* 1977). Lastly, light attenuation coefficients were calculated for all 33 study sites upon placement and retrieval of NDSs to determine whether variation in epilithic biomasses in the Wapiti-Smoky and Athabasca rivers relates to light availability. For each site, photosynthetically active radiation (PAR; $mMol/s^{-1}/m^{-2}$) was measured using a Licor Quantum Sensor meter at 10 cm intervals over a 60 cm vertical transect. Light attenuation coefficients were calculated as the natural logarithym of PAR versus depth using linear regression.

## 3.2 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSES

### 3.2.1 Experiment 1 - effects of nutrient enrichment and herbivory on epilithic biomass

The experiment followed a two factor analysis of variance (ANOVA) design with three levels of nutrient treatment (control, nitrogen (N) enriched, phosphorus (P) enriched) and two levels of herbivory (presence and absence of malathion) (Table 2). Diffusing substrata were amended with a nitrogen (0.8 M $NaNO_3$) or phosphorus (0.5 M $KH_2PO_4$) solution, or deionized distilled water (i.e., controls); 12 of each of the 24 N, P or control NDS were treated with malathion (0.01 M malathion; Table 2). Malathion inhibits grazing of benthic algae by stream herbivores and thus comparison of treatments with and without malathion gives an indirect measure of the importance of herbivory in determining algal biomass (Winterbourn 1990). Twelve replicates of each treatment were placed in the Athabasca River on 28 February and the majority retrieved on 25 March, 1994.

The effect of nutrient enrichment and the presence of malathion on epilithic biomass (expressed as both Chl*a* and AFDM) was tested with a two factor MANOVA. When the MANOVA indicated a significant treatment effect, two-factor ANOVA's were performed separately on the Chl*a* and AFDM data and treatment means compared with Least Significance Difference (LSD) criteria (Neter *et al.* 1990).

### 3.2.2 Experiment 2- spatial patterns in nutrient limitation in the Wapiti-Smoky and Athabasca rivers.

Differences in epilithic Chl*a* from stone scrapings among sites were tested with a single factor ANOVA after $\log_{10}$ transformation to remedy inequality of variances. The analysis was performed separately for samples collected at the start (early fall) and end (late fall) of the experiment. Multiple regression using a backward-elimination approach (elimination level $F = 0.10$) was used to relate epilithic biomass to physicochemical data: bioavailable phosphorus (taken to be soluble reactive phosphorus, BIOP), bioavailable nitrogen (i.e., $NO_2$+$NO_3$+ $NH_4^+$, BION), PAR attenuation coefficient (ATTEN), and instantaneous water temperature (TEMP).

The NDS experiment followed a single factor ANOVA with four levels of nutrient enrichment (nitrogen [0.8 M $NaNO_3$], phosphorus [0.5 M $KH_2PO_4$]], nitrogen +phosphorus and control) at each of 33 sites. When single factor ANOVA tests indicated a significant difference in Chl*a* between treatments, means were compared with Least Significance Difference (LSD) criteria (Neter *et al.* 1990).

The physicochemical characteristics of nutrient limited and unlimited sites (defined from the NDS results) were described using seven characteristics: (1) the sum of the concentrations of bioavailable phosphorus and nitrogen (BIONP), (2) water-column Chl*a* (CHLA), (3) bioavailable nitrogen:bioavailable phosphorus ratio in the early fall ($NPRATIO_E$), (4) bioavailable nitrogen:bioavailable phosphorus ratio in the late fall ($NPRATIO_L$), (5) instantaneous water temperature measured at the start and end of the experiment, (6) PAR attenuation coefficients measured at the start and end of the experiments (ATTEN), and (7) epilithic Chl*a* sampled from stones. Statistical comparison (i.e., between nutrient-unlimited and nutrient-limited sites) were performed using univariate (*t*-tests) to describe differences between the two group types (i.e., nutrient-unlimited and nutrient limited). A multivariate discriminant function analysis was then performed to determine how well the five physicochemical characteristics could discriminate between the two groups. A stepwise discriminant function analysis was initially performed to eliminate statistically redundant variables (Tabachnick and Fidell 1983) using an entry and removal criteria of $F = 0.15$. Non-redundant variables identified by the stepwise procedure were entered into a canonical discriminant function model analysis (Tabachnick and Fidell 1983) to determine if the physicochemical characteristics of nutrient-unlimited and nutrient-limited sites differed, and to quantify the predictability of group membership. Discriminant function analyses were performed using PC-SAS (SAS Inst. Inc. 1988).

Table 2. Experimental design used to investigate the effects of nutrient enrichment and presence of the herbivore-deterrent malathion on algal biomass downstream of the combined Hinton and Weldwood of Canada Ltd. bleached kraft mill on the Athabasca River between 28 February - 25 March, 1994.

| Factor | Levels | |
|---|---|---|
| 1 - Nutrient treatment | 1. | Control |
| | 2. | Nitrogen |
| | 3. | Phosphorus |
| 2 - Herbivore-deterrent treatment | 1. | Malathion present |
| | 2. | Malathion absent |

For all analyses, we determined whether data were normally distributed by applying Shapiro-Wilks tests. Homogeneity of variances were tested with F-tests (two sample *t*-tests), Bartletts test and graphical examination of residuals (following ANOVA tests). Where variances were heterogeneous, data were transformed to satisfy data normality and homogeneity of variance assumptions. Non-parametric tests were used if transformed data did not fulfil test assumptions. All statistical comparisons were conducted with SAS (SAS 1987) with an alpha of 0.05 as argued by Carmer and Walker (1982). Results are presented as means ($\bar{x}$) ± 1SE unless stated otherwise.

# 4.0 RESULTS

## 4.1 EXPERIMENT 1- THE EFFECTS OF NUTRIENT ENRICHMENT AND HERBIVORY ON EPILITHIC BIOMASS.

Preliminary analyses indicated that raw (i.e., untransformed data) Chl*a*, AFDM, water depth and velocity data were not normally distributed (Shapiro-Wilks tests, $P < 0.05$) and analyses were performed using $\log_{10}$ transformed data. Chl*a* and AFDM on NDS were significantly affected by the presence of malathion ($P < 0.001$), but not nutrient treatment ($P = 0.56$) or the interaction of these factors ($P = 0.30$) (two factor MANOVA; Figure 2). Algal biomass on malathion-enriched NDS (Chl*a*= 15.42±1.98, AFDM = 0.894±0.083; $N = 33$) was about twice that on NDS without malathion (Chl*a* = 6.23±0.97, AFDM = 0.449±0.045; $N = 26$) (Figure 2).

Water depths measured upon placement and retrieval of NDS did not differ significantly between malathion amended NDS ($P = 0.58$), nutrient-enriched NDS ($P = 0.74$), or the interaction of these factors ($P = 0.71$) (ANOVA). Similarly, there was no significant difference in water velocities between malathion amended NDS ($P = 0.38$), nutrient-enriched NDS ($P = 0.48$), or the interaction of these factors ($P = 0.75$) (ANOVA). These results suggest that differences in algal biomass, measured either as Chl*a* or AFDM, between nutrient and malathion treatments are not due to differences in water depths or velocities. Water temperatures were low (1.08±0.021 °C, $N = 589$) and varied little (i.e., up to 2 °C) throughout the experiment. Algal cellular division rates at these temperatures are typically low (Bothwell 1988).

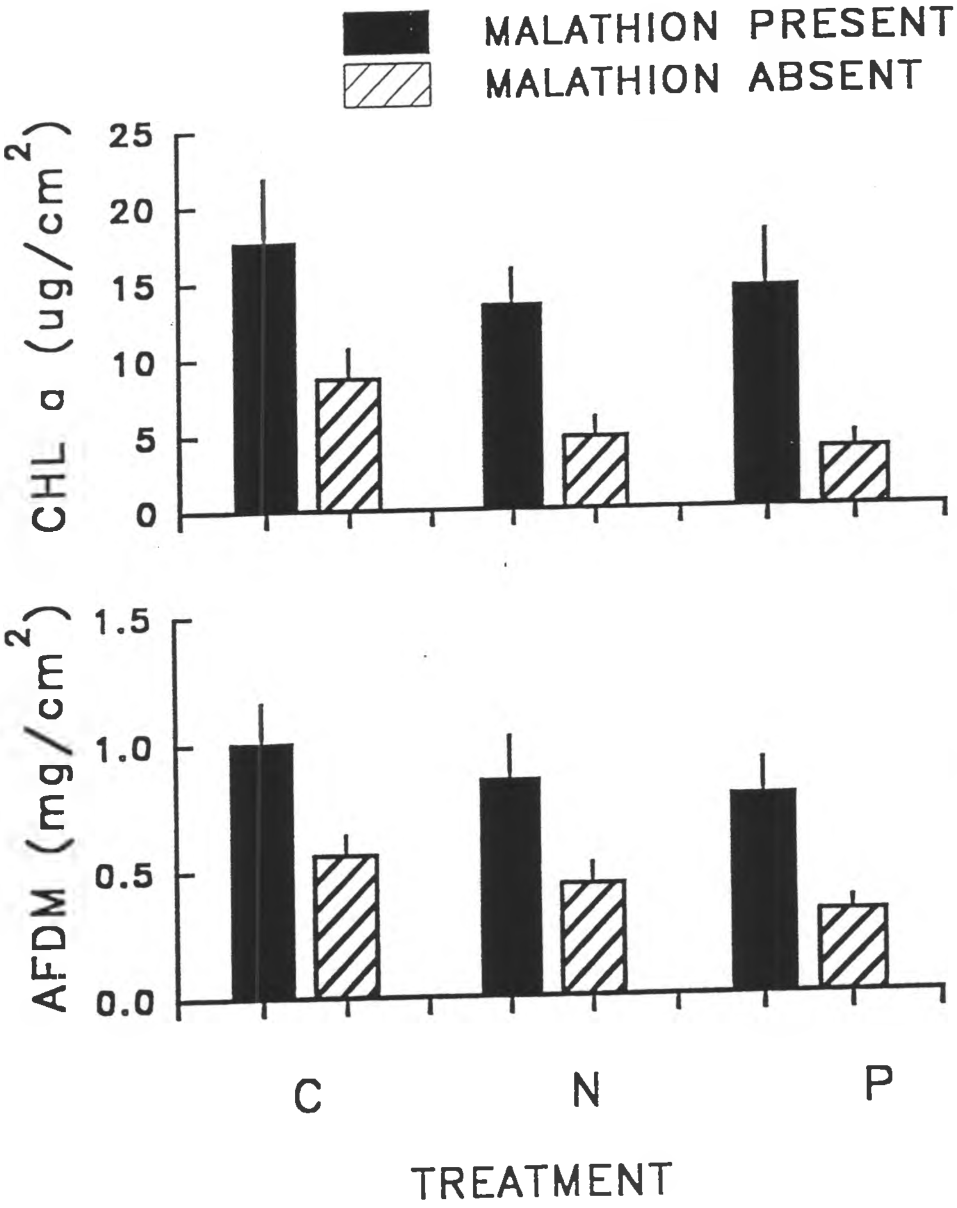

Figure 2. Mean ($\bar{x}$ ± 1SE) epilithic biomass measured as chlorophyll *a* concentration ($\mu$g Chl*a*/cm$^2$) and ash free dry mass (mg AFDM/cm$^2$) on nutrient and malathion amended diffusing substrata after 25 days downstream of the combined effluent from the town of Hinton and the Weldwood of Canada Ltd. bleached kraft mill, 28 February - 25 March, 1994. Treatments: C = control, N = nitrogen added, P = phosphorus added. Hatched histograms = malathion absent, solid histograms = malathion present.

Table 3. Comparison of mean ($\bar{x}$±1SE) initial water depths and velocities among nutrient diffusing treatments placed in the Athabasca River downstream of the combined effluent from Hinton and Weldwood of Canada Ltd. bleached kraft mill on 28 February, 1994. C = control, N = nitrogen enriched, P = phosphorus enriched.

| NDS Treatment | Water depth (cm) | Water velocity (cm/s) |
|---|---|---|
| | Nutrient enriched; malathion absent | |
| C | 38.36±1.75 | 61.91±2.69 |
| N | 38.75±1.23 | 57.92±3.24 |
| P | 38.58±1.64 | 64.67±3.89 |
| | Nutrient enriched; malathion present | |
| C | 36.33±2.16 | 59.75±4.92 |
| N | 38.25±1.74 | 58.25±2.81 |
| P | 38.31±1.78 | 59.80±2.45 |

## 4.2 EXPERIMENT 2 - SPATIAL PATTERNS IN NUTRIENT LIMITATION IN THE WAPITI-SMOKY AND ATHABASCA RIVERS.

Spatial patterns in epilithic biomass in the Wapiti-Smoky and Athabasca rivers

Epilithic biomass (expressed as Chl*a* from stones) varied significantly among the 33 sites in early (ANOVA on $\log_{10}$ transformed data, $P < 0.0001$) and late fall ($P < 0.0001$) (Fig. 3). Epilithic biomasses were relatively low in early fall in the Wapiti-Smoky rivers. In both early and late fall, epilithic Chl*a* was significantly higher ($P < 0.05$) downstream of the Grande Prairie sewage treatment plant (STP) (Site 2) and the Weyerhaeuser pulp mill (Site 3) compared to the most upstream site (Site 1). Moreover, epilithic biomasses at Sites 2 and 3 were not significantly different from each other and from those observed downstream of the confluence of the Wapiti-Smoky rivers (i.e., Sites 4 and 5) (Fig. 3). On average, epilithic biomasses were two fold higher in late compared to early fall (Fig. 3).

Overall, epilithic biomasses were higher in the Athabasca than the Wapiti-Smoky rivers (Fig. 3). In early and late fall, epilithic Chl*a* concentrations downstream of the Jasper STP (Site 7) were up to 15 fold higher than upstream (Site 6) but returned to background levels (i.e., Site 6) by Site 9. This suggests that the zone of enrichment from the Jasper STP extends for at least 4 km (to Site 8) to a maximum of < 12 km (Site 9). Similarly, epilithic biomasses downstream of Hinton (Site 11) were up to four-fold higher than immediately upstream (Site 10). That epilithic biomasses at all sites between Hinton and Whitecourt (Sites 11 - 14, and 16) were significantly higher than upstream of Hinton (Site 10) suggests either that the zone of enrichment may be extensive (i.e., up to 200 km) or that the river receives additional nutrient inputs in this reach to sustain nutrient unlimited conditions for epilithon growth.

In the Whitecourt region, epilithic Chl*a* on stones was higher ($P < 0.05$) downstream of the effluent discharge from ANC (Site 15), MWPL (Site 17) and the STP (Site 18) compared to upstream locales (Sites 13 and 14). Algal biomass was also significantly higher in the McLeod River (Site 16) compared to the two sites located upstream of Whitecourt. Thus, increased algal biomass at Site 15 resulted solely from enrichment from ANC, whereas enrichment at Sites 17 and 18 (downstream of MWPL, the Whitecourt STP) resulted from cumulative inputs from ANC, MWPL, the Whitecourt STP and the McLeod River. Elevated epilithic biomasses were less pronounced 20 km or more downstream (Sites 19, 20 and 21) of Whitecourt suggesting that the cumulative effects of numerous nutrient inputs in this region of the Athabasca River are relatively localized (Fig. 3).

In contrast to the marked enrichment effects from nutrient point-sources in the middle-upper reaches of the Athabasca River, loading from the Lesser Slave River with its effluent from the Slave Lake Pulp mill was not associated with a significant increase in epilithic biomass. In fact, epilithic Chl*a* concentrations downstream of the confluence of the Lesser Slave River (Site 23) were slightly (early fall) or significantly ($P < 0.05$; late fall) lower than upstream (Site 22). The most marked difference in epilithic biomasses was observed at the sites located immediately upstream and downstream of the

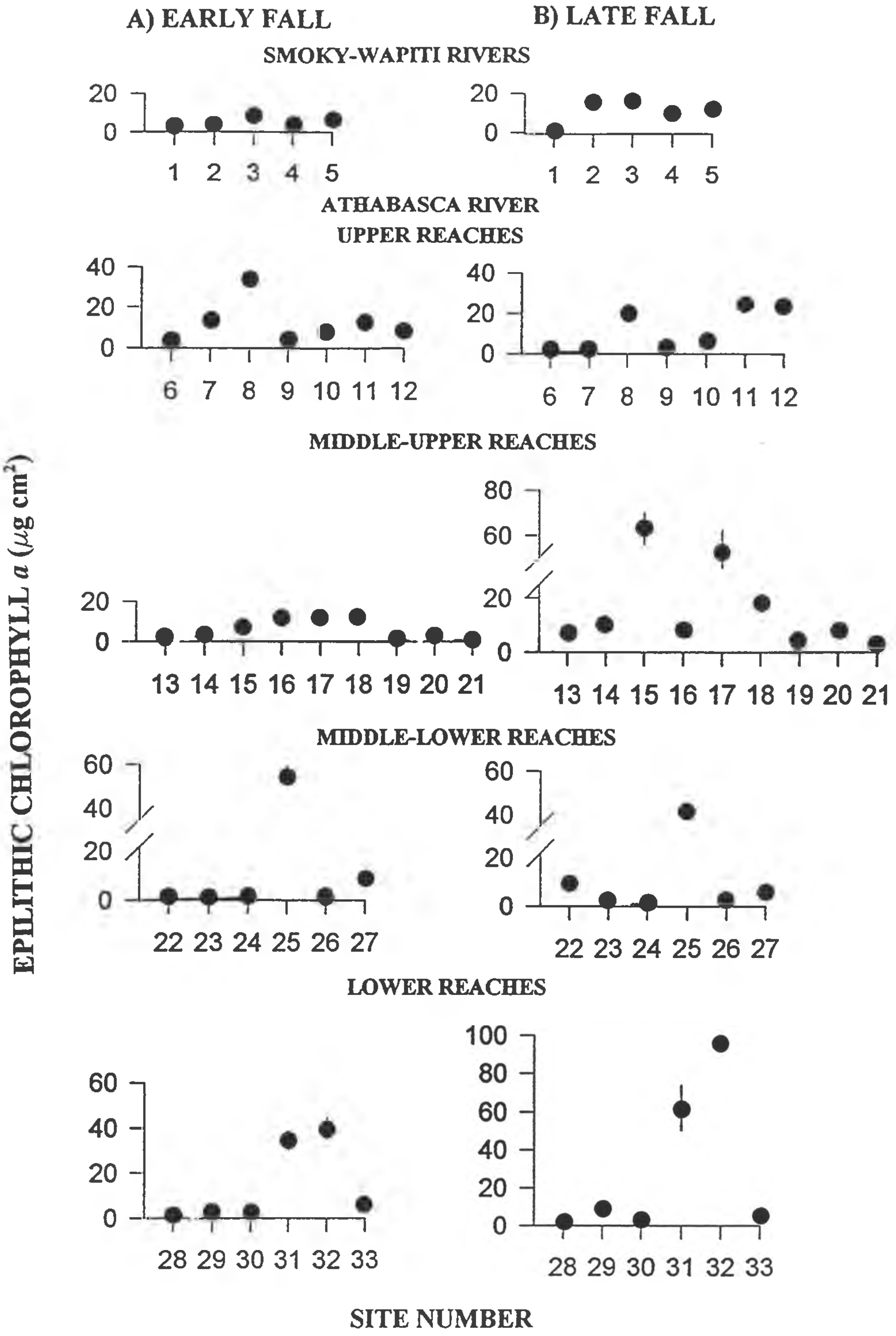

Figure 3. Mean (x̄ ± 1SE) epilithic chlorophyll *a* concentration (μg Chl*a*/cm$^2$) at 33 sites on the Wapiti-Smoky and Athabasca rivers in early fall (September-October) and late fall (October-November), 1994. For some sites, error bars are contained withiin symbols.

Athabasca STP (Sites 24 and 25). On average, epilithic biomasses were about 45-fold greater downstream compared to upstream of the STP. However, epilithic biomass declined rapidly and was significantly lower 3 km downstream of the STP, suggesting that the nutrient enrichment effect is highly localized. While epilithic biomasses decreased downstream of the Athabasca STP, epilithic biomass was 16 fold higher downstream of AlPac (Site 27) compared to upstream (Site 26) (Fig. 3).

Epilithic biomasses on stone surfaces in the lower reaches of the Athabasca River were significantly higher downstream of nutrient point-source loadings (Fig. 3). In early and late fall, epilithic Chl*a* concentrations were relatively low immediately upstream of Fort McMurray (Sites 28 and 30) and in the Clearwater River (Site 29) compared to downstream of the Fort McMurray STP (Site 31 and 32) where epilithic Chl*a* concentrations were 40 - 100 times greater ($P < 0.05$) than upstream (Site 28). Chl*a* concentrations, however, declined dramatically and by Fort McKay (Site 33, 49 km downstream of the Fort McMurray STP) and did not differ significantly ($P < 0.05$) from concentrations observed upstream of the Fort McMurray STP. Thus, the zone of enrichment from the Fort McMurray STP appears to be less than 49 km.

## Predicting large scale patterns in epilithic biomass

Multiple regression analyses showed that epilithic Chl*a* was significantly correlated with bioavailable phosphorus and bioavailable nitrogen in early ($P < 0.001$) and late fall ($P < 0.0001$) such that:

Early fall: $\text{EPIL} = 3.395 + 0.809\ \text{BIOP} + 0.005\ \text{BION}$ ($r^2 = 0.38$).

Late fall: $\text{EPIL} = 10.38 + 0.256\ \text{BIOP} + 0.10\ \text{BION}$ ($r^2 = 0.57$).

where EPIL is epilithic Chl*a* ($\mu$g/cm$^2$), BIOP is bioavailable phosphorus (i.e., SRP), and BION is bioavailable nitrogen ($NO_2$+$NO_3$ $\mu$g/L). Concentrations of BIOP and BION are expressed in $\mu$g/L.

## Patterns in nutrient limitation

Spatial patterns in nutrient limitation in the Wapiti-Smoky and Athabasca rivers were strongly affected by the presence or absence of point-source nutrient inputs (Figs 4 - 7, Table 5). Epilithic communities upstream of point-source inputs from the Town of Grande Prairie STP and the Weyerhaeuser pulp mill (Site 1) were limited by both N and P availability (Fig. 4, Table 5) such that Chl*a* concentrations on NDS enriched with both P and N (i.e., N+P) were significantly higher than on control and P alone enriched substrata. Algal communities were also N+P limited immediately downstream of the Grande Prairie STP (Site 2). In contrast, epilithic biomasses did not differ significantly among the four nutrient treatments downstream of the Weyerhaeuser pulp mill and the STP (Site 3) indicating that communities here were not nutrient limited. In the Smoky River 12 km downstream of the Wapiti confluence (Site 4), epilithic communities were N limited (Fig 4, Table 5). Farther downstream of the Wapiti-Smoky Rivers (Site 5), the community reverted to N+P limitation as was observed upstream of all point-source inputs (Site 1).

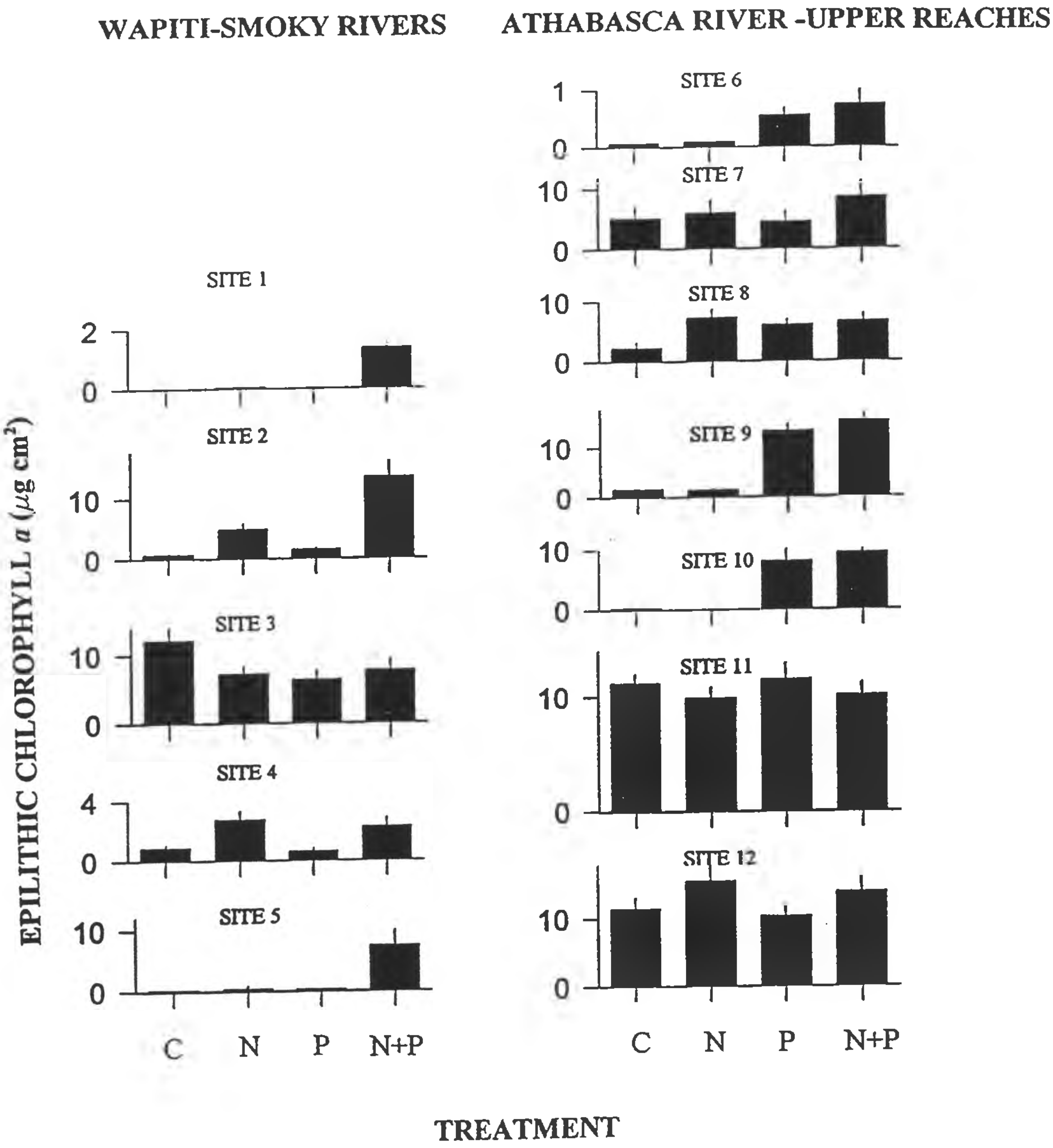

Fig. 4. Mean ($\bar{x}$ ± 1SE) epilithic chlorophyll *a* concentration (Chl*a*/cm$^2$) on control and nutrient-enriched diffusing substrata in the Wapiti-Smoky rivers and upper reaches of the Athabasca River, fall 1994. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added.

Table 4. Summary and interpretation of results of ANOVA on the effects of nutrient enrichment at 33 sites in the Wapiti-Smoky and Athabasca rivers, fall 1994. Data were $\log_{10}$ transformed to remedy inequality of variances and fulfill normality assumption of ANOVA. [1]interpreted as being nutrient unlimited, [2]interpreted as being nitrogen limited, [3] interpreted as being phosphorus limited. N = nitrogen, P = phosphorus.

| Site | ANOVA | *P* | Pairwise comparisons | Interpretation |
|---|---|---|---|---|
| 1 | $F_{(3,30)} =$ 98.43 | <<0.05 | C=P<N=N+P | N and P limited |
| 2 | $F_{(3,36)} =$ 38.08 | <<0.05 | C<P<N<N+P | N and P limited |
| 3 | $F_{(3,34)} =$ 2.46 | =0.08 | - | Nutrient unlimited |
| 4 | $F_{(3,36)} =$ 6.24 | <<0.05 | C=P; C=N+P; N=N+P | N limited[2] |
| 5 | $F_{(3,36)} =$ 45.93 | <<0.05 | C=P<N=N+P | N limited |
| 6 | $F_{(3,29)} =$ 6.80 | <<0.05 | C=N<P=N+P | P limited |
| 7 | $F_{(3,35)} =$ 1.09 | >>0.05 | - | Nutrient unlimited |
| 8 | $F_{(3,31)} =$ 5.90 | <<0.05 | C<P=N=N+P | Nutrient unlimited[1] |
| 9 | $F_{(3,36)} =$ 55.50 | <<0.05 | C=N<P=N+P | P limited |
| 10 | $F_{(3,31)} =$ 38.71 | <<0.05 | C=N<P=N+P | P limited |
| 11 | $F_{(3,34)} =$ 0.47 | >>0.05 | - | Nutrient unlimited |
| 12 | $F_{(3,34)} =$ 1.20 | >0.05 | - | Nutrient unlimited |
| 13 | $F_{(3,36)} =$ 3.64 | >0.05 | N<C=P=N+P | Nutrient unlimited[1] |
| 14 | $F_{(3,35)} =$ 1.89 | >0.05 | - | Nutrient unlimited |
| 15 | $F_{(3,34)} =$ 14.49 | <<0.05 | C=P<N=N+P | N limited |
| 16 | $F_{(3,36)} =$ 17.20 | <<0.05 | C=P<N=N+P | N limited |
| 17 | $F_{(3,34)} =$ 37.79 | <<0.05 | C=P<N=N+P | N limited |
| 18 | $F_{(3,35)} =$ 111.05 | <<0.05 | C=P<N=N+P | N limited |
| 19 | $F_{(3,34)} =$ 32.35 | <<0.05 | C=P<N=N+P | N limited |
| 20 | $F_{(3,36)} =$ 6.62 | <0.05 | C=P<N=N+P | N limited |
| 21 | $F_{(3,36)} =$ 11.56 | <<0.05 | C=P<N=N+P | N limited |
| 22 | $F_{(3,36)} =$ 35.00 | <<0.05 | C=P<N=N+P | N limited |
| 23 | $F_{(3,26)} =$ 2.92 | =0.053 | C=N<P=N+P | P limited[3] |
| 24 | $F_{(3,34)} =$ 1.57 | >0.05 | - | Nutrient unlimited |
| 25 | $F_{(3,27)} =$ 1.07 | >0.05 | - | Nutrient unlimited |
| 26 | $F_{(3,36)} =$ 1.67 | >0.05 | - | Nutrient unlimited |
| 27 | $F_{(3,32)} =$ 9.72 | <0.05 | C=N=P; N+P>C,N,P | N and P limited |
| 28 | $F_{(3,35)} =$ 53.14 | <<0.05 | C<N,P,N+P; N=P; N+P>C,N,P | N and P limited |
| 29 | $F_{(3,32)} =$ 0.87 | >>0.05 | - | Nutrient unlimited |
| 30 | $F_{(3,35)} =$ 0.40 | =0.065 | C<N<P=N+P | P limited[3] |
| 31 | $F_{(3,37)} =$ 0.53 | >>0.05 | - | Nutrient unlimited |
| 32 | $F_{(3,29)} =$ 0.44 | >>0.05 | - | Nutrient unlimited |
| 33 | $F_{(3,36)} =$ 0.64 | >>0.05 | - | Nutrient unlimited |

In contrast to N+P limitation observed upstream of Grande Prairie, epilithic communities upstream of Jasper in the Athabasca River were P limited (Fig. 4, Table 5). Epilithic biomasses on P and N+P enriched treatments were significantly higher than those on control and N enriched substrata (Table 5). In contrast, epilithic accrual did not differ significantly among nutrient treatments downstream of Jasper (Site 7), indicating nutrient unlimited conditions. Epilithic communities were also nutrient unlimited 4 km downstream (Site 8), but reverted to P limitation 12 km downstream of the STP discharge (Site 9). Phosphorus limitation was also observed immediately upstream (Site 10) of the combined Hinton effluent discharge, where algal accrual on P and N+P substrata was significantly greater than on control and N enriched substrata. Similar to Jasper, epilithic communities were P limited upstream of Hinton (Site 10) but were nutrient unlimited 1 km (Site 11) and 20 km (Site 12) downstream of the Hinton combined effluent.

Spatial patterns in nutrient limitation varied strongly among sites in the middle-upper reaches of the Athabasca River (Fig.5, Table 4). Accrual of epilithic biomass on NDS upstream of ANC (Sites 13 and 14) was particularly low ($< 0.6\ \mu g$ Chl*a*/cm$^2$). However, despite these low biomasses, epilithic Chl*a* concentrations at these sites did not differ significantly among treatments indicating that communities were not nutrient limited. The presence of nutrient unlimited sites upstream of Whitecourt is markedly different to that of N limitation at sites further downstream (Sites 18 - 21). At these sites, epilithic communities were strongly N limited and epilithic biomass on NDS was six to 25 fold higher on N and N+P compared to control and P enriched substrata (Fig. 5). Epilithic communities were also N limited in the McLeod River (Site 16).

Middle-lower reaches of the Athabasca River displayed a diversity of patterns in nutrient limitation. Epilithic communities immediately upstream of the confluence of the Lesser Slave River (Site 22) were N limited but were P limited 3 km downstream of the confluence (Site 23) (Fig. 6, Table 4). At further distances downstream, algal communities shifted from nutrient unlimited (Sites 24 - 26) to N+P limitation (Site 27).

Spatial patterns in nutrient status in the lower reaches of the Athabasca River were related to known sources of nutrient loading (Fig. 6, Table 2). Epilithic communities were N+P limited upstream of Fort McMurray (Site 28) but were nutrient unlimited 1 (Site 31) and 3 km (Site 32) downstream of the Fort McMurray STP (Table 4). The shift in nutrient status from N+P limited to nutrient unlimited conditions results from nutrient enrichment from the Fort McMurray STP as well as inputs from the Clearwater River (Site 29) which was also nutrient unlimited. Epilithic communities were also not limited by nutrient availability immediately downstream of Fort McKay (Site 33).

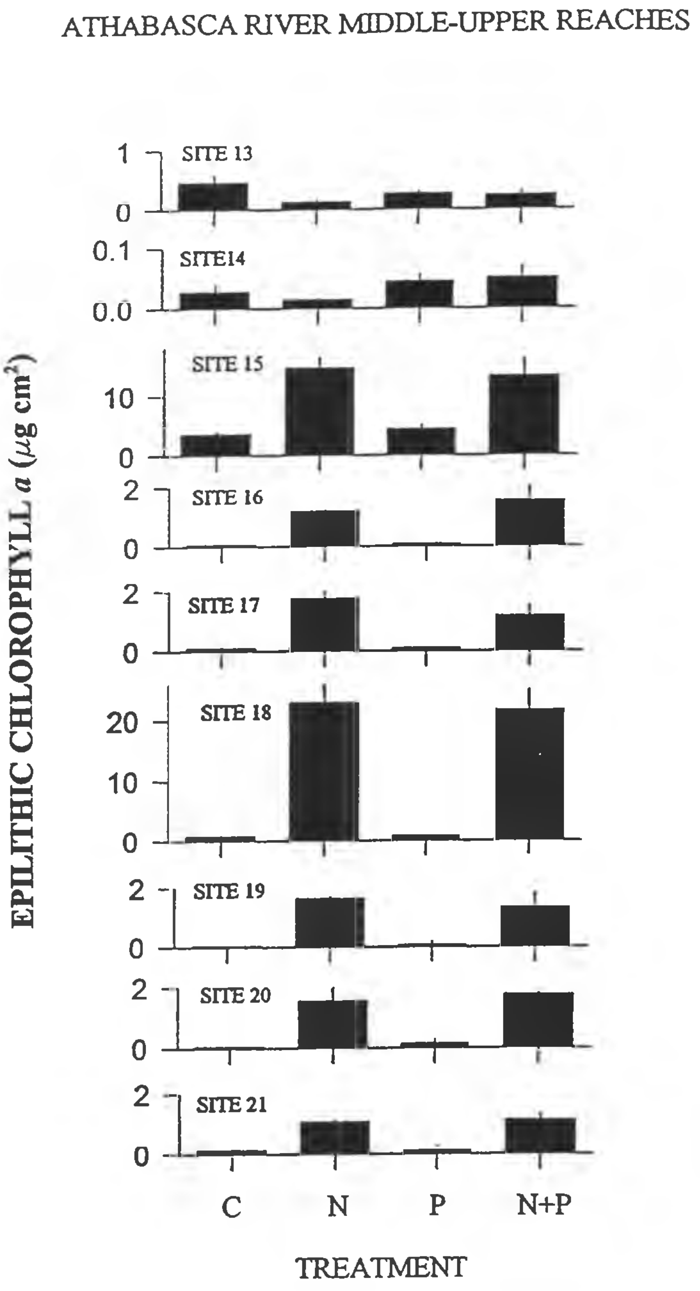

Figure 5. Mean ($\bar{x}$ ± 1SE) epilithic chlorophyll *a* concentration (μg Chl*a* cm²) on nutrient-enriched diffusing substrata in the middle-upper reaches of the Athabasca River, fall 1994. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added.

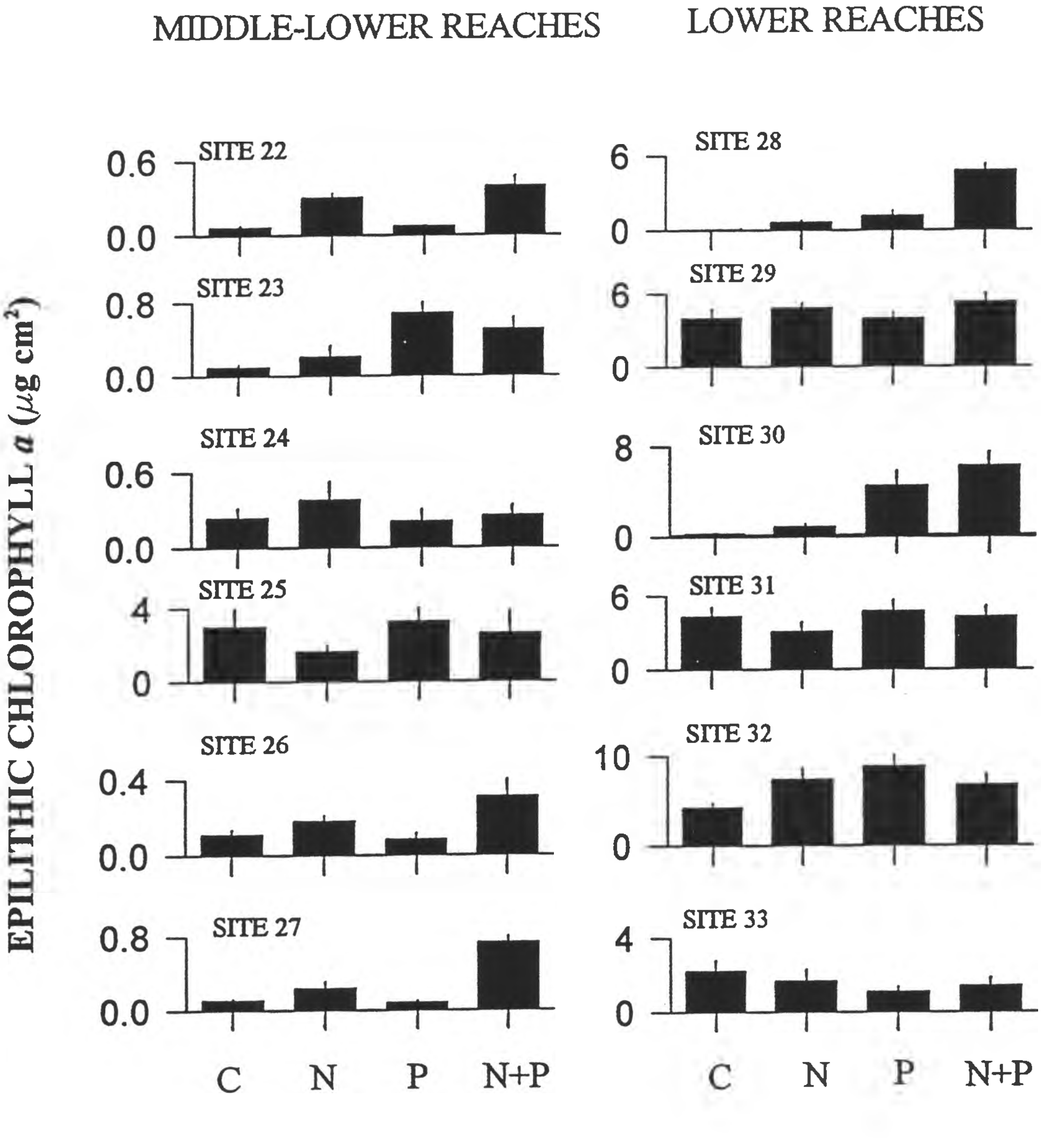

Figure 6. Mean ($\bar{x}$ ± 1SE) epilithic chlorophyll *a* concentration (μg Chl*a*/cm$^2$) on nutrient-enriched diffusing substrata in the middle-lower and lower reaches of the Athabasca River, fall 1994. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added.

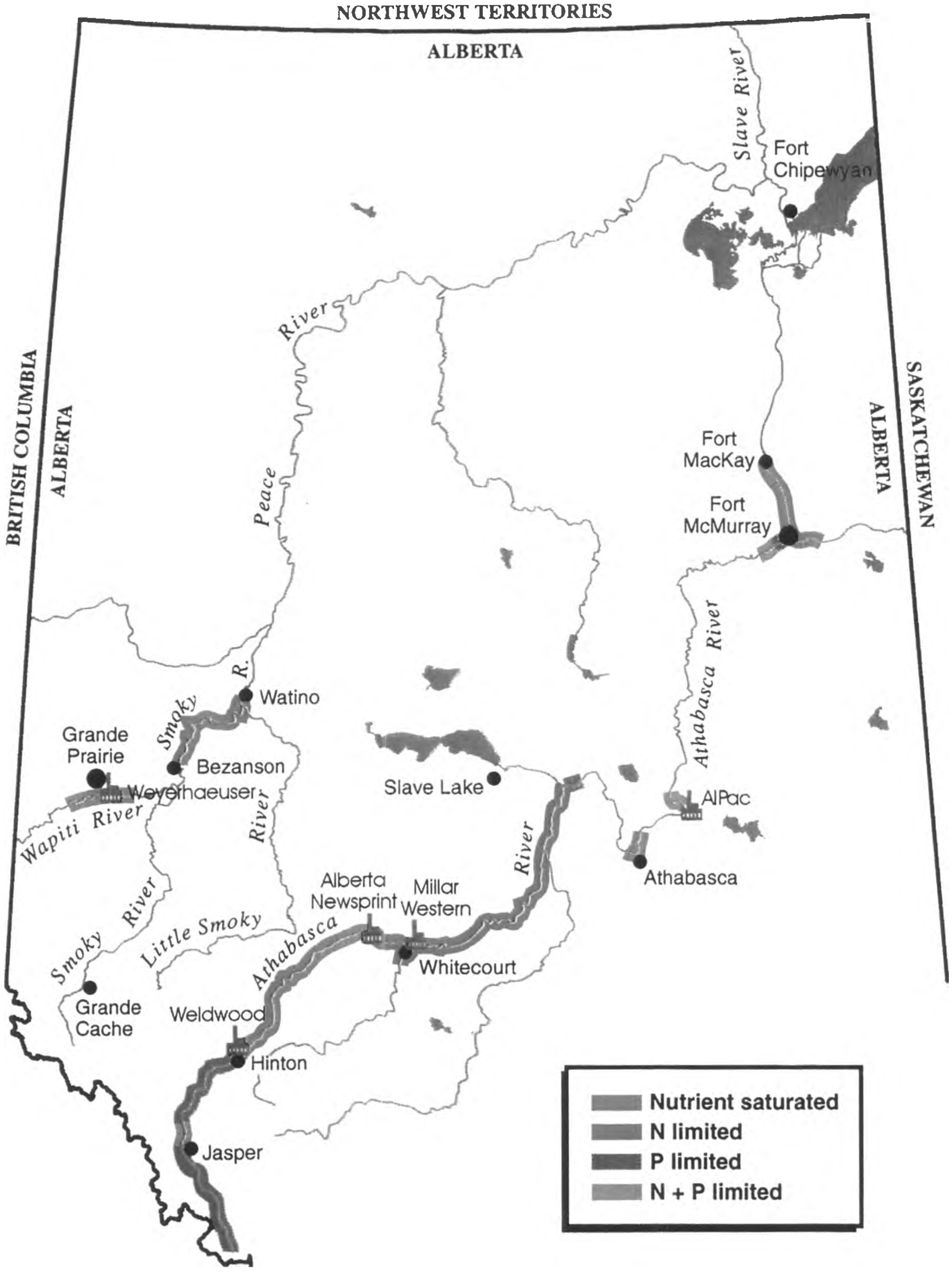

Figure 7. Nutrient status of study reaches in the Wapiti-Smoky and Athabasca rivers, fall 1994.

## Discriminating between nutrient limited and nutrient unlimited sites

Nutrient diffusing substrata identified 19 sites as being nutrient limited (five P limited, 10 N limited, four N+P limited) and 14 nutrient unlimited sites (Table 4). Two sample t - tests showed that nutrient unlimited sites had significantly higher ($P < 0.05$) concentrations of bioavailable N+P and water column Chla compared to nutrient limited sites. Water temperature, bioavailable N:P ratios in the early and late fall, light attenuation coefficients and epilithic Chl*a* did not differ between nutrient unlimited and nutrient limited sites ($P > 0.05$, Table 5).

Stepwise discriminant function analysis indicated that six of the seven physicochemical characteristics (temperature, water-column Chl*a*, early fall and late fall N:P ratios, light attenuation, and epilithic Chl *a*) were statistically redundant (i.e., statistically nonsignificant). Thus, only one of the discriminators (bioavailable N+P) was entered into the canonical discriminant function analysis. The canonical discriminant function analysis identified bioavailable N+P as a significant discriminator between nutrient limited and nutrient unlimited sites. A homogeneity of within covariance matrices test indicated that matrices were significantly different ($\chi^2 = 16.35$, df = 1, $P < 0.001$). Thus, we applied a canonical analysis using a quadratic discriminant function (i.e., the within group covariance matrix). This analysis indicated that bioavailable N+P was a significant ($P < 0.01$) predictor of nutrient limited and unlimited sites and that there was a relatively strong relationship between discriminant scores and group membership ($r = 0.46$). Nutrient unlimited sites had higher concentrations of bioavailable N+P compared to nutrient limited sites (Table 5). Overall the quadratic discriminant function had a classification success of 70% (23 of 33). The model correctly identified five of the 14 (30%) nutrient unlimited sites as being nutrient unlimited and all (N = 18) of the nutrient limited sites as being nutrient limited.

Table 5. Mean ($\bar{x}$ ±1SE) physicochemical characteristics of nutrient unlimited ($N$=15) and nutrient limited ($N$= 18) sites in the Wapiti-Smoky and Athabasca rivers, Alberta, fall 1994. BIONP = bioavailable nitrogen + phosphorus ($\mu$g/l), NPRATIO$_E$ = bioavailable nitrogen : bioavailable phosphorus ratio for early fall, NPRATIO$_L$ = bioavailable nitrogen:bioavailable phosphorus ratio for late fall, CHLA = water-column chlorophyll *a* ($\mu$g/l), TEMP = mean instantaneous water temperature (°C), ATTEN = light attenuation coefficient (m$^{-1}$), EPIL = epilithic chlorophyll *a* (g/cm$^2$). All values are untransformed data. *T*-tests performed on log$_{10}$ transformed data except TEMP.

| Variable | Unlimited | Limited | Test statistic | *P* |
|---|---|---|---|---|
| BIONP | 1078.6+673.44 | 46.61+5.96 | 2.69 | <0.05 |
| CHLA | 3.80+1.08 | 1.30+0.19 | 2.30 | <0.05 |
| NPRATIO$_E$ | 79.4+62.0 | 15.57+3.80 | 0.40 | >0.05 |
| NPRATIO$_L$ | 77.3+62.0 | 8.90+2.59 | 1.80 | >0.05 |
| TEMP | 4.81+0.49 | 4.91+0.28 | 0.18 | >0.05 |
| ATTEN | 1.17+0.17 | 1.01+0.11 | 0.70 | >0.05 |
| EPIL | 24.74+6.66 | 12.99+4.05 | 1.66 | >0.05 |

# 5.0 DISCUSSION

## 5.1 SPATIAL PATTERNS IN EPILITHIC BIOMASS

Epilithic biomass in rivers is influenced by a suite of environmental factors including light availability, hydrologic regimes, nutrient concentrations and removal rates by stream grazers (Rounick and Gregory 1981, Bothwell 1988, Hershey *et al.* 1988, Biggs 1989, Peterson *et al.* 1993, Lowell *et al.* 1994, Peterson *et al.* 1995). In nutrient limited systems, nutrient enrichment can result in significant increases in epilithic biomass as well as in the biomass and numerical abundance of the grazer communities (Perrin *et al.* 1987, Lamberti *et al.* 1989, Peterson *et al.* 1993).

To evaluate the impact of herbivory on epilithic biomass an *in situ* experiment was undertaken immediately downstream of the municipal-pulpmill discharge to the Athabasca River at Hinton. When malathion, a cholinesterase inhibitor that reduces colonization by grazers (Winterbourn 1990, Peterson et al. 1993, Dube 1995) was added to diffusing substrata, Chl*a* accrual on malathion-amended NDS (Chl*a* = 15.42±1.98, AFDM = 0.894±0.083) was about twice that on malathion-absent NDS (Chl*a*= 6.23±0.97, AFDM = 0.449±0.045). Algal accrual was not significantly affected by nutrient treatments (i.e., nitrogen enriched, phosphorus enriched and nutrient absent controls) indicating that algal growth is not nutrient limited downstream of Hinton. The lack of a nutrient response downstream of Hinton is consistent with the findings by Scrimgeour *et al.* (1995) for fall 1993. These results indicate that effluent loading from Hinton results in nutrient unlimited conditions 1 km downstream of the outfall but that grazing by invertebrate herbivores (Scrimgeour *et al.* 1991) reduces this biomass from the maximum achievable.

Increased epilithic biomass from addition of phosphorus and nitrogen has been widely documented (Perrin *et al.* 1987, Hart and Robinson 1990, Bothwell 1992, Hill *et al.* 1992, Peterson *et al.* 1993). For example, addition of nitrogen and phosphorus to the Keogh River, British Columbia, increased epilithic Chl*a* accrual rates by more than an order of magnitude (Perrin *et al.* 1987). Similarly, addition of phosphorus to the Kuparuk River, Alaska over a four year period affected all trophic levels by increasing some microbial processes, epilithic biomass and productivity, and invertebrate and fish growth rates (Peterson *et al.* 1993). Predicting the magnitude of the epilithic biomass response to nutrient enrichment, however, is difficult because of the interplay between 'bottom-up' and 'top down' effects (Hinterleitner-Anderson *et al.* 1992, Peterson *et al.* 1993, 1995). For example, in the Kuparuk River, enrichment responses varied dramatically among years and the strong "bottom-up" effects observed in years 1 and 2 were less apparent in years 3 and 4 when strong 'top-down' feedbacks (notably chironomid and mayfly grazing) became prominent (Peterson *et al.* 1993). Thus, the magnitude of the epilithic biomass response to enrichment was stronger in years 1 and 2 but lower in years 3 and 4 when much of the epilithic biomass was converted to secondary production by herbivorous invertebrates (Peterson *et al.* 1993, 1995).

Our evaluation of large scale patterns in epilithic biomass in the Wapiti-Smoky and Athabasca rivers showed that epilithic biomasses were significantly greater downstream of point-source inputs of nutrients in both river systems. Thus, in the Wapiti-Smoky rivers, epilithic biomass on stone surfaces was significantly higher downstream of the Grande Prairie STP and the Weyerhaeuser pulp mill compared to the farthest upstream site. Similarly, epilithic Chl*a* concentrations were significantly higher immediately downstream of the Jasper STP and the Hinton effluent outfall compared to upstream reference sites. Epilithic Chl*a* was also significantly higher downstream of the Alberta Newsprint Company, Millar Western Pulp Limited and the Whitecourt STP compared to upstream of Alberta Newsprint Company. Similarly, biomasses were 50, 16, and 35-50 fold higher downstream of the Athabasca STP, Alberta Pacific Forest Industries and Fort McMurray STP than upstream reference sites. In contrast, the Lesser Slave River with its effluent from the Slave Lake Pulp mill was not associated with significantly higher epilithic biomasses in the Athabasca River downstream of the confluence. In fact, epilithic Chl*a* concentrations downstream of the confluence of the Lesser Slave River were either not different (early fall) or significantly lower (late fall) than upstream.

Our results showing increased epilithic biomasses immediately downstream of point-source nutrient inputs is consistent with those from other surveys of the Athabasca (Hamilton *et al.* 1985, Anderson 1989, Terrestrial & Aquatic Environmental Managers Ltd. 1991a, Luoma and Shelast 1994) and Wapiti (Terrestrial & Aquatic Environmental Managers Ltd. 1990, 1991b, Noton 1992, Terrestrial & Aquatic Environmental Managers Ltd. 1992) rivers. In the Athabasca River, increased epilithic biomass has been typically observed downstream of Hinton and Whitecourt, and similarly on the Wapiti-Smoky rivers downstream of the Grande Prairie STP and the Weyerhaeuser pulp mill (Terrestrial & Aquatic Environmental Managers Ltd. 1990, 1991b, Noton 1992, Terrestrial & Aquatic Environmental Managers Ltd. 1992).

Multiple regression analysis showed that epilithic Chl*a* was significantly correlated ($P < 0.001$) with bioavailable P and bioavailable N. That both bioavailable N and P were statistically significant in the models suggests that epilithon is affected by the availability of both nutrients, rather than solely N or P. However, while these models are highly significant statistically, they explained only 38 and 60 % of the total spatial variation in epilithic biomass in the Wapiti-Smoky and Athabasca rivers in the early and late fall, respectively. Some of the unexplained variation in epilithic biomass may be due to differences in herbivore grazing rates and light availability among sites. In fact, our initial nutrient diffusing experiment downstream of Hinton showed that epilithic biomasses were two-fold higher when malathion, an insecticide that reduces colonization by herbivores, was present compared to when it was absent. A portion of the unexplained variation may be also be due to differences in light availability among sites. In its broadest sense, light availability can be viewed as the dual product of day length and transmittance through the water column to the river substratum. While we quantified PAR light attenuation coefficients at each site (i.e., the transmittance component), we did not measure the length of time that each site received light. Further studies are required to increase the amount of variation explained by physicochemical and landscape characteristics if a multiple regression approach is to be used as a predictive management tool.

## 5.2 SPATIAL PATTERNS IN NUTRIENT LIMITATION

Large-scale patterns in nutrient limitation were investigated by placing NDS at 33 sites in the Wapiti-Smoky and Athabasca rivers in the fall, 1994. In these systems, spatial patterns in nutrient limitation were strongly affected by the location of point-source nutrient inputs. Epilithic communities were typically nutrient unlimited at sites located immediately downstream of point- source inputs but nutrient limited at sites located immediately upstream or at considerable distances downstream of these inputs. For instance, epilithic communities were N+P limited upstream of Grande Prairie and also immediately downstream of the Grande Prairie STP but were nutrient unlimited downstream of the Weyerhaeuser pulp mill discharge. In the Athabasca River, algal communities were P limited immediately upstream of Jasper and Hinton but were nutrient unlimited at sites immediately downstream. In the Whitecourt area, algal communities were nutrient unlimited upstream of Alberta Newsprint Company (Sites 13 & 14) but were strongly nitrogen limited downstream of Alberta Newsprint Company, Millar Western Pulp Ltd., Whitecourt STP and upstream and downstream of Fort Assiniboine. Epilithic communities were also N limited in the McLeod River tributary. Patterns in nutrient limitation were more complex at sites located in the middle-lower reaches of the Athabasca river. The shift from nutrient-limited to nutrient-unlimited conditions was not apparent upstream and downstream of the Athabasca STP or the Alberta Forest products Ltd. In fact, epilithic communities were nutrient-unlimited both upstream and downstream of the Town of Athabasca. Shifts from N+P or P limitation to nutrient unlimited conditions were clearly apparent in the Lower reaches of the Athabasca River (upstream and downstream of Fort McMurray and Fort McKay).

Canonical discriminant analysis to test for concordance between the known nutrient status of study sites (i.e., nutrient limited versus nutrient unlimited based on the NDS results) and physicochemical parameters of light attenuation, water column Chl*a*, epilithic Chl*a*, mean water temperature and the summed concentrations of bioavailable N and P showed that bioavailable N+P had an overall classification success of 70% (the initial stepwise function analysis indicated that the other physicochemcial properties were statistically redundant). Thus, the model correctly identified 70% of the nutrient-limited sites as being nutrient-limited and nutrient-unlimited sites as being nutrient-unlimited, with nutrient unlimited sites having higher concentrations of bioavailable N+P. However, the model was most successful in classifying nutrient-limited sites (classification success = 100%) but less successful in correctly classifying nutrient-unlimited sites (33%). That the model was less powerful in classifying nutrient unlimited sites suggests that other physicochemical variables, such as day length, herbivory and hydrological factors may also be important in identifying nutrient-unlimited sites. Finally, validation of the discriminant function model, either spatially or temporally, needs to be completed before the utility of the model can be fully understood.

Our analysis of spatial patterns in epilithic biomass and nutrient limitation in the Wapiti-Smoky and Athabasca rivers revealed two apparent inconsistencies. First, the discriminant function analysis indicated that epilithic Chl*a* was not a significant discriminator between nutrient-limited and nutrient-unlimited sites. This is an apparent contraction to our observation that epilithic biomasses were typically greater downstream of point-source nutrient inputs compared to upstream reference sites.

This contradiction may be because nutrient diffusing substrata only show whether epilithic communities respond to nutrient enrichment (i.e., nutrient limited). A significant difference between N, P, N+P enriched and control substrata indicates that the site is nutrient limited in one form or another. In contrast, the absence of a treatment effect results when nutrient concentrations are sufficiently high to satisfy cellular and biofilm requirements or other factors, such as light availability, are limiting. Epilithic biomass would be expected to be high under nutrient-unlimited conditions only when other factors are not limiting. Thus, epilithic biomass will be a useful discriminator between nutrient limited and unlimited sites only when other factors are not limiting growth.

The second inconsistency, is the observation from the discriminant function analysis that bioavailable nitrogen:bioavailable phosphorus ratios (N:P ratios) were not significant predictors of nutrient limited and nutrient unlimited sites in either early and late fall. In general, N:P mass ratios below <9 suggest nitrogen limitation, > 9 suggest phosphorus limitation and N:P ratios between 10-20 suggest joint limitation (see Allan 1995). Our results showed that mean N:P mass ratios ranged between 9 - 16 at nutrient limited sites suggesting phosphorus or joint N and P limitation. In fact, 14 of the 19 sites were N limited (*N* = 10) or colimited (*N* = 4). That N:P ratios were not significant predictors of nutrient limited versus unlimited sites is not surprising because our analysis discriminated between two classes of nutrient limitation (nutrient limited versus unlimited) rather than the three types of limitation (i.e., N, P, or colimitation). Increased numbers of N, P, and colimited sites are required to complete the latter analysis.

Our description of spatial and temporal patterns in epilithic biomass and spatial patterns in nutrient limitation have important implications for the management of the Wapiti-Smoky and Athabasca rivers. First, high epilithic biomasses typically occurred immediately downstream of industrial and municipal point-source inputs. Second, in the majority of cases, such inputs significantly altered the nutrient status of river reaches, shifting the epilithic community from nutrient-unlimited to nutrient limited conditions. Further, these effects were found to be relatively complex and likely influenced by other environmental factors, such as herbivory. Our results indicate that nutrient limitation in the Wapiti-Smoky and Athabasca rivers is a mosaic of nutrient-unlimited sites interspersed with nitrogen, phosphorus and nitrogen+phosphorus nutrient limited sites. Thus, management of epilithic biomass in the Wapiti-Smoky and Athabasca rivers must be viewed in terms of the availability of both nitrogen and phosphorus rather than solely by the abundance of one nutrient. While considerable research needs to be completed to satisfy information deficiencies, they should include studies to identify temporal patterns in nutrient limitation in these systems.

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# 7.0 APPENDICES

Appendix 1. Water quality measurements in the Athabasca River during Experiment 2. DO = dissolved oxygen (mg/l), Chl *a* = chlorophyll *a*, $NO_2$+$NO_3$-N = nitrite+nitrate, $NH_4$ = ammonium, TP = total phosphorus, TDP = total dissolved phosphorus, = soluble reactive phosphorus, - = data not available, Temp = instantaneous water temperature. * missing data, value derived from either early or late fall.

| SITE | DATE | Bioavailable $NO_2$+$NO_3$ | $NH_4$ | TDN | Bioavailable SRP | TP | TDP | Bioavailable N:P | CHLa | PH | DO | TEMP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Early Fall | | | | | | | | | | | | |
| 1 | 29/9 | 4.3 | 11.4 | 91.9 | 6.6 | 13.8 | 10.4 | 2.38 | 0.41 | 8.26 | 13.28 | 10.0 |
| 2 | 30/9 | 4.6 | 9.4 | 68.4 | 3.4 | 9.0 | 4.2 | 4.12 | 0.54 | 8.28 | 13.26 | 9.0 |
| 3 | 29/9 | 7.2 | 11.3 | 81.2 | 17.2 | 29.6 | 19.9 | 1.08 | 0.82 | 8.26 | 13.32 | 9.0 |
| 4 | 29/9 | 20.2 | 8.0 | 66.2 | 6.6 | 21.6 | 8.2 | 4.27 | 2.15 | 8.19 | 13.73 | 10.0 |
| 5 | 29/9 | 14.7 | 11.2 | 93.2 | 2.6 | 13.9 | 5.0 | 9.96 | 1.61 | 8.22 | 13.47 | 9.0 |
| 6 | 27/9 | 40.3 | 43.8 | 64.0 | 1.2 | 18.8 | 1.5 | 70.08 | 0.24 | 7.47 | 12.37 | 11.0 |
| 7 | 28/9 | 40.6 | 21.6 | 69.1 | 1.6 | 17.6 | 1.7 | 38.88 | 0.21 | 7.86 | 12.39 | 11.0 |
| 8 | 27/9 | 41.7 | 58.2 | 166.7 | 2.3 | 16.6 | 1.5 | 43.43 | 0.51 | - | 12.91 | 9.0 |
| 9 | 27/9 | 49.5 | 43.3 | 59.2 | 2.7 | 27.2 | 1.6 | 34.37 | 1.00 | 7.90 | 12.14 | 10.0 |
| 10 | 26/9 | 32.8 | 7.9* | 73.8 | 1.9 | 13.5 | 2.0 | 21.42 | 0.38 | - | 12.97 | 12.5 |
| 11 | 26/9 | 35.3 | 8.7* | 73.7 | 4.0 | 23.1 | 4.4 | 11 | 0.83 | - | 12.78 | 12.0 |
| 12 | 26/9 | 21.6 | 3.9* | 64.59 | 2.3 | 17.3 | 2.8 | 11.09 | 0.70 | - | 13.55 | 12.0 |
| 13 | 3/10 | 29.0 | 9.4 | 108.4 | 13.5 | 46.5 | 17.3 | 2.84 | 3.13 | 7.75 | 15.16 | 5.5 |
| 14 | 3/10 | 37.1 | 41.9 | 42 | 3.9 | 65.0 | 6.7 | 20.26 | 4.90 | 7.36 | 14.17 | 5.5 |
| 15 | 3/10 | 24.4 | 31.3 | 132.3 | 3.6 | 52.8 | 5.9 | 15.47 | 3.98 | 8.08 | 14.19 | 7.0 |
| 16 | 5/10 | 4.7 | 17.6 | 152.4 | 3.6 | 21.3 | 7.7 | 6.19 | 1.16 | 8.25 | 13.54 | 8.0 |

Appendix 1 - early Fall (continued)

| SITE | DATE | Bioavailable $NO_2$+$NO_3$ | $NH_4$ | TDN | Bioavailable SRP | TP | TDP | Bioavailable N:P | CHLa | PH | DO | TEMP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Early Fall | | | | | | | | | | | | |
| 17 | 4/10 | 8.1 | 62.4 | 122.7 | 4.6 | 27.2 | 7.8 | 15.33 | 1.20 | 8.18 | 14.02 | 7.5 |
| 18 | 4/10 | 29.7 | 53.0 | 85.9 | 4.1 | 61.2 | 7.8 | 20.17 | 3.10 | 8.13 | 14.4 | 7.5 |
| 19 | 4/10 | 31.4 | 58.8 | 45.4 | 4.3 | 61.7 | 6.8 | 20.98 | 3.30 | 8.09 | 14.13 | 7.5 |
| 20 | 4/10 | 3.3 | 24.2 | 63.3 | 4.5 | 47.7 | 8.3 | 6.11 | 2.94 | 8.07 | 13.96 | 7.5 |
| 21 | 4/10 | 3.2 | 42.6 | 60.8 | 5.0 | 47.5 | 10.2 | 9.16 | 3.41 | 8.04 | 14.14 | 7.0 |
| 22 | 7/10 | 6.5 | 2.2 | 52.6 | 3.4 | 24.2 | 4.1 | 2.56 | 1.07 | 8.03 | 13.87 | 9.0 |
| 23 | 7/10 | 7.5 | 9.6* | 109.5 | 4.1 | 31.9 | 4.8 | 4.17 | 1.72 | 8.04 | 13.6 | 9.0 |
| 24 | 10/10 | 6.1 | 43.1 | 240.8 | 7.4 | 143.8 | 12.8 | 6.65 | 25.10 | 8.17 | 14.63 | 10.0 |
| 25 | 7/10 | 128.4 | 61.2 | 334.1 | 42.0 | 94.3 | 46.9 | 4.51 | 11.50 | 8.14 | 14.21 | 10.0 |
| 26 | 8/10 | 9.5 | 34.6 | 116.7 | 5.4 | 20.3 | 6.6 | 8.17 | 4.14 | 8.17 | 13.66 | 9.5 |
| 27 | 8/10 | 8.8 | - | 108.7 | 3.8 | 18.1 | 6.1 | - | 4.03 | 8.16 | 13.62 | 8.0 |
| 28 | 9/10 | 3.4 | 28.5* | 187.7 | 1.9 | 19.9 | 5.5 | 16.79 | 4.96 | 8.20 | 13.78 | 6.5 |
| 29 | 9/10 | 27.7 | 31.5* | 341 | 15.1 | 57.9 | 20.5 | 3.92 | 4.90 | 7.88 | 13.21 | 7.0 |
| 30 | 9/10 | 4.0 | 21.1* | 212.8 | 1.5 | 22.2 | 5.8 | 16.73 | 4.06 | 8.11 | 14.54 | 7.0 |
| 31 | 9/10 | 35.5 | 5960.7* | 439 | 6.8 | 35.6 | 12.0 | 881.79 | 4.58 | 8.16 | 14.7 | 7.0 |
| 32 | 9/10 | 8.0 | 194.0 | 202.6 | 2.7 | 30.2 | 6.6 | 74.81 | 4.62 | 8.14 | 14.31 | 7.0 |
| 33 | 9/10 | 5.1 | 9.6 | 218.4 | 4.5 | 31.2 | 8.4 | 3.27 | 4.75 | 8.10 | 14.19 | 6.5 |

Appendix 1. - continued

| SITE | DATE | Bioavailable $NO_2$+$NO_3$ | NH4 | TDN | Bioavailable SRP | TP | TDP | Bioavailable N:P | CHLa | PH | DO | TEMP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Late fall | | | | | | | | | | | | |
| 1 | 30/10 | 1.1 | 4.2 | 88.9 | 3.1 | 11.0 | 4.9 | 1.71 | 0.37 | 7.93 | 15.82 | 1.5 |
| 2 | 30/10 | 7.8 | 4.7 | 103.5 | 5.4 | 11.8 | 7.0 | 2.31 | 0.40 | 7.89 | 14.08 | 1.5 |
| 3 | 30/10 | 5.8 | 11.3* | 94.3 | 25.3 | 33.7 | 27.7 | 0.68 | 0.62 | 8.02 | 13.68 | 1.5 |
| 4 | 29/10 | 7.7 | 13.3 | 79.5 | 12.0 | 20.4 | 13.2 | 1.75 | 0.92 | 7.91 | 15.54 | 1.5 |
| 5 | 30/10 | 14.7* | 0.8 | 115.2 | 5.2 | 21.2 | 7.4 | 2.98 | 0.58 | 8.05 | 17 | 2.0 |
| 6 | 10/10 | 75.6 | 11.7 | - | 2.1 | 7.5 | 3.3 | 41.57 | 0.15 | 7.89 | 14.08 | 2.5 |
| 7 | 10/10 | 67.6 | 24.7 | - | 5.1 | 13.0 | 7.4 | 18.10 | 1.39 | 7.94 | 14.11 | 2.5 |
| 8 | 10/10 | 48.9 | 8.0 | - | 5.0 | 12.4 | 6.6 | 11.38 | 1.01 | 8.15 | 14.93 | 2.5 |
| 9 | 10/10 | 71.4 | 8.7 | - | 14.6 | 22.2 | 16.2 | 5.49 | 0.47 | 8.00 | 14.58 | 2.0 |
| 10 | 10/10 | 64.8 | 7.9 | - | 2.4 | 12.8 | 4.2 | 30.29 | 0.46 | 8.11 | 15.45 | 2.0 |
| 11 | 10/10 | 63.7 | 8.7 | - | 10.4 | 23.9 | 12.3 | 6.96 | 0.46 | 8.16 | 15.51 | 3.0 |
| 12 | 10/10 | 51.4 | 3.9 | - | 5.8 | 13.3 | 7.6 | 9.53 | 0.59 | 7.89 | 16.93 | 2.5 |
| 13 | 3/11 | 37.7 | 1.3 | 66.8 | 1.1 | - | 0.0 | 35.45 | 0.39 | 8.04 | 13.98 | 0.1 |
| 14 | 3/10 | 30.5 | 41.9* | 69.9 | 2.5 | - | 0.0 | 28.96 | 0.54 | 8.04 | 13.65 | 0.1 |
| 15 | 3/10 | 25.0 | 31.3* | 74.8 | 5.4 | - | 0.0 | 10.43 | 1.28 | 8.03 | 14.92 | 0.1 |
| 16 | 29/10 | 3.9 | 17.6* | 208.3 | 3.9 | 19.7 | 3.7 | 5.51 | 0.80 | 7.98 | 16.95 | 2.5 |
| 17 | 4/10 | 4.0 | 14.0 | 144.4 | 2.1 | - | - | 8.57 | 0.33 | 8.06 | 13.1 | 0.1 |

Appendix 1 - Late Fall (continued)

| | | Bioavailable | | | Bioavailable | | | Bioavailable | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SITE | DATE | $NO_2$+$NO_3$ | NH4 | TDN | SRP | TP | TDP | N:P | CHLa | PH | DO | TEMP |
| Late fall | | | | | | | | | | | | |
| 18 | 4/11 | 16.3 | 53.0* | 92.1 | 15.2 | - | - | 4.56 | 0.73 | 7.69 | 12.92 | 0.1 |
| 19 | 30/10 | 3.2 | 3.2 | 70.4 | 10.2 | 18.0 | 9.0 | 0.63 | 0.37 | 7.88 | 13.66 | 0.5 |
| 20 | 30/10 | 3.4 | 3.0 | 92.7 | 5.8 | 28.6 | 6.7 | 1.10 | 0.56 | 8.01 | 16.77 | 0.1 |
| 21 | 30/10 | 9.3 | 32.8 | 95 | 15.4 | 25.3 | 17.2 | 2.73 | 0.58 | 7.91 | 11.13 | 0.1 |
| 22 | 24/10 | 7.3 | 11.5 | 102.7 | 1.3 | 12.7 | 4.9 | 14.46 | 0.63 | 7.36 | 15.75 | 0.1 |
| 23 | 24/10 | 4.2 | 9.6 | 106.7 | 5.8 | 17.6 | 9.7 | 2.38 | 0.50 | 7.96 | 15.89 | 0.1 |
| 24 | 23/10 | 4.0 | 26.7 | 322.5 | 4.2 | 75.8 | 11.5 | 7.31 | 7.86 | 8.33 | 15.19 | 0.1 |
| 25 | 23/10 | 414.0 | 226.6 | 865.2 | 127.4 | 178.8 | 146.7 | 5.03 | 2.28 | 8.39 | 16.56 | 0.1 |
| 26 | 23/10 | 0.8 | 7.2 | 146.7 | 3.1 | 25.0 | 10.1 | 2.58 | 2.20 | 8.39 | 14.00 | 0.1 |
| 27 | 23/10 | 0.6 | - | 224.6 | 2.9 | 18.4 | 9.2 | - | 2.50 | 8.44 | 14.82 | 1.0 |
| 28 | 27/10 | 4.3 | 28.5 | 174.2 | 2.0 | 15.9 | 5.0 | 16.40 | 1.57 | 7.89 | 14.9 | 2.0 |
| 29 | 27/10 | 23.3 | 31.5 | 222.9 | 11.4 | 37.5 | 15.6 | 4.81 | 4.16 | 7.56 | 14.3 | 2.0 |
| 30 | 25/10 | 8.8 | 21.1 | 189 | 4.1 | 24.4 | 9.0 | 7.29 | 2.10 | 7.89 | 15.95 | 2.0 |
| 31 | 25/10 | 35.5* | 5960.7 | 7290 | 6.8* | 465.6 | - | 881.79 | 11.60 | 7.79 | 15.98 | 2.0 |
| 32 | 25/10 | 487.4 | 4198.9 | 4496 | 77.2 | 328.2 | 234.0 | 60.70 | 7.12 | 7.83 | 16.04 | 2.0 |
| 33 | 25/10 | 21.3 | 13.6 | 196.7 | 4.1 | 26.0 | 9.3 | 8.51 | 2.63 | 7.83 | 15.59 | 2.0 |

Appendix 2. Terms of reference for Northern River Basins Study contract No. 2614-D1:

# NORTHERN RIVER BASINS STUDY

# DRAFT

## Schedule A - TERMS OF REFERENCE

**Project 2614-D1:** **Identification of Spatial and Temporal Patterns in Nutrient Limitation in the Peace-Athabasca rivers.**

## I. Background and Objectives

The addition of nutrients into river ecosystems can impair ecosystem health by altering water quality, restricting river use by both humans and biota, and affecting the integrity of aquatic communities. The effect of nutrient additions on rivers is determined by a diversity of factors including when (i.e., the temporal component) and where (i.e., the spatial component) nutrients are added to the watershed. Understanding spatial and temporal patterns in nutrient limitation is crucial for the timing of nutrient releases and setting nutrient loading guidelines to minimize negative impacts.

The utility of nutrient diffusing substrata (NDS) as an *in-situ* tool to identify spatial and temporal patterns in nutrient limitation in the Athabasca River was explored by the NRBS in 1993/94 (NRBS project #2614-C1). An initial set of field experiments were conducted to: 1) develop an innovative NDS bioassay design that would be suitable for use in the Peace and Athabasca Rivers, and 2) quantify nutrient release rate coefficients from NDS. Field experiments were subsequently performed to determine spatial patterns in nutrient limitation and identify the effects of nutrient additions on algal and macroinvertebrate communities at four locations in the Athabasca River. Experiments conducted upstream and downstream of the Weldwood Ltd. of Canada bleached kraft pulp mill at Hinton indicated that while phosphorus is the nutrient limiting algal communities upstream of Hinton, algal communities were not limited downstream. In contrast, equivocal responses to phosphorus enrichment suggest that algal biomass was not limited by phosphorus additions either immediately upstream or downstream of the proposed effluent discharge from the ALPAC pulp mill.

Results from these experiments suggest that the effects of nutrient additions in the Peace and Athabasca rivers will likely be complex and depend upon the location of point discharges within the watershed as well as the quantity and seasonality of these discharges.

The purpose of this project is therefore to conduct large-scale field experiments extending throughout the basin, and encompassing a range in nutrient inputs (industrial, municipal, agricultural, natural) to predict the effects of nutrient additions on the aquatic ecosystem. Results from this study are critical for establishing nutrient-loading guidelines for present and future developments in order to preserve aquatic life (NRBS Question #6) and for informed management and sustainable development (NRBS Questions 5, 13b, 14).

## II. Project Requirements

### A. Experimental design, data compilation and interpretation

1. Undertake *in-situ* riverbed nutrient addition experiments in summer and fall 1994 to determine the effects of nutrient additions on algal biomass (Chlorophyll *a)* and macroinvertebrate community density at a minimum of 14 locations (as identified in Appendix A) located upstream and downstream of point and non-point nutrient discharges on the Wapiti and Athabasca rivers.

2. Compare algal and benthic macroinvertebrate responses to riverbed nutrient additions using nutrient diffusion substrate.

3. Quantify algal biomass and sort benthic macroinvertebrates for taxonomic identification.

4. Where appropriate, screen data sets and comment on data quality.

5. Perform appropriate statistical tests to determine the effects of riverbed nutrient additions on algal biomass and the density of benthic macroinvertebrate communities upstream and downstream of point and non-point discharges on the Wapiti and Athabasca rivers.

## III. Reporting Requirements

Based on the information obtained from the *in situ* riverbed nutrient addition experiment, produce a report describing the effects of riverbed nutrient additions on algal biomass and species diversity of benthic macroinvertebrate communities upstream and downstream of point and non-point nutrient discharges on the Wapiti and Athabasca rivers.

1. A description of the location of the study sites.

2. Details of the experimental design; data presentation and interpretation.

3. A critical evaluation of the performance of the role of nutrient diffusion technique in identifying the effects of nutrient additions on algal and benthic macroinvertebrate communities.

4. Recommendations for additional experiments to identify spatial and temporal patterns of nutrient limitation in the Peace-Athabasca rivers by means of *in-situ* nutrient additions.

5. Where relevant, present a comparison of results from riverbed nutrient addition experiments performed in the Peace-Athabasca river system to riverbed nutrient addition experiments performed elsewhere.

6. Provide ten copies of the draft report, along with an electronic copy, to the Project Liaison Officer by March 31, 1995.

7. Three weeks after the receipt of review comments on the draft report, the contractor is to submit ten cerlox bound copies and two unbound, camera-ready originals of the final report, along with an electronic copy, to the Project Liaison Officer. The style and format of the final report is to conform to that outlined in the NRBS Style Manual. A copy of the Style Manual will be supplied to the contractor by the NRBS. The final report will also include a table geo-referencing (latitude and longitude) the location of all sampling sites.

8. The final report is to include the following: an acknowledgement section that indicates any local involvement in the project, Project Summary, Table of Contents, List of Tables, List of Figures and an Appendix with the Terms of Reference for this project.

Text for the report should be set up in the following format:

a) Times Roman 12 point (Pro) or Times New Roman (WPWIN60) font.
b) Margins; are 1" at top and bottom, 7/8" on left and right.
c) Headings; in the report body are labelled with hierarchical decimal Arabic numbers.
d) Text; is presented with full justification; that is, the text aligns on both left and right margins.
e) Page numbers; are Arabic numerals for the body of the report, centred at the bottom of each page and bold.

- If photographs are to be included in the report text they should be high contrast black and white.

- All tables and figures in the report should be clearly reproducible by a black and white photocopier.

- Along with copies of the final report, the Contractor is to supply an electronic version of the report in Word Perfect 5.1 or Word Perfect for Windows Version 6.0 format.

- Electronic copies of tables, figures and data appendices in the report are also to be submitted to the Project Liaison Officer along with the final report. These should be submitted in a spreadsheet (Quattro Pro preferred, but also Excel or Lotus) or database (dBase IV) format. Where appropriate, data in tables, figures and appendices should be geo-referenced.

9. All figures and maps are to be delivered in both hard copy (paper) and digital formats. Acceptable formats include: DXF, uncompressed E00, VEC/VEH, Atlas and ISIF. All digital maps must be properly geo-referenced.

10. All sampling locations presented in report and electronic format should be geo-referenced. This is to include decimal latitudes and longitudes (to six decimal places) and UTM coordinates. The first field for decimal latitudes / longitudes should be latitudes (10 spaces wide). The second field should be longitude (11 spaces wide).

## IV. Project Administration

The Scientific Authority for this project is:

Dr. Patricia Chambers
National Hydrology Research Institute
11 Innovation Blvd.
Saskatoon, Saskatchewan S7N 3H5
phone: (306) 975-5742
fax: (306) 975-5143

Questions of a scientific nature should be directed to her.

The NRBS Study Office Project Liaison Officer for this project is:

Ken Crutchfield
Associate Science Director
Office of the Science Director
Northern River Basins Study
690 Standard Life Centre
10405 Jasper Avenue
Edmonton, Alberta T5J 3N4
phone: (403) 427-1742
fax: (403) 422-3055

Administrative questions related to this project should be directed to him.

## SAMPLING LOCATIONS

Athabasca River Sites

1. 1 km upstream of Hinton township
2. 1 km downstream of Hinton township
3. Obed Coal Bridge
4. Knight Bridge
5. Upstream of Whitecourt
6. Downstream of Whitecourt
7. Upstream of ALPAC
8. Downstream of ALPAC
9. Upstream of Ft. McMurray
10. Downstream of Ft. McMurray

Wapiti River Site

1. Upstream of Grande Prairie
2. Downstream of Grande Prairie
3. Downstream of Grande Prairie Sewage Outfall and Weyerhaeuser Canada Pulp Mill
4. Towards confluence with Smoky River.