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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.

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(Date)

IDENTIFICATION OF SPATIAL AND TEMPORAL PATTERNS IN NUTRIENT LIMITATION, ATHABASCA RIVER, OCTOBER TO DECEMBER, 1993

STUDY PERSPECTIVE

A particular area of concern related to municipal and industrial effluent discharges in the northern river basins is the effect of nutrients (nitrogen and phosphorus) 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 benthic biota and production, reproduction and survivorship of fish. Nutrients may also affect dissolved oxygen concentrations as a result of enhanced plant growth, which is, in turn, decomposed by bacteria that consume oxygen. The changes to the biological communities resulting from the addition of nutrients and their subsequent effect on the chemical and physical components of the ecosystem is referred to scientifically as eutrophication. Understanding the impacts of

Related Study Questions

4a) What are the contents and nature of the contaminants entering the system and what is their distribution and toxicity in the aquatic ecosystem with particular reference to water, sediments and biota?
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?

nutrients on the aquatic environment is therefore critical for managing industrial and municipal effluent discharges to the Peace, Athabasca and Slave rivers in order to minimize eutrophication and safeguard ecosystem health.

The long-term goal of this study is to identify which reaches of the Athabasca, Wapiti and Smoky rivers are nutrient limited (i.e., would respond to added nutrients) and at what time of year. Laboratory and field experiments were initially performed to develop an innovative nutrient diffusing substrata that would be suitable for use in the Athabasca River. Subsequent field experiments were conducted at four sites on the Athabasca River between October and December, 1993, to identify the effects of nutrient additions on bottom-dwelling algae and invertebrate communities. The four test sites were upstream and downstream of the combined effluent discharge from Hinton and Weldwood Canada Ltd., and upstream and downstream of the discharge from the Alberta-Pacific pulp mill.

Nutrient diffusing substrata consisting of porous clay pots were attached to the bottom of the Athabasca River. These clay pots were filled with a jelly-like substance called agar that was prepared with either deionized water alone (the control) or nitrogen, phosphorus and/or a secondary treated bleached kraft mill effluent. The responses of benthic algae and invertebrate communities to nutrient enrichment varied between sites. In the fall, there was greater algae growth and higher invertebrate densities on the clay pots enriched with phosphorus or phosphorus + nitrogen upstream of Hinton. There was no response to nutrient enrichment downstream of the Hinton-Weldwood combined effluent discharge because the already high nutrient concentrations in the water. At this same downstream site, algal growth was lower in winter than in fall, probably due to lower water temperatures. The effects of nutrient loading from Alberta-Pacific on benthic communities were less evident, suggesting that other factors may be important in regulating benthic production in this reach. When 100% bleached kraft mill effluent (BKME) was added to the nutrient diffusing substrata, the enrichment response was reduced such that algal growth was 5-fold lower on phosphorus + 100% BKME than on phosphorus alone. BKME also appeared to reduce the number of invertebrates.

The results from this study demonstrate that nutrient diffusing substrata are a valuable technique for assessing the impact of nutrient loading on benthic communities in the Athabasca River. This approach allows the identification of river reaches that would respond to nutrient additions and during which season. The experiments further support the hypothesis that prolonged nutrient additions could result in localized increases in primary and secondary production in northern rivers. Further studies with nutrient diffusing substrata are underway to assess nutrient status at 34 sites on the Athabasca, Wapiti and Smoky rivers.

REPORT SUMMARY

Spatial patterns in nutrient limitation were investigated using nutrient diffusing substrata in the Athabasca River, Alberta between October-December 1993. Laboratory and field experiments were initially performed to: 1) develop an innovative nutrient diffusing substrata (NDS) bioassay design that would be suitable for use in the Athabasca River, and 2) quantify nutrient release rates from NDS. Three related field experiments were subsequently conducted in the Athabasca River to determine spatial patterns in nutrient limitation and identify the effects of nutrient additions on epilithic and macroinvertebrate communities at four sites: upstream and downstream of the combined effluent discharge from the Town of Hinton and the Weldwood of Canada Ltd. bleached kraft pulp mill at Hinton, and upstream and downstream of the proposed discharge from the Alberta Pacific pulp mill (ALPac) located north-east of the Town of Athabasca.

Initial laboratory and field experiments indicated that NDS consisting of a porous clay pot (height = 6 cm, width = 11 cm, volume = 325 ml), filled with agar prepared with the test compound (i.e., nitrogen, phosphorus and/or secondary treated bleached kraft mill effluent) sealed with a 4 mm polypropylene base (diameter = 12 cm) and attached to the river substratum with plastic pegs were suitable for use in the Athabasca River. Laboratory estimates of nutrient release rates from NDS containing 0.5 M KH₂PO₄ and 0.5 M NaNO₃ placed in 2 L beakers showed that NO₂+NO₃-N and soluble reactive phosphorus (SRP) release rates declined 10-fold in an exponential fashion over a 32 day period. Further, concentrations of NO₂+NO₃-N and SRP-P measured at 2-5 hour intervals over a 24 h period indicated that release rates were linear over this period. These results indicate that NDS could be placed into the Athabasca River for at least 32 days because release rates up to this time are likely higher than that required to saturate epilithic requirements.

Results from an in-situ experiment to determine the effects of localized nutrient enrichment showed that responses of epilithic and invertebrate communities to nutrient enrichment varied with both site and time of year. Upstream of the Hinton-Weldwood combined effluent discharge, phosphorus availability limited epilithic biomass accrual in the fall such that epilithic biomass and total invertebrate density were significantly higher on phosphorus (P) and phosphorus+nitrogen (N) enriched substrata compared to control and N - enriched substrata. In contrast, neither epilithic biomass or total faunal density were significantly affected by nutrient treatment downstream of the Hinton outfall suggesting that nutrient loadings from the combined Hinton effluent exceed epilithic requirements. Phosphorus availability may also limit epilithic accrual upstream and downstream of the Alberta Pacific pulp mill. In general, phosphorus-enriched substrata supported higher epilithic biomasses and were colonized by higher numbers of invertebrates, however low epilithic biomasses and invertebrate densities confounded interpretation of these trends. In situ experiments with NDS containing phosphorus and effluent from the Weldwood of Canada Ltd. bleached kraft mill indicated that the presence of compounds other than nutrients have the potential to affect epilithic biomass accrual and total invertebrate density. Bleached kraft mill effluent (BKME) reduced the enrichment response to phosphorus such that algal biomass was about 5-fold lower on phosphorus+100% effluent than phosphorus alone. BKME also appeared to reduce the number of invertebrates. However, the response was less clear as the P+1% BKME, P+10% BKME and P+100% BKME treatments but not the P+50% BKME were significantly different from P addition alone. Further studies are underway to relate concentrations of BKME within NDS to in-river concentrations.

Experiments to determine seasonal differences in the response to nutrient addition showed that epilithic biomass on NDS from the Athabasca river downstream of the Hinton-Weldwood outfall was markedly lower in winter (November - December) than in fall (October). However, there was no difference in the pattern of nutrient responses; epilithic biomass downstream of the Hinton was not affected by nutrient addition in either season.

In conclusion, our results showed that NDS are a valuable technique for assessing the impact of BKME and nutrient loadings on benthic communities in the Athabasca River. Experiments conducted in the fall indicate that nutrient loading from the combined Hinton-Weldwood effluent exceed epilithic requirements. The absence of a significant nutrient addition effect in the winter may have arisen because low water temperatures reduced algal cellular division rates. The effects of nutrient loading from ALPac on benthic communities are equivocal and other factors, such as light limitation, may play an important role in regulating benthic production. Further studies are required to establish the impact of nutrient loading from anthropogenic sources and tributaries and the seasonality of these impacts on benthic communities in the Athabasca River.

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1.0 INTRODUCTION

Bleached kraft mill effluent (BKME) can be a major pollutant of some lakes, rivers and coastal areas in Europe, Canada, United States of America, Australia and New Zealand (Walden 1976, Leung and Sell 1982, Davis et al., 1988, Scrimgeour et al. 1989, Owens 1991, Bothwell et al. 1992). Pulp mill effluent can affect aquatic ecosystems by altering abundance, biomass and species diversity of benthic invertebrate and fish communities (Kelso 1977, Vander Wal 1977, Weinbauer et al., 1980, Majack and Waterhouse 1983, Scrimgeour 1989, Amblard et al., 1990; but see Harris 1993 for an exception to these patterns). The ecological effects of pulp mill effluent are typically attributed to high biochemical oxygen (BOD), and the presence of coloured substances, suspended sediments, nutrients, and organic contaminants (Walden 1976, Davis 1988, Scrimgeour 1989, Owens 1989, Owens 1991).

Our ability to predict the effects of BKME on aquatic ecosystems is generally poor because of the lack of a strong experimental approach to isolate the individual effects and mechanisms of action of the over 250 compounds that comprise BKME. Although the commonly observed increase in epilithic biomass downstream of BKME discharges (e.g., Bothwell 1992) is typically attributed solely to increased nutrient loadings, the presence of contaminants could contribute to increased epilithic biomass by reducing the ability of herbivorous insects to graze these layers.

Rivers in the Peace-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 (Terrestrial and Aquatic Environmental Managers 1990, Anderson 1989, 1991, Swanson et al. 1992, Tones 1994). Recent expansions and the addition of new pulp mills in the region has raised concerns on whether additional effluent loads will adversely affect the quality of these aquatic resources. The mandate of the Northern River Basins Study, a joint Federal and Provincial 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-C1 of the Northern River Basins Study to identify spatial and temporal patterns in nutrient limitation in the Athabasca River. Our primary objectives were to: 1) identify the nutrient limiting epilithic biomass, 2) identify spatial and temporal patterns in responses of epilithon and invertebrate communities to nutrient additions, and 3) determine whether the presence of compounds other than nutrients have the potential to affect epilithic biomass and the abundance of benthic invertebrates. These objectives were addressed by conducting three related experiments using nutrient diffusing substrata at four sites in the Athabasca River between October - December, 1993.

2.0 STUDY AREA

Field work was conducted at four sites on the Athabasca River, Alberta between July - December, 1993: approximately 1 km upstream (Site 1) and 1 km downstream (Site 2: 53° 25 47S, 112° 55 26S) of the combined effluent discharge from the Weldwood of Canada Ltd. Hinton Division bleached kraft mill and Town of Hinton, and approximately 3 km upstream (Site 3: 54° 57 52S, 112° 55 26S) and 4 km downstream (Site 4: 54° 57 45S, 112° 50 49N) of the Alberta Pacific BKME discharge point. The combined Hinton-Weldwood effluent is a known source of nutrient loading to the Athabasca River (Anderson 1989, 1991 and references therein). Upstream of the Hinton effleunt discharge, total dissolved phosphorus concentrations and total dissolved nitrogen to total dissolved phosphorus ratios (TDN:TDP) average 2±1 ug/L and 110±64:1 ($\bar{x} \pm$ SD, n = 7), respectively during winter low flows (1988 - 1992). Daily inputs of nitrogen and phosphorus from the mill's aerated stabilization basin averaged 566±187 kg total nitrogen ($\bar{x} \pm$ SD, n = 40; 1991) and 97±62 kg total phosphorus ($\bar{x} \pm$ SD, n = 40; 1991) (Tones 1994).

The ALPac mill began operations in September 1993. Total dissolved phosphorus concentrations and TDN:TDP ratios averaged 10 ug/L and 43, respectively, 44 km upstream of the mill during winter low flows (n = 45; 1982 - 1992). Daily inputs from the mills aerated stabilization basins averaged 192 kg total kjeldahl nitrogen (n = 3) and 388 kg total phosphorus (n = 8) for October 1993.

3.0 <u>DEVELOPMENT OF A NUTRIENT DIFFUSING SUBSTRATA DESIGN</u> FOR USE IN THE ATHABASCA RIVER

3.1. MATERIALS AND METHODS

Diffusing substrata consisting of either plastic or clay 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, Dr. Linda Corkum, Department of Zoology, University of Windsor, Windsor, Ontario, pers comm). Few studies however have used NDS in large rivers and the utility of existing designs in these systems is not known. We conducted a preliminary field experiment to determine the utility of two NDS designs for use in the Athabasca River (Figures 1 and 2). Our primary objectives were to choose a design that would be sufficiently robust to withstand the stresses of attachment to a river substratum that consisted of large cobbles and to select attachment pegs that would be used to firmly secure NDS to the river bottom.

Two general NDS designs were evaluated for use in the Athabasca River (Figures 1 and 2). The first design consisted of a 9 cm diameter petri dish which was filled with agar

Figure 1. Plastic nutrient diffusing substrata design



Figure 2. Clay nutrient diffusing substrata design.



prepared with the test compound (Figure 1). A 25 cm² area was removed from the top petri dish and replaced with 250 um Nitex mesh to allow nutrients to diffuse from the agar into the surrounding water. Wire loops attached to the petri dish base were used to secure the NDS to the river substratum by either extending an additional piece of wire from the wire loops around existing river cobble or by placing pegs through the wire loops into the river substratum. The small size, lightweight nature and shape of this design ensures rapid and inexpensive construction and easy storage and transportation. The Nitex mesh also provides a surface for algal attachment that can easily be removed and preserved for latter quantification of chlorophyll a and algal taxonomy (e.g., Winterbourn 1990, Winterbourn et al. 1992). However, the small volume of agar contained within the petri dish limits the length of time that they can be incubated in the river before nutrient release rates approach zero. Nutrient release rate coefficients of phosphorus and nitrogen, measured as soluble reactive phosphorus (SRP-P) and NO₃-N respectively, from NDS of this design decline rapidly to low levels after a 12-day incubation period (Dr. Linda Corkum, Department of Zoology, University of Windsor, Windsor, Ontario, pers comm). While the small volume of agar within the NDS could be increased by using larger petri dishes, it is unlikely that this would increase its structural strength sufficiently for use in large, fast flowing rivers.

The second NDS design evaluated for use in the Athabasca River consisted of a clay pot (outer diameter = 11 cm, height = 6 cm, internal volume = 325 ml) (Figure 2). Nutrient diffusing substrata consisting of clay pots (e.g., Fairchild and Lowe 1984, Fairchild et al 1985, Lowe et al. 1986) or clay saucers (e.g., Hill and Knight 1988) have been used widely to investigate responses of periphyton and invertebrate communities to nutrient enrichment in lakes and streams. In contrast to the plastic NDS design, the clay NDS is substantially larger, more robust but also considerably more expensive to purchase and prepare. Previous studies that have used clay NDS sealed them with a plastic petri dish (e.g., Fairchild and Lowe 1984). While this may be sufficient when attaching NDS to soft substrata, the plastic base would likely break when attached to larger river substratum consisting of cobbles and boulders. Thus, we sealed the clay NDS with a 12 cm diameter polypropylene disk (thickness = 4 mm). This material is relatively inert and extremely robust. Aquarium safe silicone sealant was used to attach the polypropylene base to the clay pot because it provides a sufficiently strong seal to withstand field stresses but can be removed easily in the laboratory for cleaning. Like the petri dish NDS design, the clay NDS can be attached to the river substratum using two pegs placed either side of the NDS.

To determine which NDS type would be suitable for use in the Athabasca River, ten NDS of each type (i.e., plastic and clay) were placed into the Athabasca River downstream (true left bank immediately downstream of the Weldwood Haul Bridge) of the Weldwood mill for a 2-week period in August 1993. Substrata were subsequently retrieved and inspected for damage.

3.2. <u>RESULTS</u>

Only 16 (7 plastic and 9 clay) of the 20 NDS were found. It is not known whether differences in the number of each NDS type retrieved reflect the increase visibility of the clay NDS design compared to the plastic design or that more of the plastic NDS were scoured from the substratum. Irrespective of differences in the number of NDS retrieved, a higher percentage of plastic NDS were damaged compared to clay NDS (71.4% and 22%, respectively). Our impression was that damage occurred when NDS were installed in the river. Further, NDS attached to the river bottom with large plastic pegs (length, width, height = $20 \times 2 \times 2$ cm) appeared to be better secured to the river bottom than those secured with

shorter and narrower metal (length, width, height = $20 \times 2 \times 1$ cm) and fibreglass (length, width, height = $20 \times 1 \times 1$ cm) pegs. Thus, results from the initial field experiment indicated that the best overall NDS design to investigate nutrient limitation in the Athabasca River would consist of a clay NDS, fitted with a polypropylene base, and attached to the river substratum with large plastic pegs.

4.0 <u>OUANTIFICATION OF NUTRIENT RELEASE RATE COEFFICIENTS</u>

4.1. MATERIALS AND METHODS

Laboratory experiments were performed to determine the length of time that clay NDS could be placed into the Athabasca River before nutrient release rates declined appreciably.

Nutrient diffusing substrata were initially soaked in deionized-distilled water for 1 week then dried and filled with a hot agar solution containing agar (12 g / L agar in autoclaved deionized-distilled water) and sufficient KH_2PO_4 or NaNO₃ mixed with deionized-distilled water to make up 0.5 M solutions. 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 nutrients were added. The agar-nutrient solution was then poured into NDS and allowed to cool completely before attachment of the polypropylene base with aquarium safe silicone. Because the agar solution is partially absorbed into the clay NDS walls, 350 ml was typically poured into each NDS.

Release rates of nitrite $(NO_2-N) + nitrate (NO_3-N)$ and SRP-P were estimated from NDS containing 0.5 M NaNO₃ and 0.5 M KH₂PO₄ (4 replicates of each). Individual NDS were placed into 2 L glass beakers filled with 1 L of deionized-distilled water. Beakers were maintained at 18°C and agitated 8-10 times per day. Water from each beaker was replaced daily and concentrations of NO₃+NO₂-N and SRP measured at 1-5 day intervals over a 32 day period. Sampling of water from individual 2 L beakers consisted of pouring a 250 ml water sample from each beaker into a polyethylene bottle. Samples were stored at 5°C in the laboratory and analyzed within 3 days. A microbial biofilm formed on the inside of each beaker during the experiment. To minimize uptake of NO₃+NO₂-N and SRP-P by this bacterial community, beakers were replaced with acid-washed beakers every 4 days. Concentrations of SRP-P and nitrite+nitrate-N were measured on a Technicon II autoanalyzer following the molybdenum blue and the cadmium reduction techniques, respectively (APHA 1975).

A second laboratory experiment was performed to test the hypothesis that nutrient release rates were constant over 24 h. Nutrient release rates obtained from this experimental design can be underestimated if release rates are not constant during the 24h period before water is exchanged. This can result if nutrient concentrations in the water surrounding the NDS equals that inside of the NDS (i.e., the lack of a diffusion gradient). The absence of a concentration gradient would overestimate the length of time that NDS could be placed into a river without appreciable nutrient losses by reducing the amount of nutrients released from the agar into the surrounding water. To test this hypothesis, a 5 ml sample of water was removed with a autopipette from each beaker on 9 occasions over a 24 h period. Samples were placed in acid washed 50 ml test tubes, stored in the laboratory for 1 day and analyzed for NO_3+NO_2-N and SRP-P as stated previously. Regression analyses were used to determine whether release rates of NO_3+NO_2-N and SRP-P (uM) were linear over 24 h.

4.2 **RESULTS**

4.2.1 <u>Quantification of Nutrient Release Rate Coefficients</u>

Release rates of NO₃+NO₂-N and SRP-P decreased logarithmically over 1 32 d (Figure 3). Linear equations for release rates of \log_{10} transformed release rates ($\log_{10} y = a + bx$), of NO₃+NO₂-N and SRP-P against time (d) are:

 $log_{10} NO_3 + NO_2 - N = 2.66 - 0.03$ Time (d) ($F_{(1.52)} = 48.89$, P < 0.001, $r^2 = 0.49$; $log_{10} SRP - P = 2.58 - 0.02$ Time (d) ($F_{(1.52)} = 33.44$, P < 0.001, $r^2 = 0.40$.

These results indicate that NDS can be placed into the Athabasca River for at least 28 days before appreciable loss of nutrients occurs. This potential incubation period is generally longer than reported for NDS elsewhere (e.g., Pringle and Bowers 1984, Fairchild and Lowe 1986) but less than suggested for standard artificial substrate exposure times (Biggs 1988). The potentially long incubation period for the clay NDS design is advantageous when algal colonization and growth rates are low due to low epilithic seston levels and low water temperatures.

Concentrations of NO_2+NO_3-N and SRP-P in laboratory beakers could potentially overestimate field incubation periods if nutrient release rates decreased during the 24 h period. Release rates would decrease through time if a concentration gradient was not present between the NDS and the surrounding water. Our results indicated that this was not the case. In fact, NO_2+NO_3-N and SRP-P concentrations in the water increased linearly over 24 h (Figure 4) such that:

NO₂+NO₃-N = -6.96 + 8.78 Time (h) ($F_{(1,34)}$ = 409.6, P < 0.0001, r² = 0.92); SRP-P = 31.25 + 14.37 Time (h) ($F_{(1,34)}$ = 255.3, P < 0.0001, r² = 0.89).

Figure 3. Mean ($\times \pm 1$ SE) soluble reactive phosphorus (SRP-P) and nitrite and nitrate (NO₂+NO₃-N) release rates (uM/L/day) from nutrient diffusing substrata containing either 0.5 M KH₂PO₄ or 0.5 M NaNO₃ agar solutions into distilled-deionized water at 18°C.



Figure 4. Mean concentrations ($\times \pm 1$ SE) of soluble reactive phosphorus (SRP-P) and nitrite and nitrate (NO₂+NO₃-N) (uM/L) from nutrient diffusing substrata containing either 0.5 M KH₂PO₄ or 0.5 M NaNO₃ agar solutions into distilled-deionized water at 18°C.



5.0 MAIN FIELD EXPERIMENTS

5.1 EXPERIMENTAL DESIGN

Three experiments were performed between October - December 1993 to investigate the effects of nutrient enrichment on epilithic and benthic invertebrate communities in the Athabasca river (Table 1). The aim of Experiment 1 was to test the hypothesis that nutrient enrichment would increase benthic Chl a concentration and invertebrate density. NDS enriched with nitrogen, phosphorus or nitrogen and phosphorus plus controls were placed at all four sites in the Athabasca River (Table 1). If nutrient loading from the Weldwood or ALPac mills are sufficiently high to saturate epilithic requirements then epilithic biomasses and total invertebrate densities should not be significantly affected by nutrient enrichment downstream of the mill (Sites 2 and 4) but they should be significantly increase in response to nutrients upstream (Sites 1 and 3).

Experiment 2 was performed upstream of the Weldwood of Canada Ltd. pulp mill (i.e., Site 1) and was designed to discriminate between the effects of nutrient additions on epilithic and invertebrate communities and those produced by other compounds present in the effluent. This was accomplished by adding mixing agar and different concentrations of phosphorus (0.5 M KH₂PO₄ present or absent) and Weldwood of Canada Ltd. secondary treated bleached kraft mill effluent (0% to 100% BKME) (Table 1). If the effects on epilithic biomass and invertebrate density are produced solely by the presence or absence of phosphorus then there should be: 1) a significant difference between control (no nutrients added) and all other treatments, and 2) no significant difference between phosphorus added treatments irrespective of BKME concentration.

Experiment 3 was designed to investigate the seasonality of benthic responses to nutrient additions. NDS were placed in the river at Sites 1 and 2 on 15 November, 1993. However, they could not be retrieved from Site 1 because formation of an ice jam during freeze-up increased water levels by 60 cm and made retrieval hazardous. Thus, Experiment 3 was completed only at Site 2 (i.e., downstream of the Weldwood mill, Hinton) and tested the hypothesis that epilithic biomass and invertebrate density were affected by nutrient treatment at this site. As predicted in Experiment 1, epilithic biomass downstream of the combined Hinton-Weldwood discharge should be not be significantly affected by nutrient addition if nutrient loads from the combined discharge are sufficiently high to saturate epilithic requirements. Accrual of epilithic biomass over the experimental period should be minimal at this time of year because of low water temperatures and short day length.

5.2. GENERAL MATERIALS AND METHODS.

5.2.1. Field

Nutrient diffusing substrata were filled with a hot agar solution and sealed with a polypropylene base as stated previously (Section 3.1). The effluent treatments for Experiment 2 were made by dissolving agar either in Weldwood of Canada Ltd. secondary-treated BKME or 100% deionized-distilled water to produce four treatments containing 0.5 M KH_2PO_4 and either 1%, 10%, 50% or 100% BKME (Table 1).

Nutrient diffusing substrata were attached to the river bottom with plastic pegs. At each site, three replicate NDS of each treatment were attached to the river substratum in each of three discrete riffles. To avoid interference of compounds leached from upstream NDS, NDS in each riffle were separated by a minimum of 1.5 m along the direction of flow. Water depth and mean current velocity (measured at 0.6 times water depth) were measured at the start and end of each experiment with a Marsh-McBirney model 2000 portable flowmeter immediately upstream (10 cm) of where NDS were attached to the riverbed. Finally, thermographs were placed in the river substratum at each site to quantify thermal regimes and to determine whether any potential differences in epilithic biomass accrual among sites could be affected by differences in water temperature.

Epilithic biomass on natural substrata at each site was estimated at the start of each experiment by scraping a 9.6 cm^2 area from the top of 10 stones collected from three riffles. Stone scrapings were immediately frozen and epilithic biomass determined in the laboratory using an ethanol extraction and measured with a Turner designs model 10 series fluorometer.

Substrata were retrieved 22 - 23 days after placement in the Athabasca River (Table 1). Epilithic biomass did not appear to be homogeneously distributed on NDS. Epilithic layers were most abundant on the downstream facing surface and almost undetectable on the upstream facing surface. Thus, epilithic biomass was determined by sampling the downstream surface of each NDS. Epilithon was sampled by delimiting a 9.6 cm² on the downstream facing surface and removing this layer with a stout brush. The sample was then placed into a scintillation vial, frozen immediately and stored in the dark before analysis. The 9.6 cm² epilithic sample was split into two equal portions so that epilithic biomass could be expressed as Chl a (subsample 1) and ash free dry mass (AFDM) (subsample 2). Epilithic biomass measured as chlorophyll a was measured after ethanol extraction with a model a Turner designs model 10 series fluorometer and ash free dry mass (AFDM) determined as the difference between the sample dry mass minus ash weight. Initial examination of epilithic samples indicated that the mass of epilithic biomass as Chl a because this is a widely used technique and permits comparisons with other studies that have used nutrient diffusing substrata to quantify responses of epilithon to nutrient enrichment.

Invertebrate fauna associated with NDS were sampled with a U net sampler (mesh size = 0.25 mm) (Scrimgeour et al., 1993). NDS were approached from downstream to minimize disturbance of invertebrates, 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 includes 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 and Scrimgeour

1993) and thus should be considered as part of the NDS invertebrate 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.

5.2.2 Laboratory

Water quality measurements were taken at each site during the sampling program. Instantaneous estimates of pH and water temperature were recorded with a Fisher Scientific Accumet 1000 series handheld 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 chlorophyll a, total phosphorus (TP), dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP-P) 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. Chlorophyll a was determined spectrophotometrically technique based upon the ethanol extraction technique of M. Ostrovsky (Biology Department, Alleghany College, Meadville, P.A. unpubl.) (Bierhuizen and Prepas 1985). 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-P as stated previously. Samples for NO₂, NO₃ and NH₄ were collected in 50 ml polystyrene bottles, stored on ice in the field and then refrigerated at 4°C in the laboratory. Nitrite and nitrate samples were filtered through prewashed 0.45 um HAWP millipore membrane filters. Nitrite, nitrate and NH₃ concentrations were analyzed on Technicon autoanalyzer (Stainton et al. 1977).

5.2.3 Statistical analyses

The effect of nutrient enrichment on epilithic biomass and total invertebrate density were tested with a single factor analysis of variance (ANOVA) for each site. Differences between upstream and downstream sites in water velocity and water depth was tested with two factor ANOVA with site (i.e., upstream versus downstream) and nutrient treatment (i.e., control, nitrogen enriched, phosphorus enriched, nitrogen and phosphorus enriched NDS) as factors. When single factor ANOVA tests indicated a significant treatment effect, means were compared with Least Squares Difference (LSD) criteria and significant interactions in two factor ANOVA tests with orthogonal contrasts (Neter et al., 1990).

Mean water depth and velocity, and mean epilithic biomass on stones collected upstream and downstream of the Hinton combined effluent and upstream and downstream of ALPac were compared with two-sample t-tests. 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 (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 $(x) \pm 1SE$ unless otherwise stated.

Table 1. Field experiments conducted in the Athabasca River, Alberta, 1993. C = control (agar only), N = agar containing 0.5 M NaNO₃, P = agar containing 0.5 M KH₂PO₄, N+P = agar containing 0.5 M NaNO₃ and KH₂PO₄, P+1.0% BKME = agar containing 0.5 M KH₂PO₄ dissolved in 1% secondary treated bleached kraft mill effluent (BKME) from the Weldwood of Canada Ltd., Hinton Division pulp mill, P+10%BKME = 0.5 M KH₂PO₄ dissolved in 10% effluent, P+50% BKME = 0.5 M KH₂PO₄ dissolved in 10% effluent.

Experiment	Site	Start Date	Duration (days)	Treatments
1	1 2 3 4	4 Oct 4 Oct 6 Oct 6 Oct	23 23 22 22	C, N, P, N+P C, N, P, N+P C, N, P, N+P C, N, P, N+P
2	1	4 Oct	23	C, P, P+1.0% BKME, P+10% BKME, P+50% BKME, P+100% BKME
3	2	15 Nov	20	C, N, P, N+P

5.3. **RESULTS**

5.3.1 Experiment 1.

<u>Water chemistry.</u> Water chemistry differed markedly among the four sites (Table 2). While there were minor differences in pH among sites, chlorophyll <u>a</u> concentrations on 5 - 6 October were up to 10-fold greater at Sites 3 and 4 compared to sites 1 and 2. Chlorophyll <u>a</u> concentrations however were more similar among sites approximately 23 days later when nutrient diffusing substrata were retrieved from the river.

Concentrations of $NO_2 + NO_3$ were consistently higher at Sites 1 and 2 than Sites 3 and 4, although differences in ammonium among sites were less consistent. Likewise, total phosphorus concentrations did not differ consistently among sites (Table 2). In early October TDP concentrations were up to four times lower at Site 1 than other sites, but were below detection limits at all sites approximately 23 days later. Similarly, SRP-P concentrations were almost two fold higher at all sites 22-23 days later, they were almost two fold higher at Sites 3 and 4 compared to Sites 1 and 2 (Table 2).

The relatively high bioavailable N:P ratios and low concentrations of total dissolved and soluble reactive phosphorus suggests that phosphorus may be the nutrient limiting primary production at Site 1 (Tables 2 & 3). In contrast, although bioavailable N:P ratios were high at Site 2 (i.e., downstream of the Weldwood of Canada pulp mill), the high concentrations of nitrogen and phosphorus suggests nutrient availability may not limit primary production. The relatively high nutrient concentrations at Sites 3 and 4 suggest that primary production is not nutrient limited at these sites.

Responses of epilithon and invertebrates to nutrient treatments at Sites 1 and 2. Epilithic biomass on upper stone surfaces was significantly greater at Site 2 (46.2 ± 10.0 ug Chl a/cm², N = 10) than Site 1 (9.2 ± 1.6 ug Chl a/cm², N = 10) (two sample t-test on \log_{10} transformed data, t = 2.9, P < 0.05) (Figure 5). This difference in epilithic biomass can not be attributed to differences in either mean water velocity (upstream: 0.28 ± 0.02 m/s, N =10; downstream: 0.27 ± 0.04 , N = 10, two sample t-test, t = 0.89, P > 0.05) or depth between sites (upstream: 20.7 ± 1.74 cm, N = 10; downstream: = 20.1 ± 1.84 , N = 10, t-test, t = 0.82, P > 0.05).

reactive photometers	osphorus,	- = not	measur	ed, BD	= belov	w detection	on (TDP dete	ction =	0.5 ug/L)	, Temp = instantaneous water	
Date	Site	Temp (°C)	D.O. (mg/L	Hd (Chl <u>a</u> (ug/L	Bioava NO ₃ +N) (ug/L)	ilable N 102 NH4 (ug/L)	TP (ug/l	TDP (J/gu) (J	Bioavailable P SRP Bioavailable (ug/L) N:P	
						Experii	ment 1				
05/10/93	1	8.9	8.9	8.3	0.4	32.5	12.1 11.4	0.9	0.5	89.2	
05/10/93	2	8.2	8.9	8.2	0.7	33.5	18.9 20.6	3.6	1.1	47.6	
6/10/93	ę	6.9	9,5	8.4	57	4.1	12.1 27.8	4.3	1.4	11.6	
06/10/93	4	8.2	9.6	8.2	6.9	4.2	3.6 25.8	4.2	1.4	5.6	
27/10/93	1	4.8	9.7	8.1	1.1	67.5	16.0 25.9	BD	1.6	52.2	
27/10/93	7	5.2	9'6	8.3	0.7	60.9	14.3 11.5	BD	1.5	50 1	
29/10/93	3	0.5	10.8	8.2	1.0	5.0	12.4 17.2	BD	2.9	6,0	
29/10/93	4	0.8	10.4	8.2	1.0	3.2	12.2 15.6	BD	2.8	5-5	

Figure 5. Mean $(\overline{x} \pm 1SE)$ chlorophyll a concentration (ug/cm²) on upper stone surfaces at Site 1 (i.e., upstream of the combined Hinton and Weldwood of Canada pulp mill discharge) and Site 2 (i.e., downstream of the combined mill discharge) in the Athabasca River, 4 October 1993.


Chlorophyll <u>a</u> concentrations on NDS were significantly affected by nutrient addition at Site 1 (single factor ANOVA on \log_{10} transformed data $F_{(3,25)} = 12.6$, P < 0.0001) (Figure 6). Chlorophyll <u>a</u> concentrations on NDS enriched with either P or N+P were significantly higher than on N-enriched, or untreated (i.e., control) (Least squares difference tests, P < 0.05). The absence of a significant difference between control and N-enriched substrata, and between enriched with P and N+P indicates that phosphorus is the nutrient limiting epilithic growth at Site 1 in the Athabasca River.

Total invertebrate density at Site 1 was also significantly affected by nutrient addition (single factor ANOVA on \log_{10} transformed data $F_{(3,25)} = 3.9$, P < 0.05) (Figure 7). Total faunal density was significantly higher on NDS enriched with P, and N+P compared with controls. However, total faunal density did not differ significantly between control and N-enriched substrata (Figure 7).

In contrast, neither chlorophyll a concentration (single factor ANOVA on \log_{10} transformed data $F_{(3,28)} = 0.9$, P > 0.05; Figure 8) or total faunal density (single factor ANOVA on \log_{10} transformed data $F_{(3,30)} = 1.4$, P > 0.05; Figure 9) were significantly affected by nutrient addition at Site 2. Chlorophyll a concentrations and total invertebrate densities on NDS were markedly higher downstream compared to upstream of the Hinton-Weldwood combined discharge (Figures 6 - 9). Taken together, these results suggest that phosphorus limits epilithic biomass upstream of Hinton while neither nitrogen nor phosphorus limit epilithic biomass downstream. Interestingly, P enrichment at Site 1 resulted in similar Chl a levels on NDS compared to levels observed downstream of the mill.

The higher epilithic biomass on stones and control NDS at Site 2 may be due to higher concentrations of SRP-P downstream of the mill (Table 2) or to higher water temperatures. Water temperature downstream of Hinton (i.e., Site 2, 4.4 ± 0.1 °C, N = 529) was significantly higher than that upstream (i.e., Site 1, 3.9 ± 0.1 °C, N=529) (Wilcoxon rank test, Z = 8.5, P < 0.0001) (Figure 10).

Mean water depths were not significantly different among sites or nutrient treatments either at the start or end of experiments among sites (upstream versus downstream of Hinton, P > 0.05; nutrient treatment ANOVA, P > 0.05) (Table 4). Similarly, water depths measured when NDS were retrieved from the river (i.e., final measurements) were not significantly affected by Site ($F_{(1,55)} = 2.5$, P > 0.05), nutrient treatment ($F_{(3,55)} = 0.5$, P > 0.05) or the interaction of these terms ($F_{(3,55)} = 0.3$, P > 0.05) (Table 4).

Figure 6. Mean ($\bar{x} \pm 1$ SE) chlorophyll <u>a</u> concentration (ug/cm²) on control and nutrient-enriched diffusing substrata after 22 days at Site 1 in the Athabasca River, 26 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 7. Mean ($\overline{\times} \pm 1SE$) total invertebrate density (No./NDS) on control and nutrient- enriched diffusing substrata after 22 days at Site 1 in the Athabasca River, 26 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 8. Mean ($\times \pm 1SE$) chlorophyll a concentration (ug/cm²) on control and nutrient- enriched diffusing substrata after 23 days at Site 2 in the Athabasca River, 27 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 9. Mean ($\overline{\times} \pm 1$ SE) total invertebrate density on control and nutrient- enriched diffusing substrata after 23 days at Site 2 in the Athabasca River, 27 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 10. Hourly water temperature (°C) in the Athabasca River at Site 1 (upstream of Hinton = dashed line) and Site 2 (downstream of Hinton = solid line) during Experiment 1, 4 October - 27 October, 1993. Comparisons of mean water temperature between sites was based on the period 1200h julian day 277 to 1300h on julian day 299 when hourly water temperature data was for each site (see Methods section).



effluent used in some NDS treatments in Experiment 2. Abbreviations as shown in Table 2. Dates represent when water samples were Table 3. Water quality measurements in the Athabasca River during experiments 2 & 3 and in secondary treated bleached kraft mill collected and can differ by 1 day compared to when nutrient diffusing were placed and retrieved from the Athahasca river

Disconsilable	:					Bioa	vailable N	7			Bioavailable P	
Date	Site	Temp (°C)	D.O. (mg/L	Hd ('	Chl a ug/L	NO ₃ + (ug/L	NO ₂ NH ₄	TP (ug/L)	TDP (J/gu)	SRP (ug/L)	N:P	
						Exp	eriment 2					
05/10/93	-	8.9	8.94	8.3	0.4	32.5	12.1	11.4	6.0	0.5	89.2	
27/10/93	1	4.8	69.6	8.1	1.1	67.5	16.0	25.9	BD	1.6	52.2	
						Exp	eriment 3					
16/11/93	6	0.1	11.0	9.3	0.68	86.9	51.0	26.8	3.5	2.6	53.0	
07/12/93	7	0.8	12.0	8.4	0.66	ī	40.9	26.8	3.5	2.0	4	
						EMu	ient chara	cteristic	S			
15/10/93	ı	ā		,	ų.	263	1800	543	263	199		

Table 4. Comparison of mean ($x \pm 1SE$) water depths (cm) among nutrient diffusing substrata treatments placed on the riverbed at Sites 1 and 2 (i.e., upstream and downstream of the combined Hinton and Weldwood of Canada pulp mill discharge) during Experiment 1 in the Athabasca River. C = control, N = nitrogen added treatment, P = phosphorus added treatment, N+P = nitrogen and phosphorus added treatments. Numbers in brackets are number of replicates.

	Initi]	Fina	**********************
Treatment	Site 1	Site 2	Site 1	Site 2
(1)	33.7±2.2 (7)	33,6±1,9 (8)	12.7±1.5 (7)	14.4±1.2 (7)
7	32, 1±3, 3 (8)	34.4±1.9 (8)	13.5±3.4 (6)	15.0±1.6 (5)
	36.4±3.0 (8)	35.0±2.3 (8)	14 1±1.7 (8)	15.3±1.7 (7)
A+P	34.5±1.5 (8)	39 4±1 9 (8)	13 6±2 1 (8)	18.0±1.9 (8)

Similarly, neither initial nor final water velocities were significantly different among sites (ANOVA, P > 0.05), nutrient treatment (P > 0.05) or the interaction of these terms (P > 0.05) (Table 5). These results indicate that differences in chlorophyll <u>a</u> and total invertebrate densities upstream and downstream of Hinton can not be attributed to differences in water depth or velocity.

Responses of epilithon and invertebrates to nutrient treatments at Sites 3 and 4. Epilithic biomass on upper stone surfaces was significantly higher upstream (Site 3: $2.3\pm.02$ ug Chl a/cm², N = 10) than downstream (Site 4: 1.5 ± 0.2 ug Chl a/cm², N = 10) of the ALPac discharge (two sample t-test, t = 2.9, P < 0.05) (Figure 11). These biomasses were also markedly lower than those observed at Sites 1 or 2 (Figure 5). Differences in epilithic biomass between sites 3 and 4 can not be attributed to differences in either water velocity (upstream = 0.27 ± 0.03 m/s N =10; downstream = 0.29 ± 0.03 , N = 10, t-test, t = 0.57, P > 0.05) or depth (upstream = 19.9 ± 1.82 cm, N =10, downstream = 21.1 ± 1.62 cm, N = 10, t-test, t = 0.63, P > 0.05).

Table 5. Comparison of mean ($\bar{x} \pm 1SE$) water velocities (m/s) among nutrient diffusing substrata treatments placed on the riverbed at Sites 1 and 2 (i.e., upstream and downstream of Hinton) during Experiment 1 in the Athabasca River. Treatment: C = control, N = nitrogen added treatment, P = phosphorus added treatment, N+P = nitrogen and phosphorus added treatments.

		-Initial		inal
Treatment	Site 1	Site 2	Site 1	Site 2
0	(2) 20 0+52 0	0 60+0 08 (8)	0 03+0 04 7	
)		(a) an'n=rcn'n	() +0.0+C2.0	$(1) cn'n \pm 17'n$
N	0.64±0.07 (8)	0.79±0.07 (8)	0.26±0.05 (6)	0.24 ± 0.03 (5)
Р	0.76±0.05 (8)	0.71±0.08 (8)	0.22±0.02 (8)	0.22±0.04 (7)
N+P	0.72±0.07 (8)	0.72±0.07 (8)	0.31±0.05 (8)	0.17±0.03 (8)

Figure 11. Mean ($\bar{x} \pm 1$ SE) chlorophyll <u>a</u> concentration (ug/cm²) on upper stone surfaces at Site 3 (upstream of the ALPac discharge) and Site 4 (downstream of the ALPac discharge) in the Athabasca River, 6 October 1993.



Chlorophyll a concentrations on NDS were significantly affected by nutrient addition at Site 3 (single factor ANOVA on \log_{10} transformed data, $F_{(3,25)} = 7.6$, P < 0.001) (Figure 12), however there was no consistent pattern. Compared to control substrata chlorophyll a concentrations were significantly higher than on P-enriched NDS yet not significantly different on N+P substrata. Chlorophyll a concentrations did not differ significantly between nitrogen enriched and control substrata (Least squares comparisons, p < 0.05) (Figure 12). Although these results suggest that P may be the limiting nutrient at Site 3, the generally low levels of chlorophyll a combined with sample variation may make it difficult to observe consistent responses to nutrient enrichment.

Total invertebrate density at Site 3 was significantly affected by nutrient treatment (single factor ANOVA on \log_{10} transformed data $F_{(3,24)} = 5.8$, P < 0.005) (Figure 13). Densities were significantly higher on P-enriched than control and N-enriched substrata. However, there was no significant difference between total invertebrate densities on P-enriched and P+N-enriched substrata (Least squares difference tests, P > 0.05; Figure 13).

Chlorophyll <u>a</u> concentrations at Site 4 were significantly affected by nutrient addition (single factor ANOVA on \log_{10} transformed data $F_{(3,25)} = 6.0$, P < 0.005) (Figure 14). Chl <u>a</u> concentrations on P-enriched substrata was significantly higher than on all other treatments (Least squares difference tests, P < 0.05). In contrast, total invertebrate density was not significantly affected by nutrient treatment (single factor ANOVA on \log_{10} transformed data $F_{(3,24)} = 0.6$, P > 0.05) (Figure 15) indicating that nutrient enrichment effects on epilithon were not consistent with those observed for the total NDS invertebrate community. Lastly, mean water temperature at Site 3 (4.9 ± 0.04 , n = 697) was significantly higher than at Site 4 although the absolute difference is minimal (< 1.5 °C) (4.8 ± 0.04 , N = 697) (Wilcoxon rank test, Z = 2.14, P < 0.05) (Figure 16)

Figure 12. Mean ($\times \pm 1SE$) chlorophyll a concentration (ug cm²) control and nutrient- enriched diffusing substrata after 22 days at Site 3 in the Athabasca River, 28 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 13. Mean ($\times \pm 1SE$) total invertebrate density (No./NDS) control and nutrient- enriched diffusing substrata after 22 days at Site 3 in the Athabasca River, 28 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Neither initial nor final water depth or velocity differed between sites (i.e., upstream versus downstream of AlPac, ANOVA on \log_{10} transformed data, P > 0.05), nutrient treatment (ANOVA on \log_{10} transformed data, P > 0.05) or the interaction of these terms (ANOVA on \log_{10} transformed data, P > 0.05) (Tables 6 and 7). These results indicate that differences in Chl a and total invertebrate densities upstream and downstream of the AlPac mill discharge may be temperature related and not be attributable to differences in initial or final depths or velocities.

Figure 14. Mean ($\overline{x} \pm 1$ SE) chlorophyll a concentration (ug/cm²) on control and nutrient-enriched diffusing substrata after 22 days at Site 4 in the Athabasca River, 27 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 15. Mean ($\bar{x} \pm 1SE$) total invertebrate density (No./NDS) on control and nutrient-enriched diffusing substrata after 22 days at Site 4 in the Athabasca River, 27 October, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



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Figure 16. Water temperature (°C) in the Athabasca River measured at 15 minute intervals at Site 3 (i.e., upstream of the ALPac discharge = dashed line) and Site 4 (i.e., downstream of the AlPac discharge = solid line) during Experiment 1 in the Athabasca River, 4 Oct - 27 Oct, 1993. Water temperatures were highly variable after julian day 298 when reduced flow may have partially exposed thermographs causing diel cycles of > 5°C. Comparison of mean water temperature between sites was based on the 14 day period between julian days 279 (1200h) to 293 (1815h) when quarter hourly water temperature data were available for each site.



Table 6. Comparison of mean ($\overline{x} \pm 1SE$) water depths (cm) among nutrient diffusing substrata treatments placed on the riverbed at Sites 3 and 4 (i.e., upstream and downstream of the Alberta Pacific pulp mill discharge) during Experiment 1 on the Athabasca River. C = control, N = nitrogen added treatment, P = phosphorus added treatment, N+P = nitrogen and phosphorus added treatments.

	Init	tial		Final
Treatment	Site 3	Site 4	Site 3	Site 4
C	47.9±2.1 (7)	48.8±1.5 (5)	25.0±1.2 (7)	26.0±2.4 (8)
Z	48,0±1,7 (8)	45.5±3.9 (8)	27, 4±1, 8 (7)	22.0±3.8 (8)
Р	43.0±1.9 (8)	48.4±1.1 (8)	24.1±1.9 (8)	22,4±1.8 (8)
N+P	43.0±1.6 (9)	48.5±2.9 (6)	23.6±2.1 (7)	24.3±2.0 (6)

Sites 3 and 4 (i.e., upstream and downstream of the Alberta Pacific pulp mill) during Experiment 1 in the Athabasca River. C = control, N Table 7. Comparison of mean (x \pm 1SE) water velocities (m/s) among nutrient diffusing substrata treatments placed on the riverbed at = nitrogen added treatment, P = phosphorus added treatment, N+P = nitrogen and phosphorus added treatments.

		Initial		-Final
Treatment	Site 1	Site 2	Site 1	Site 2
U	0.54±0.04 (7)	0.57±0.05 (5)	0 30±0 01 (7)	0 27+0 03 (5)
Z	0.61±0.04 (8)	0.55±0.02 (8)	0.27±0.03 (7)	0.29±0.03 (8)
Ь	0.62±0.05 (8)	0.62±0.05 (8)	0.32±0.02 (8)	0.28±0.01 (8)
A+P	0.61±0.04 (9)	0.52±0.03 (6)	0.26±0.03 (7)	0.31±0.01 (6)

5.3.2 Experiment 2.

<u>Water chemistry</u> Water temperature at Site 1 declined sharply between 5 October - 27 October (Table 3) and coincided with increases in concentrations of NO_2+NO_3-N , NH_4 , TP, SRP-P. Concentrations of phosphorus were low compared to nitrogen sources (Table 3).

Responses of epilithon and invertebrates to nutrient treatments at Site 1. Chlorophyll a concentrations on NDS were significantly affected by nutrient treatment (single factor ANOVA on \log_{10} transformed data $F_{(5,42)} = 9.9$, P < 0.0001) (Figure 17). Comparison of treatment means showed that P-enriched NDS that contained 0% to 50% secondary-treated BKME were not significantly higher than controls (i.e., no added nutrients). In contrast among phosphorus added treatments, Chl a concentrations on NDS containing 100% BKME + 0.5 M KH₂PO₄ were significantly lower than other P-enriched treatments and not significantly different from untreated controls. While the BKME used to dissolve agar contained phosphorus (Table 3), the amounts are minor compared to the total amount of phosphorus added (i.e., 0.5 M KH₂PO₄). Thus, it is unlikely that any differences in epilithic biomass or invertebrate density between phosphorus-enriched and phosphorus + BKME enriched treatments reflect differences in nutrient levels.

Total invertebrate density was also significantly affected by nutrient treatment (single factor ANOVA on \log_{10} transformed data, $F_{(5.38)} = 3.8$, P < 0.01) (Figure 18). Densities were greatest on NDS enriched solely with P. There was no significant difference between control densities and those from NDS containing BKME.

Significant differences in epilithic biomasses among treatments can not be attributed to differences in initial or final mean water depths or velocities among treatments (single factor ANOVA on: 1) water depths, P > 0.05; 2) water velocities, P > 0.05; or 3) final mean water depths ANOVA on \log_{10} transformed data $F_{(5,42)} = 1.5$, P > 0.01) (Table 8). In contrast, mean water velocity measured immediately after the NDS were removed was significantly affected by nutrient treatment (ANOVA on \log_{10} transformed data $F_{(5,42)} = 4.5$, P > 0.05. Substrata enriched with phosphorus and 50% BKME (P+50%BKME) were retrieved from significantly higher current velocities than P+10%BKME, P+50%BKME and control substrata. Further, there were significant differences in mean current velocities between P+1%BKME and control NDS; control NDS and P+0%BKME; P+0%BKME and P+50%BKME (Table 8).

Figure 17. Mean ($\overline{x} \pm 1$ SE) chlorophyll a concentration (ug/cm²) on control, P-enriched, and P + bleached kraft mill effluent enriched diffusing substrata after 23 days at Site 1 in the Athabasca River, 27 October, 1993. Secondary-treated BKME obtained from Weldwood of Canada Ltd. Treatments: 0 nutrients+0% effluent; P(0.5M KH₂PO₄) + 0% BKME; P(0.5 M KH₂PO₄) + 1% BKME; P(0.5M KH₂PO₄) + 10% BKME; P(0.5 M KH₂PO₄) + 50% BKME; P(0.5 M KH₂PO₄) + 100% BKME. Treatments with the same letter are not significantly different.



Figure 18. Mean ($\bar{x} \pm 1SE$) total invertebrate density (No./NDS) on control, phosphorus and secondarytreated bleached kraft mill effluent enriched diffusing substrata after 23 days at Site 1 in the Athabasca River, 27 October, 1993. Abbreviations as shown in Table 15.



TREATMENT

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Experiment 2 in the Athabasca River. C = control, P = phosphorus added treatment, E = secondary-treated bleached kraft mill effluent.Number in brackets = no. of replicates. Table 8. Comparison of mean (x \pm 1SE) water depths (cm) and velocities (m/s) among NDS treatments placed at Site 1 during

	Wate	sr depth	Wat	er velocity	
Treatment	Initiai	Final	Initial	Final	
C	39.1±3.5 (8)	17 8±2.3 (8)	0.68±0.08 (8)	0.13±0.02 (8)	
P+0%E	36.4±3.0 (8)	14.1±1.7 (8)	0.76±0.05 (8)	0.22±0.02 (8)	
P+1%E	35.3±4.6 (8)	24.6±5.4 (5)	0.63±0.09 (8)	0.29±0.08 (5)	
P+10%E	35.9±3.3 (8)	14.1±3.4 (8)	0.67±0.04 (8)	0.18±0.02 (8)	
P+50%E	36.8±2.5 (8)	12.3±2.0 (7)	0.73±0.04 (8)	0.14 ± 0.02 (7)	
P+100%E	32.5±2.7 (8)	$11.0\pm1.9(7)$	0.64±0.10 (8)	0.30 ± 0.05 (7)	

5.3.3 Experiment 3.

<u>Water chemistry.</u> Dissolved oxygen concentrations at Site 2 in December were high and exceeded 10 g/L on both sampling occasions (Table 3). Concentrations of NO_2 + NO_3 -N, TP and SRP-P were also relatively high during December compared to October (Table 3).

Responses of epilithon and invertebrates to nutrient treatments at Site 2. Epilithic biomass on NDS was not significantly affected by nutrient treatment (single factor ANOVA on \log_{10} transformed data, $F_{(3,28)} = 0.91$, P > 0.05) (Figure 19). Chlorophyll a accrual over the 20 day incubation period was considerably lower than at the same site in October-November (Experiment 1). Low chlorophyll a concentrations on NDS in December may have resulted from lower water temperatures (mean hourly temperature = 0.34 °C; Figure 20) as compared to October (4.36 °C) when cell division rates would be low. The absence of a significant nutrient treatment effect on epilithic biomass can not be explained by differences in initial (single factor ANOVA, $F_{(3,31)} = 0.67$, P > 0.05) or final (ANOVA, $F_{(3,31)} = 0.7$, P > 0.05) water depths or velocities (ANOVA initial velocities: $F_{(3,31)} = 0.5$, P > 0.05; final depths, $F_{(3,31)} = 0.74$, P > 0.05) (Table 9).

Figure 19. Mean ($\bar{x} \pm 1$ SE) chlorophyll a concentration (ug/cm²) on control and nutrient-enriched diffusing substrata after 20 days at Site 2 in the Athabasca River, 15 November - 6 December, 1993. Treatments: C = control, N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added. Treatments with the same letter are not significantly different.



Figure 20. Water temperature (°C) in the Athabasca River measured at 30 minute intervals at Site 2 (i.e., downstream of Hinton) during Experiment 3, 15 November - 6 December, 1993. Mean water temperature was based on the period 1200h julian day 319 to 1675h on julian day 340.



Table 9. Comparison of mean ($x \pm 1SE$) water depths (cm) and velocities (m/s) among NDS treatments placed on the substratum at Site 2 (i.e., downstream of Hinton) during Experiment 3 in the Athabasca River. C = control, N = nitrogen added treatment, P = phosphorus added treatment, N+P = nitrogen and phosphorus added treatments.

	Wate	er depth	Wate	r velocity
[reatment	Initial	Final	Initial	Final
0	51.9±2.7 (9)	39 6±2.9 (9)	0.82±0.10 (9)	0.72±0.10 (9)
7	52.3±2.2 (7)	40.6±1.9 (7)	0.68±0.08 (7)	0.64 ± 0.10 (7)
	54.3±4.2 (8)	42,9±4.2 (8)	0.86±0.04 (8)	0.73±0.05 (8)
4+P	48.0±3.2 (8)	36.3±3.2 (8)	0.92±0.09 (8)	0.80±0.08 (8)

6.0 **DISCUSSION**

Our results have shown that NDS are a useful technique for assessing impacts of nutrient and contaminant loading on benthic communities in rivers. Field experiments in the Athabasca River, Alberta demonstrated that NDS consisting of a porous clay pot filled with agar and attached to the riverbed with stakes remained intact for at least three weeks in current speeds up to 0.8 m/s. Nutrient diffusing substrata were colonized by epilithon and macroinvertebrates. Laboratory experiments showed that NO₂+NO₃-N and SRP-P were released in quantities that likely exceed algal cellular requirements for at least 32 days (> 60 ug/L/day of NO₂+NO₃-N and SRP-P). Following on our successful laboratory and field trials, we deployed NDS at 4 sites on the Athabasca River in fall and winter to assess the impact of nutrient loading from BKME (i.e., the combined Hinton and Weldwood combined effluent; effluent released by ALPac) relative to upstream reference sites.

Responses of epilithic and benthic invertebrate communities to localized nutrient enrichment using NDS were variable depending upon location in the river and season. For example in the fall, Chl a concentrations and total invertebrate densities on P-enriched NDS were significantly higher than controls upstream (i.e., Site 1) but not downstream of Hinton (i.e., Site 2). The lack of a significant difference in Chl a and invertebrate density between P-enriched NDS and controls downstream of Hinton was also observed when NDS were deployed in the winter (Experiment 3). These comparisons indicate that phosphorus availability limits algal biomass upstream but not downstream of Hinton.

In contrast to the strong evidence for phosphorus limitation upstream but not downstream of Hinton, epilithic responses to P-enriched NDS were equivocal upstream (i.e., Site 3) and downstream (Site 4) of ALPac. For example, Chl a on P-enriched NDS exceeded that on controls, but there was no difference between N+P-enriched and controls at both sites. Further, although total invertebrate density on P-enriched NDS was significantly higher than on control substrata at Site 3, there was no difference between total invertebrate density on P-enriched and N+P-enriched despite a four-fold difference in Chl a concentrations; total invertebrate density did not differ significantly between any of the enrichment treatments at Site 4 despite significant differences in epilithic biomass.

Our experiments provide strong support that epilithic biomass in the Athabasca River can be limited by the availability of phosphorus. Although our experiments were conducted over relatively short periods of time, the results suggest that prolonged nutrient additions alone could result in localized increases in primary and secondary production in the Athabasca River. This hypothesis is consistent with results from several recently reported longer term nutrient fertilization studies (e.g., Hershey et al. 1988, Hart 1990, Johnson et al. 1990, Hinterleitner-Anderson et al. 1992, Peterson et al. 1985, 1993). For instance, long-term whole river fertilization of a pristine Alaskan river over 4 consecutive summers initially increased algal biomass and in later stages (i.e., summers 3 and 4 of fertilization) was associated with increased densities of some herbivorous insects, and growth of adult grayling (<u>Thvmallus arcticus</u>) (Peterson et al. 1993). Similarly, fertilization of the Keogh River, British Columbia, increased periphyton accumulation rates (Perrin et al. 1987) and size of steelhead trout (<u>Oncorhvnchus mykiss</u>) and coho salmon fry (<u>Oncorhvnchus kisutch</u>) (Johnson et al. 1990). Phosphorus additions in stream-side artificial streams also significantly increased individual mass of some invertebrate species (Hart and Robinson 1990). However, some rivers are N, not P, limited (Bothwell 1992).

Epilithic and invertebrate communities on P-enriched NDS varied with river location independent of nutrient enrichment. Thus, epilithic biomass on phosphorus-enriched substrata was almost four-fold higher upstream of the combined Hinton and Weldwood outfall than upstream of the ALPac outfall (i.e., Site 3). Although the mechanisms producing these differences are not fully understood, differences in epilithic biomasses between these sites may reflect differences in light attenuation because effluent from the kraft process can be highly coloured (Owens 1991, McCubbin 1992). Differences in epilithic biomass are unlikely due to differences in water temperature since mean water temperatures differed little between sites (Experiment 1: Site 1 = 3.9 °C, Site 3 = 4.9 °C). Moreover, these differences would be expected to have enhanced algal growth at Site 3 compared to Site 1.

Accrual of epilithic biomass on NDS placed downstream of the Hinton municipal and Weldwood of Canada pulp mill also differed seasonally. For instance, epilithic biomass for all treatments ranged from 9.5 ± 2.7 to 15.9 ± 2.4 ug Chl a cm², in October but was 34 to 132 times lower in December $(0.1\pm0.02 \text{ to } 0.3\pm0.10 \text{ ug Chl a/cm}^2)$. Differences in chlorophyll a accrual between the October and December experiments may reflect differences in water temperature (See Bothwell 1988). Mean water temperature at Site 2 was substantially lower in December ($\times \pm 1SE = 0.3\pm0.01$ °C) than in October (4.4 ± 0.1 °C) and should have reduced algal division rates. Reduced day length and low reduced insolation, due to low sun angle, may have also contributed to these differences.

Effluent from bleached kraft mills contain a diversity of organic contaminants including resin and fatty acids, and chlorinated dioxin, furan, phenol, guaiacol and catechol compounds (Hall et al. 1991, McCubbin and Associates Ltd. 1992, Swanson et al. 1992, National council of the paper industry for air and stream improvement 1993). These compounds can potentially affect epilithic biomass dynamics due to their lethal and sub-lethal effects. For instance, the presence of contaminants could potentially increase epilithic biomass downstream of pulp mill discharges by reducing the ability of herbivorous insects to graze algal mats. Under this scenario, increased epilithic biomass downstream of a pulp mill effluent would result from increased nutrient availability combined with the negative balance between the herbivore communities numerical response and contaminant-mediated feeding inhibition.

Our results indicate that the presence of BKME combined with 0.5 M KH₂PO₄ significantly affected epilithic biomass (Experiment 2). At BKME concentrations of 1%, 10%, 50% algal biomass, expressed as Chl <u>a</u>, was not significant (P > 0.05) from that of P addition alone. However, at BKME of 100%, the enrichment response to added P was significantly reduced such that algal biomass was 2 fold lower in the P+100% BKME than the P only treatment. Total invertebrate density was also significantly affected by effluent concentration. While only the P+10% BKME treatment had significantly lower (P < 0.05) invertebrate densities than the P only treatment, densities from the P+1% BKME was significantly different at a slightly higher alpha (P = 0.07). This suggests that BKME has the potential to affect benthic algal biomass. While the relationship between a BKME concentration of 100% in the diffusion substrata and the concentration leached into the surrounding water has yet to be determined, it is likely that concentrations inhibitory to benthic production are much greater than typical 2% effluent:river water dilution.

Ecological effects of BKME on aquatic ecosystems typically have consisted of field surveys to identify longitudinal patterns in water chemistry, benthic algal, invertebrate and fish communities upstream and downstream of a mill point source discharge. These surveys are then combined with laboratory or field experiments to identify the underlying mechanisms involved. This is a potentially powerful approach because the biotic patterns identified from the analysis of field surveys can be used to test specific hypotheses to identify cause and effect relationships. In reality, this approach has suffered from one of two methodological weaknesses. First, the search for cause-effect relationships is often compromised because experiments are pseudo-replicated (See Hurlbert 1984 for different types of pseudo-replication). Second, numerous experiments designed to investigate the effects of BKME on epilithon and invertebrates have been performed with too few replicates such that the probability of identifying a treatment effect is minimal because of low statistical power (i.e., committing a type II error) (e.g., Amblard et al. 1990, Hall et al. 1991). The use of NDS also overcomes the two methodological weaknesses (i.e., psuedoreplication and low replicate numbers). Preliminary results from studies with NDS on the Athabasca River identified that effluent loading from combined municipal and pulp mill effluent saturated nutrient requirements of the epilithic community compared to the nutrient limited condition upstream of the effluent outfall in the fall. Further, studies are required to determine seasonality and spatial variability in benthic responses to nutrient enrichment.

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9.0 APPENDICES

Appendix 1. Terms of reference for Northern River Basins Study contract No. 2614-C1: Identification of Spatial and Temporal Patterns in Nutrient Limitation in the Peace-Athabasca Rivers.

NORTHERN RIVER BASINS STUDY

TERMS OF REFERENCE

Project 2614-C1: Identification of Spatial and Temporal Patterns in Nutrient Limitation in the Peace-Athabasca Rivers

I. Objective

The objective of this project is to identify spatial patterns of nutrient limitation in the Peace-Athabasca rivers by conducting *in-situ* nutrient addition experiments upstream and downstream of the Weldwood and Alpac pulp mill sites in September-October, 1993.

II. Requirements

A. Experimental design, data compilation and interpretation

- 1. Design and undertake *in-situ* riverbed nutrient addition experiments in September-October 1993 to determine the effects of nutrient additions on algal biomass (Chlorophyll *a* & ash-free dry mass) and benthic biomass and species diversity upstream and downstream of the Weldwood and Alpac pulp mill sites.
- 2. Compare algal and benthic macroinvertebrate responses to riverbed nutrient additions using agar nutrient diffusion substrate.
- 3. Quantify algal biomass and to sort and prepare 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 biomass and species diversity of benthic macroinvertebrate communities upstream and downstream of the Weldwood and Alpac pulp mill sites.
- B. Review Report

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 the Weldwood and Alpac pulp mills in the fall season. The report will include:

- 1. A description of the physical characteristics of the study sites.
- 2. Details of the experimental design, and 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.

III. Reporting Requirements

1. Provide ten copies of the draft report to the component coordinator by March 15, 1993.

2. 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 or the final report to the Component Coordinator. An electronic copy of the report, in Word Perfect 5.1 format, is to be submitted to the Project Liaison Officer along with the final report. Text for the final report is to be in 12 point Times Roman font. The final report is to contain a table of contents, list of figures (if appropriate) list of tables, acknowledgements, executive summary and an appendix containing the Terms of Reference for this contract.

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 pertaining to this project of a scientific nature should be directed to her.

The NRBS Study Office Component Coordinator for this project is:

Greg Wagner 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.

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