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Northern River Basins Study









NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 92

IMPACTS OF CONTAMINANTS AND NUTRIENTS IN BLEACHED KRAFT MILL EFFLUENT ON BENTHIC INSECT AND PERIPHYTON COMMUNITIES: ASSESSMENTS USING ARTIFICIAL STREAMS, ATHABASCA RIVER, 1993 AND 1994





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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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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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(Dr. Fred J. Wrona, Science Director) — (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. A. Larkin — 14 Feb 1996

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

Whereas the Study Board is satisfied that this publication has been reviewed for scientific content and for immediate health implications,
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 — 23 Feb /96

(Lucille Partington, Co-chair) — (Date)

Robert McLeod. — 23 Feb/96.

(Robert McLeod, Co-chair) — (Date)

IMPACTS OF CONTAMINANTS AND NUTRIENTS IN BLEACHED KRAFT MILL EFFLUENT ON BENTHIC INSECT AND PERIPHYTON COMMUNITIES: ASSESSMENTS USING ARTIFICIAL STREAMS, ATHABASCA RIVER, 1993 AND 1994

STUDY PERSPECTIVE

An important initiative of the Northern River Basins Study is the development of methods for assessing the effects of industrial effluents on the health and integrity of the aquatic ecosystem. These effluents contain a wide array of compounds, and their effects can be stimulatory (in the case of nutrients) or inhibitory (in the case of some natural tree compounds). Previous studies have shown that high levels of nutrients contained in pulp mill effluents can result in significant increases in the primary productivity of some rivers, but these enhancement effects can mask the toxic effects of contaminants on riverine biota. Because of the complex interaction between effluent compounds and the receiving environment, difficulties arise when biomonitoring studies attempt to predict accurately the impacts of effluents on the aquatic food chain. This project makes use of an innovative artificial stream system to assess the combined effects of nutrients and contaminants in effluent discharges on the aquatic food chain in the upper Athabasca River near Hinton.

Related Study Questions

1a) *How has the aquatic ecosystem, including fish and /or other aquatic organisms been affected by exposure to organochlorines or other toxic compounds?*

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?*

13b) *What are the cumulative effects of man made discharges on the water and aquatic environment?*

The objective of this project was to differentiate between the effects of bleached kraft mill effluent (BKME) and nutrient (nitrogen and phosphorus) additions on benthic algae and invertebrate (primarily insects) communities. A transportable artificial stream system located alongside the Athabasca River at Hinton was used for the experiment. The measured response variables included: ash-free dry biomass and chlorophyll *a* of algae, the abundance and composition of benthic insects, and the average weight and growth of individual insects.

Similar to the trends observed in the Athabasca River downstream of Hinton, both the nutrient enrichment and 1% dilution BKME experimental treatments stimulated primary production of the algal community relative to the control treatment. The responses of algal communities were less noticeable in spring than in autumn, primarily the result of lower levels of soluble reactive phosphorus in the river water during spring. Abundances of total insects and several dominant groups (stoneflies, mayflies and midges) increased in the nutrient and effluent treatments. Insect communities in the two treatment groups were more similar to one another than the control group, suggesting that the shifts in species composition were a response to enrichment rather than toxicity. The increases in algal biomass had growth-enhancing effects on benthic insects in both the nutrient and BKME treatment groups, indicating that nutrient enrichment effects were not masked by contaminant inhibitory effects.

The experiments in this study provide the first strong evidence that the dominant effect of the BKME at Hinton is one of nutrient enrichment and stimulation of food chain productivity in the Athabasca River. This information will be used for assessing and managing the immediate and long-term effects of pulp mill effluents on biota. Results from these experiments will provide an important linkage with other NRBS studies dealing with food chain and contaminant fate modelling, and assessing ecosystem health in these rivers. Artificial stream research is a promising technique for investigating the cause and effect relationship of current pulp mill effluents, and proposed future development.

REPORT SUMMARY

This report discusses results from artificial stream experiments in relation to the effects of effluent stressors and nutrient enrichment on benthic algal and invertebrate communities. The report also (i) compares the results from autumn 1993 and 1994 to document between-year variability in the relationships between effluent additions, and effects on primary and secondary producers, and (ii) contrasts results from spring 1994 to those from autumn 1993 and 1994 to assess the importance of seasonal changes in moderating the nutrient-contaminant effects on benthic food web interactions. The research provides information relating to Question 5, "Are the substances added to the rivers by man-made discharges likely to cause deterioration of the water quality", and Question 1A, "How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?"

The artificial stream experiments were designed to decouple the effects on the riverine food web of the potentially confounding effects of nutrients and contaminants within effluent (Podemski and Culp 1995). This goal required that we design a novel riverside artificial stream system (Culp et al. 1994, Culp and Podemski 1995), and establish experimental treatments consisting of a control that received raw river water, a 1% dilution of treated effluent, and a 1% dilution of the nitrogen and phosphorus contained in the concentrated effluent. By comparing the effects of the nutrient and effluent treatments on different trophic components of the food web, we were able to provide a mechanistic understanding of the stimulatory and/or inhibitory effects of pulp mill effluents on the benthic food webs of the Athabasca River.

Our experiments and in-river observations provide strong evidence that the dominant effect of the Weldwood of Canada Ltd. effluent at a 1% dilution discharged to the upper Athabasca River is that of nutrient enrichment and stimulation of food web productivity. In autumn 1993 and 1994, both the nutrient and effluent treatments stimulated primary production of the largely diatom algal community relative to the control treatment, which was representative of conditions upstream of the effluent discharge. These experimental findings corresponded to in-river trends where periphyton biomass increased at sites downstream of the effluent outfall relative to upstream reference sites. The increased periphyton accumulation in the artificial streams occurred both on rocks with existing algal communities and on tiles which experienced rapid accumulation of algae over the duration of the experiment. The effect of effluent on periphyton communities in the upper Athabasca River changed seasonally such that the responses of algal communities, both in the river and artificial streams, were less marked during spring than in autumn. For example, whereas algal biomass on rocks upstream and downstream of the effluent outfall was similar in the spring, in autumn 1993 chlorophyll *a* concentrations were 2.5 times higher downstream. The seasonal differences in periphytic algal biomass were due to the much lower concentration of soluble reactive phosphorus (SRP) in spring 1994 rather than temperature differences. In spring, SRP concentrations resulting from 1% effluent addition were ≤ 1 µg/L compared to 2-3 µg/L with excursions to 5 µg/L during the autumn 1993 and 1994 experiments. On average, in-river SRP levels upstream of the effluent outfall were 1.5 µg/L in the spring, and 1 µg/L and 1.7 µg/L in the autumn 1993 and 1994, respectively. Thus, prior to biological

uptake, the SRP concentrations in the artificial streams averaged 2-3 µg/L in the spring, compared to 2-6 µg/L in autumn 1993 and 4-10 µg/L in autumn 1994.

The effluent-induced nutrient enrichment, and subsequent increase in periphyton biomass during autumn 1993 and 1994, elevated food availability for secondary producers inhabiting the upper Athabasca River. Abundances of total insects and several dominant taxa (stoneflies, mayflies and midges) increased in the nutrient and effluent treatments. Moreover, insect communities in the nutrient and effluent treatments were more similar to one another than to the control biota and were dominated by mayflies, stoneflies and midges, suggesting that composition shifts were a response to enrichment rather than toxicity. Communities in the Athabasca River downstream of the effluent discharge exhibited a similar shift and were likewise dominated by mayflies, stoneflies and midges. Although these community shifts are clearly effluent-induced, the effluent-exposed communities included many taxa (mayflies and stoneflies) that are considered to be sensitive to pollution (Rosenberg and Resh 1993). Because the Athabasca River upstream of the effluent discharge is phosphorus-limited and oligotrophic, the current effluent loads to the upper river provide levels of nutrient enrichment that increase benthic riverine productivity without the biotic changes associated with severe eutrophication. Our artificial stream studies provide experimental evidence that substantiates speculation from earlier field studies which attributed the increased insect abundance downstream of the Hinton discharge to effluent-induced nutrient enrichment (Anderson 1989, 1991). In addition, these experiments yielded the first evidence that effluent-induced increases of periphyton biomass had growth-enhancing effects on benthic insects (mayfly, stonefly and caddisfly taxa) in the upper Athabasca River. The fact that growth was similarly increased in the nutrient and effluent treatments indicates that nutrient enrichment effects where not masked by deleterious contaminant effects. Effluent exposure also produced no measurable effect of contaminants on insects at the community level with the observed changes in community structure largely reflecting the increase in abundance of taxa responding to increased periphytic resources.

The development of a unique, field-based artificial stream system for NRBS provided the means for obtaining a mechanistic understanding of the effects of effluent-related nutrient and contaminant stressors on the benthic biota of the upper Athabasca River. Causality could be assigned definitively in a field application where inferential hypothesis testing is normally very limited. By combining artificial stream results with field observation, this mechanistic understanding of stressor effects could be directly linked to *in situ* situations in the upper Athabasca River ecosystem. Future applications of artificial streams to northern rivers could include the linkage of artificial stream experiments with water quality models in order to contribute directly to the development, parameterization, and testing of models for predicting ecosystem-level responses to nutrient and contaminant addition. They would also be valuable tools for assessing the potential for additional mills along the river to raise overall contaminant and nutrient concentrations to levels that could degrade the ecosystem. Artificial stream research is a promising technique for consideration in aquatic environmental effects monitoring (EEM) programs for the pulp and paper industry because cause and effect scenarios can be investigated, and ecological indicators for riverine biota developed under experimentally controlled dose-response regimes.

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1.0 INTRODUCTION

1.1 DEFINITION OF PROBLEM

Pulp mills discharge significant amounts of complex effluents to river systems throughout Canada. Often large river systems receive multiple effluents, as is the case for the Athabasca River, Alberta, which annually receives the treated effluent from five pulp and paper mills (McCubbin and Folke 1993, Chambers 1996). Because pulp and paper mill effluents contain a complex array of compounds, their effects on aquatic organisms and food webs can be stimulatory or inhibitory (McLeay 1987, Bothwell 1992, Lowell et al. 1995). Although recent regulations have emphasized toxicity effects (Owens 1991), improvements in pulping and waste treatment processes have raised effluent quality to the point that most are only weakly toxic (Walden and Howard 1977). In contrast, the high levels of nitrogen and phosphorus contained in pulp mill effluents have resulted in significant changes in primary productivity in some rivers in western Canada and the United States (Bothwell 1992). Nutrient-enhancement effects can mask the toxic effects of contaminants on primary producers (Lozano and Pratt 1994) and, given our poor understanding of the nutrient-contaminant effects of pulp mill effluents on riverine biota, it is difficult to set rigorous regulatory guidelines (Owens 1991).

Most compounds in bleached kraft mill effluents (BKME), including wood extractives (e.g., terpenes and metals) and lignin residuals, are derived from the wood itself. Terpenes often provide terrestrial plants with resistance to insect herbivores, and can inhibit feeding and growth (Rosenthal and Janzen 1979, Harborne 1990). Similarly, chlorinated lignin residuals may affect feeding in hydropsychid caddisflies by causing the production of deformed feeding-net structures (Petersen and Petersen 1984). Thus, inhibitory compounds in BKME may lead to a suite of ecological effects, namely decreases in feeding rate, growth and fecundity of riverine grazers. Alternatively, effluents can add limiting nutrients and result in increased food resources for invertebrates which are an important nutritional base for riverine fish. This elevated production of plant material may result in higher invertebrate growth and fecundity. In the Athabasca River, abundance of many benthic invertebrates shows a marked increase for several kilometres below Hinton, Alberta, suggesting the net impact of the combined BKME and municipal sewage discharge is one of food web enrichment in this situation (Anderson 1989).

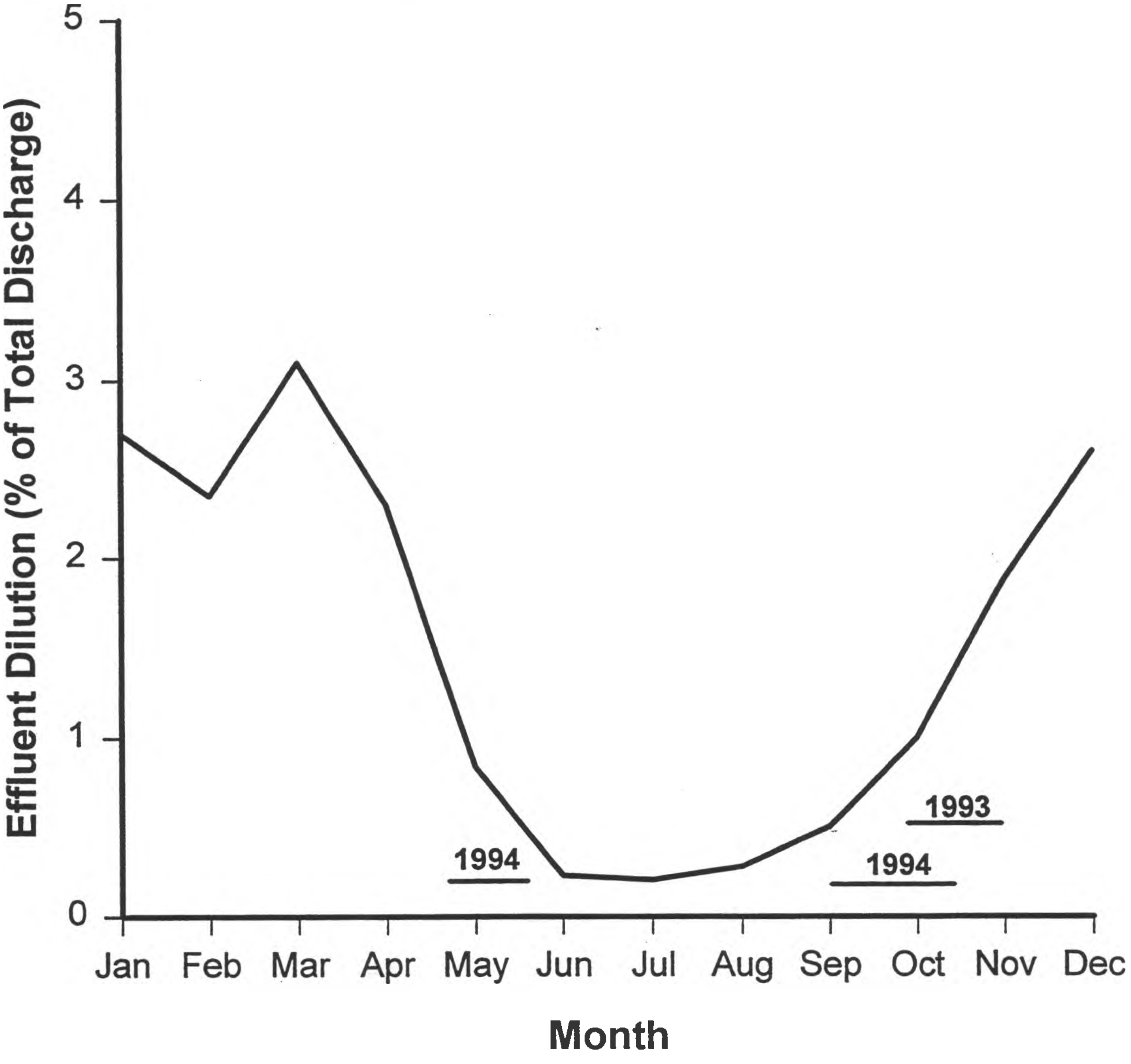
In natural environments, such as the Athabasca River, the high degree of spatial heterogeneity and the challenge of obtaining true replicates makes it difficult for biomonitoring studies to predict quantitatively the impacts of complex effluents on biota (Buikema and Voshell 1993, Forbes and Forbes 1994). Although biomonitoring provides important observations and insights into patterns of effluent effects on benthic biota, inferential hypothesis testing through field assessments is often impossible (Stewart-Oaten et al. 1992; Cooper and Barmuta 1993). To alleviate these problems, we developed an artificial stream system that simulates flowing water environments for the purpose of assessing the effects of effluent discharges on riverine food webs (Culp et al. 1994, Culp and Podemski 1995). Experimentation using artificial streams provides a promising, complimentary approach to biomonitoring assessments because artificial streams provide control over relevant environmental variables and true replication of treatments (McIntire 1993). Thus, causality (i.e., cause and effect) can be assigned definitively and the contrasting effects of nutrient and contaminant effects of BKME on benthic food webs can be disentangled, isolated and measured.

1.2 OBJECTIVES

We isolated the effects of nutrients from those of complex BKME, and tested their separate impacts on a food web comprised of primary producers (attached algae) and secondary producers (benthic insects) found in the Athabasca River upstream of Hinton, Alberta, during spring and autumn. To accomplish this, we compared the effects of the addition of a 1% dilution of BKME to our experimental community, with the effect of the addition of the nutrients (nitrogen and phosphorus) contained in the effluent. A 1% dilution was chosen because this is similar to the dilution in the Athabasca River downstream of Hinton during the spring and autumn (Fig. 1). The response variables measured included the ash-free dry biomass (AFDM) and chlorophyll *a* (Chl*a*) of periphyton, the abundance and composition of benthic insects, and the biomass of abundant invertebrates including specific caddisfly, mayfly and stonefly taxa. The experiments were carried out during autumn 1993, and spring and autumn 1994 in artificial streams located alongshore of the Athabasca River at the Hinton, Alberta, mill. These seasons were chosen for study because they are annual ice-free periods of low flow when effluent concentration is high and turbidity low, thus, they are times when nutrient enhancement is likely to increase autochthonous primary production. Although this effluent is a mixture of bleached kraft mill and municipal effluents, the combined total phosphorus (TP) loading from this mill is similar to that of other mills in the basin because the municipal loadings provide sufficient nutrients so that supplementary fertilization with phosphorus is only necessary during the winter. Therefore, the Weldwood of Canada Ltd. effluent from the Hinton facility is appropriate for investigating nutrient-contaminant interactions of BKME.

This report discusses results from artificial stream experiments in relation to the effects of effluent stressors and nutrient enrichment on benthic algal and invertebrate communities. The report also (i) compares the results from autumn 1993 and 1994 to document between-year variability in the relationships between effluent additions, and effects on primary and secondary producers, and (ii) contrasts results from spring 1994 to those from Fall 1993 and 1994 to assess the importance of seasonal changes in moderating the nutrient-contaminant effects on benthic food web interactions. The research provides information relating to Question 5, "Are the substances added to the rivers by man-made discharges likely to cause deterioration of the water quality", and Question 1A, "How has the aquatic ecosystem, including fish and/or other aquatic organisms, been affected by exposure to organochlorines or other toxic compounds?"

Figure 1. BKME Dilution in the Athabasca River Downstream of Hinton Based on 1990-1991 Discharge (Environment Canada 1994) and BKME Discharges (McCubbin et al. 1996).



2.0 STUDY AREA

Weldwood of Canada Ltd., Hinton Division, operates a large bleached kraft mill in Hinton, Alberta. The mill produces an average of 1150 metric tonnes of market pulp per day from a softwood furnish blend of 65-70% lodgepole pine, 20-25% black and white spruce, and 10% fir. The mill harvests approximately 50% of its wood supply within its forest management area, the remaining wood supply coming in the form of purchased chips. Chips from both wood supplies are combined in a continuous, 2-vessel, hydraulic MCC Kamyr digester where they are immersed in an alkaline solution containing sodium sulphide, and subjected to heat and pressure. The resulting pulp is washed, then put through a staged-bleach process consisting of oxygen delignification (O) and 100% chlorine dioxide substitution (D_{100}), caustic extraction reinforced with oxygen and peroxide (E_{OP}), chlorine dioxide (D), short-stage caustic extraction (E_S), and finally chlorine dioxide (D). Since July 30, 1993 the mill has used the bleach sequence of $OD_{100}E_{OP}(DE_SD)$ to produce elemental chlorine-free pulp (Golder 1994).

Average daily water usage by the mill is 113,000 m^3/day, which is approximately 110m^3 of effluent produced for every air dried tonne of pulp. Municipal effluent comprises about 7.5% of the volume of the mill's effluent but likely represents a higher proportion of the nutrient loading. For example, compared to Hinton, total phosphorus loading from other, slightly smaller municipalities in the basin range between 13-17 kg/d (Chambers 1996). Given a TP load of 79 kg/d from the combined mill and municipal discharge, the Hinton municipality probably contributes 15-25% of the TP loading in the Weldwood effluent. Prompted by concerns about nutrient loading to the river, the mill has been reducing nutrient additions to the stabilization basins since 1992. In the past 2 y, the basins have been fertilized only during the winter months (December-March), with municipal sewage providing sufficient nutrients during the rest of the year. (T. Andrews *per comm.*).

Effluent first passes into a 13,753 m^3 (61m diameter) primary mechanical clarifier which removes solids. Primary treated effluent then flows into a 6.5 d aerated stabilization basin that has 41 mechanical aerators (2700 Hp total, Weldwood of Canada, Ltd. 1996). When necessary, granular 12-51-0 fertilizer is added to the basins to enhance secondary treatment, which generally achieves a 91.8% BOD reduction in the effluent. After passing through a quiescent zone to reduce suspended solids, treated effluent is then mixed with non-contact cooling water from the mill and is then discharged into the Athabasca River. At low winter flows (30-35m^3/sec) the effluent comprises up to 3% of the river's discharge at complete mix (Fig. 1). Weldwood effluent is the second continuous point-source discharge to the river, sewage discharge from the town of Jasper, Alberta being the first (Chambers 1996). Even though the Weldwood mill at Hinton discharges a combined bleached kraft mill and municipal effluent, the loading of total phosphorus (79 kg/d) to the river is similar to that of other mills in the basin, such as the operations of Weyerhauser Canada Ltd. at Grande Prairie and Diashowa Maurbeni International Ltd., Peace River Pulp Division (Chambers 1996), which only discharge BKME. Thus, in terms of the loading of phosphorus, a key limiting limiting nutrient in the upper Athabasca (Chambers 1996), it would appear that the Weldwood of Canada Ltd. effluent is a good candidate for investigating the nutrient-contaminant interactions of BKME.

During this study, the Athabasca River was sampled both upstream and downstream of the mill's discharge. Sampling sites for periphyton, insects and water are indicated in Fig. 2. Upstream of the mill, the river is phosphorus limited (Scrimgeour et al. 1995). Higher periphyton biomass and invertebrate densities are observed for 22 km downstream of Weldwood's effluent release compared to upstream (Sentar Consultants Ltd. 1993).

3.0 METHODS

3.1 ARTIFICIAL STREAM SYSTEM

3.1.1 Stream Design and Construction

The experimental stream system consisted of 16 circular 0.9 m^2 tanks placed in pairs on tables, required a 9 x 5 m area of level ground for set-up, and was constructed beside the study river, thus, experiencing ambient water temperature and light regimes (Fig. 3). Water from the river was pumped into the head tank reservoir and delivered through a system of pipes to the stream microcosms. Water flow to individual streams was controlled and current in each stream created by a belt-driven propeller system. Microcosms contained a substratum made up of gravel and larger rocks and a community of algae and invertebrates from the study river.

River water was pumped via the mill's water intake and pumping system into a head tank, then gravity-fed to the streams. We delivered 2 L/min of water to each stream, therefore, water in excess of 32 L/min was required for the 16 stream system. Water depth in the tanks was maintained at 26.9 ± 0.1 cm ($\bar{x} \pm 1$ SE) by an overflow drain which returned all waste water to the river. Each stream microcosm contained 227 L, thus, hydraulic residence time was approximately 2 h. By increasing water residence time within the streams, the volume of effluents or contaminants required during an experiment was minimized. The head tank was a 378 L polyethylene tank placed on a 1.2 m high platform. Gate valves controlled water input to each stream and allowed flow rate calibration for each stream. The head tank and all water delivery lines were wrapped with heat tape and insulated to prevent freezing. Note the system withstands air and water temperatures near 0 °C.

The streams were 107 cm diameter tanks constructed of polyester fibreglass. Streams were constructed by cutting 38 cm long sections of 107 cm pipe and bonding a flat sheet of fibreglass to one end of the pipe. A 25 cm diameter section of pipe was then centred in the larger pipe and the bottom cut out to form a standpipe. Streams were placed on eight, 74 cm high tables, two to a table (Fig. 3). When assembling this system, care was taken to ensure that the tables were level. The water outflow pipe, which was screened to limit emigration of insects, passed through the standpipe and drained into pipes beneath the tables which connected to a wooden trough (Fig. 3). These drain lines were wrapped with heat tape and insulated. Waste-water from the streams was returned directly to the river.

Figure 2. Map Indicating the Location of the Weldwood Pulp Mill and Effluent Discharge at Hinton, Alberta, and Sampling Sites For Insect, Water and Periphyton Collections Along the Athabasca River.

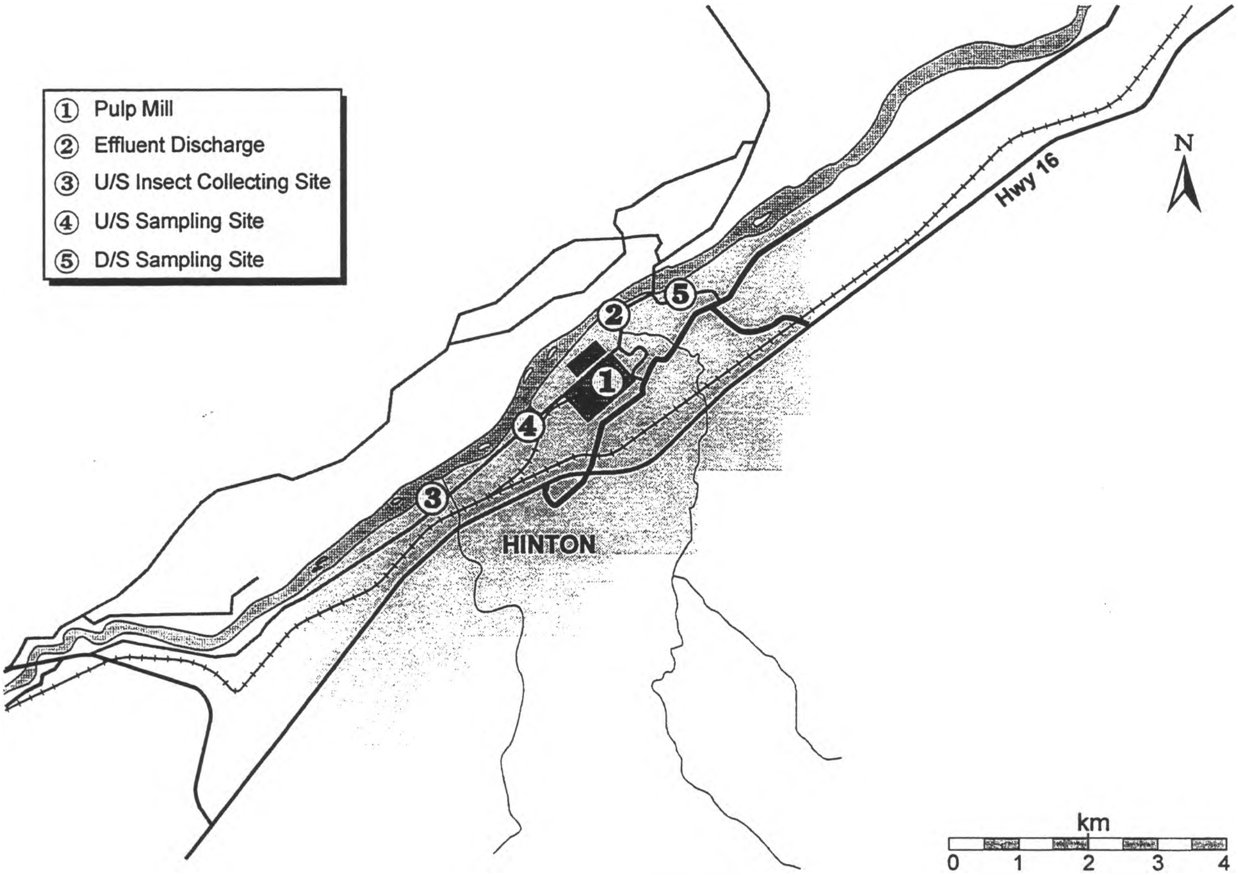
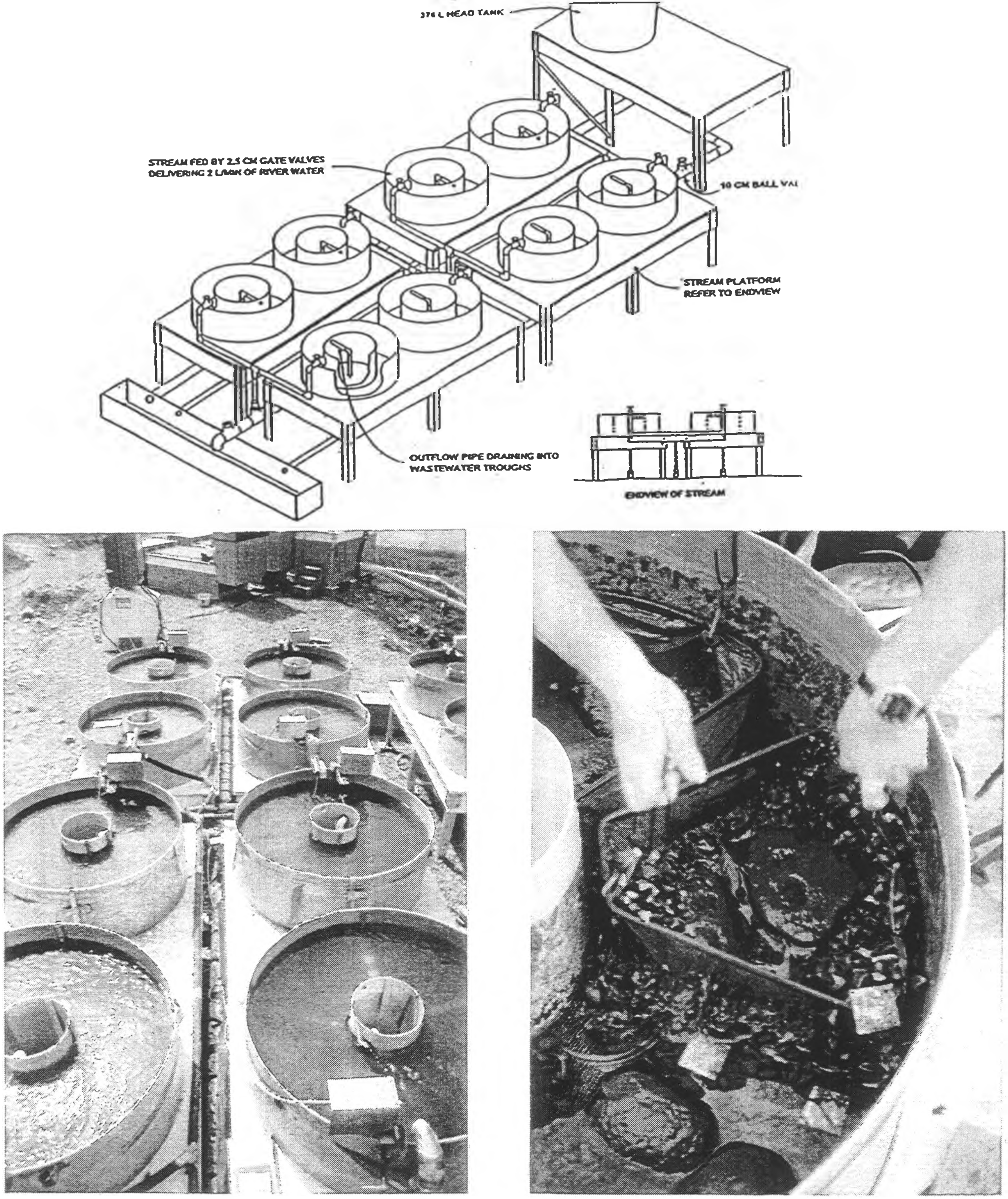


Figure 3. Oblique Line Drawing of Artificial Stream System Showing Circular Streams, Water Deliver and Waste-Water Systems (Not Drawn Exactly to Scale), and Photographs of Streams During Operation and Collection of Benthic Invertebrates Using Sampling Bags.



Current in each stream was created by a belt-driven propeller system because this type of propulsive device was relatively gentle on drifting organisms (Craig 1993). The motor assembly for each stream was comprised of a geared head motor (250 RPM, 1/40 amp) driving a 22.6 mm pulley. A belt-drive transmitted power from the motor to a 49.3 mm pulley mounted on the propeller shaft. The motor and associated electronics were mounted on an aluminum frame in a weather proof enclosure. This frame was clamped to the top edge (outside) of the stream. A 16 x 230 mm long copper strut extending downward from the aluminum frame held the propeller shaft bushing and grease seal. A grease nipple at the top of this tube allowed for lubrication of the propeller shaft and bushing. The propeller (one per stream) was a 23 cm (9 inch) diameter aluminum fan blade which rotates at a no load speed of 115 RPM.

Experimental treatment solutions were delivered independently and continuously to individual streams by peristaltic pumps (Masterflex ® L/S Nema-type 13 wash down controllers and cartridge pump heads) and a series of insulated tubes for solution delivery. Peristaltic pumps were kept in insulated boxes to keep them within approved operating temperatures. Treatment solutions such as nutrients or effluent were stored in insulated containers. Tubes carrying contaminant solutions were threaded through foam pipe insulation to the streams, then fed into the water delivery spout. Solutions and insulation of all supply lines were heated (25-30 °C) to prevent the thin supply lines (< 2 mm) from freezing.

3.1.2 Methods of Artificial Stream Operation

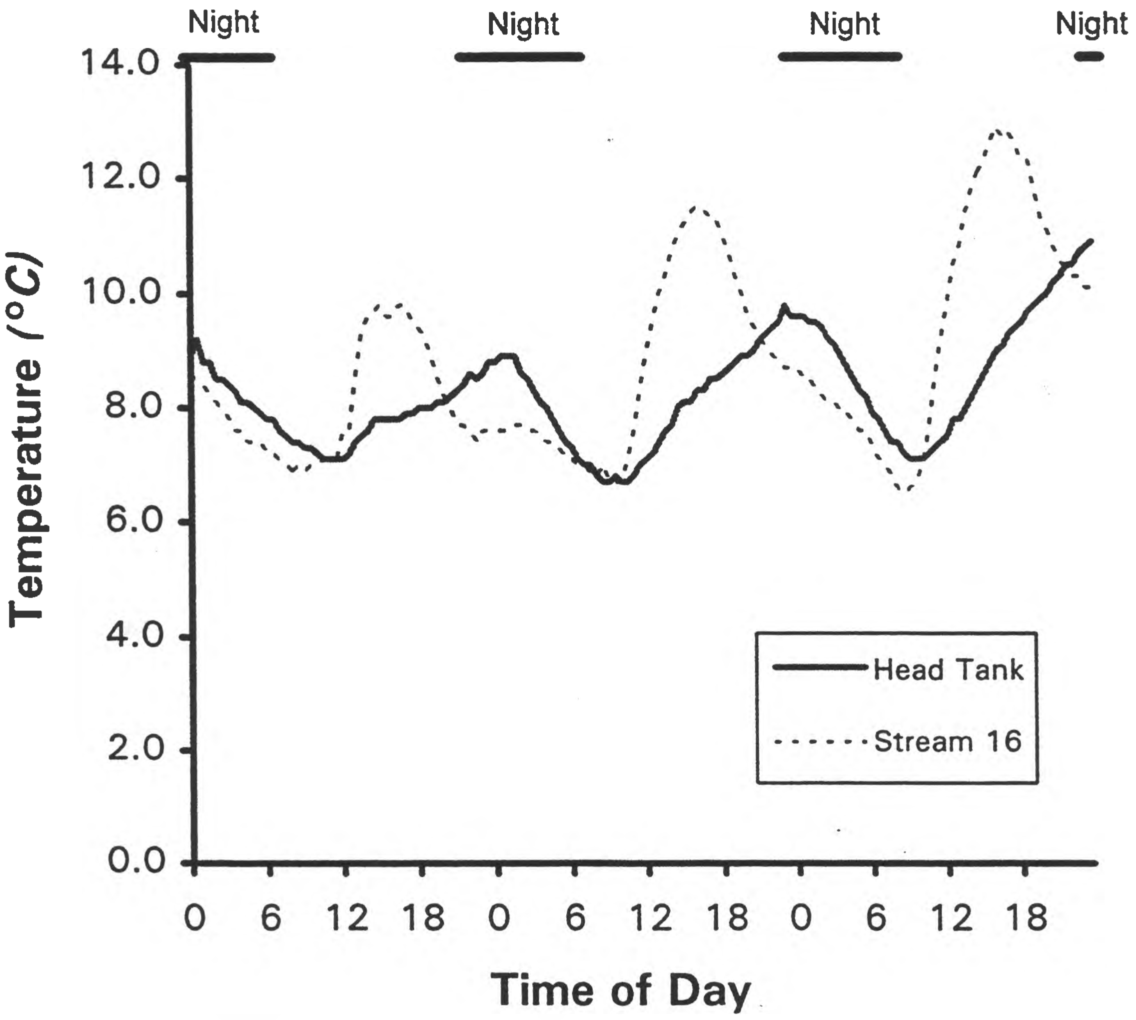
A variety of substrata types, including natural and artificial materials, can be used for stocking and/or sampling in the microcosms. In these experiments, we created a standardized benthic environment in each stream tank to simulate typical riffle areas found along the upper Athabasca River, Alberta, upstream of the Weldwood bleached kraft pulp mill operation. The bottom of each stream was covered with approximately 8 cm of thoroughly washed gravel (1-2 cm) upon which ten stones ($\bar{x}$ surface area = 508-535 cm^2) from the river were placed. The use of stones from the river provided a method of stocking the streams with a natural community of periphyton. In addition, unglazed porcelain tiles (23.5 cm^2) were used to provide a standardized substratum with which to compare periphyton development and accumulation. In autumn 1993, stones in the river were enclosed with a U-net (Scrimgeour et al. 1993), carefully lifted from the streambed and placed into a container (two stones per container) of river water so that the periphyton and invertebrates associated with the stone were not dislodged. In addition, the substratum beneath the stones was gently disturbed to collect any invertebrates under and around the base of the stone. All stones and associated invertebrates were collected from a single riffle with relatively uniform substrate composition. The stones and their biota were immediately transported to the streams and each pair of stones (and associated biota) randomly assigned to a stream for experimental tests. This random allocation of ten samples per stream ensured that invertebrate composition was initially similar among streams. The technique was modified in spring and autumn 1994 such that gravel and stones (free of invertebrates) were first added to the streams, then the periphyton was allowed to develop for 10 d before the addition of invertebrates. Invertebrates for the 1994 experiments were collected with the U-net sampler by gently disturbing the enclosed substratum, and the contents of ten such samples randomly allocated to each stream. One additional stream was not seeded with invertebrates during autumn 1993 so that the immigration of invertebrates via the inflow river water could be estimated. This stream was largely colonized by very small mayfly and midge larvae, with the total abundance of this unseeded stream being < 17% of the mean number of insects per stream in the experimental treatments.

The distribution of water velocities in the streams was characterized in the laboratory using a Nixon Instruments ® velocity meter. In this test, the substratum consisted of gravel and stones as in the Athabasca experiments. Mean velocities recorded at 21 locations around the stream were similar for all three depths: 0.20 ± 0.03 m/sec 4 cm below the water surface, 0.20 ± 0.02 m/sec at the water column mid-point, and 0.23 ± 0.02 m/sec just above the highest point of each stone. Overall mean velocity in the field experiments was similar to our laboratory tests ($\bar{x}$ = 0.26 ± 0.01 m/sec, n= 150). Visual observations of dye traces indicate that full mixing occurs within the first quarter of the stream length (Culp et al. 1994).

Water temperature was monitored by placing a Ryan ® thermograph in one of the streams and another in the head tank. Temperatures in the head tank reflected the temperature of incoming river water. In contrast, the 2 h hydraulic residence time in the streams resulted in slight heating or cooling of water in the streams depending upon ambient air temperatures. For example, over a 3 d period in autumn 1993, the streams were cooler at night and warmer during the day as compared to the incoming river water (Fig. 4). The maximum instantaneous difference between the water temperature in the river and the streams was < 5 °C.

The stream system required a moderate amount of regular maintenance as the motors had to be inspected daily for loose or misaligned belts. Loose belts were commonplace, particularly in the first 3 wks of operation, but were easily fixed. In addition, the motors were lubricated with non-toxic, Permatex Superlube ® (food grade USDA H1) every 3-6 d to prevent seizing of the propeller shafts. Debris falling into the streams, such as leaves during autumn, had to be removed daily as this material accumulated on the propellers, causing them to become unbalanced and rotate unevenly. Drain screens were brushed daily. The 10 cm water delivery lines were flushed weekly to remove any silt and sand deposits. Finally, effluent and nutrient delivery tubing had to be inspected for blockages and changed as required; this was particularly a problem in tubing carrying pulp mill effluent due to blockage apparently resulting from suspended solids and periphyton development.

Figure 4. An Example of Diel Water Temperatures In Experimental Stream And Head Tank Over A 72-H Period In Autumn 1993. The Head Tank Temperatures Are Representative of Ambient Values In The Athabasca River.



3.2 EXPERIMENTAL DESIGN AND METHODS

The experiment was repeated three times (autumn 1993, spring 1994, and autumn 1994) and included three treatments: (1) a control receiving raw river water from upstream, (2) a 1% dilution of treated mill effluent (BKME treatment), and (3) a 1% dilution of the nitrogen and phosphorus (NP treatment) found in the effluent (i.e., same nitrogen and phosphorus concentrations as were found in the 1% effluent). Although the spring 1994 experiment initially included all three treatments, technical difficulties compromised the NP treatment, thus, only the control and 1% BKME treatments could be included for data analysis. Five replicate streams were randomly assigned to each treatment. Continuous delivery of the treatment solutions was accomplished by peristaltic pumps (Masterflex ® L/S Nema, type 13 wash down controllers and cartridge pump heads). Effluent was collected daily from the mill treatment system just prior to release to the river. Samples of the effluent were collected daily and analyzed for soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen [ammonia ($N\text{-}NH_4^+$), and nitrate + nitrite ($N\text{-}NO_2$+$N\text{-}NO_3$)]. Throughout the report the dissolved inorganic nitrogen components, ammonium and nitrate + nitrate, are abbreviated as NH_4^+ and NO_2+NO_3, respectively. A solution with concentrations equivalent to the median values of the effluent samples was added to the NP streams for an 8 d period, followed by a 1 d nutrient spike application containing concentrations equivalent to the highest effluent value recorded during the previous 8 d period. This nutrient delivery schedule (8 d median, 1 d spike, 8 d median etc.,) was used because the laboratory processing time for the effluent analysis was 7-9 d. The median and spike effluent concentrations for autumn 1993 and 1994 are listed in Tables 1 and 2. A nutrient spike was used because effluent concentrations were highly variable (Fig. 5) and Bothwell (1992) showed that periphyton communities were able to utilize nutrient spikes to achieve higher long-term growth rates.

Table 1. Median and Spike Concentrations (μg/L) of SRP, NO_2+NO_3, and NH_4^+ Used Over the 28-d Experimental Period During the Autumn 1993. Note That These Are the Effluent Concentrations Used to Set the Nutrient Solution Concentrations, and That Streams Contained A 1% Dilution of These Concentrations.

DAY	SRP	NO_2+NO_3	NH_4^+
1-8	218	548	1502
9	240	761	1578
10-17	222	683	1311
18	219	251	3390
19-26	161	245	942
27	307	217	2762
28	257	151	1965

Table 2. Median and Spike Concentrations ($\mu g/L$) of SRP, NO_2+NO_3, and NH_4^+ Used Over the 39-d Experimental Period During the Autumn 1994. Note That These Are the Effluent Concentrations Used to Set the Nutrient Solution Concentrations, and That Streams Contained a 1% Dilution of These Concentrations.

DAY	SRP	NO_2+NO_3	NH_4
1-8	405	215	385
9	775	568	1679
10-17	669	470	1324
18	883	586	1650
19-26	422	518	547
27	480	576	1090
28-35	470	470	682
36	489	558	684
37-39	399	416	629

Water samples were collected from each experimental stream once every 5-7 d. All water and effluent samples were packed on ice and shipped the same day to the University of Alberta limnology laboratory for analysis. Samples for P analysis were placed in Nalgene polyethylene bottles and samples for nitrogen analysis were placed in polystyrene bottles. TDP and SRP samples were filtered through pre-washed 0.45 µm Millepore filters; TP and TDP samples were digested and analyzed by Menzel and Corwin's (1965) potassium persulfate method. NO_2+NO_3 samples were filtered through pre-washed 0.45 µm Millepore membrane filters. NH_4^+ and NO_2+NO_3 were analyzed with a Technicon autoanalyzer (Stainton et al., 1977). Analysis of alkalinity and bicarbonate were measured following standard methods (APHA 1985). In autumn 1994, effluent and water samples were also analyzed for Na^+. Sodium is a relatively conservative ion that appears in high concentrations in kraft effluents. By comparing Na^+ concentrations in upstream water, the effluent, and in the artificial streams we were able to verify the concentration of effluent in the streams. Water samples from downstream of the mill were used to estimate effluent dilution in the river.

Periphyton samples in autumn 1993 were collected every 5 d from one randomly selected stone and one tile in each replicate stream. In 1994, the sampling frequency was increased to once per 10-d period, and a composite sample of three rocks per stream was sampled in order to reduce variability. Rocks were sampled by using a scalpel to remove periphyton from within a 9.6 cm^2 template. The entire top surface (23 cm^2) of tiles was sampled. Periphyton samples were placed in vials and held on ice until frozen later the same day. Samples in the river were collected during autumn 1993 and 1994, and spring 1994, in a similar manner at sites upstream and downstream of the BKME discharge. In the laboratory, each sample was homogenized, partitioned into two parts, and each portion filtered through a GF/C filter. Chlorophyll *a* (Chl*a*) concentration was determined by extracting the filter and retained material in an 80 °C bath of 90% ethanol for 5 min, then measuring fluorescence with a Turner Designs, model 10 series fluorometer. Ash-free dry mass (AFDM) was determined by weighing the sample after drying for 24 h at 105 °C, then combusting the filter at 500 °C and determining the weight loss upon ignition.

At the end of the autumn 1993 experiment, all invertebrates were collected by washing the entire contents of each stream through a 250 μm sieve (an upper layer of 0.63 cm hardware cloth was used to remove gravel). In the 1994 experiments, sampling bags (0.1 m^2, 210 μm mesh) were installed under the gravel at the beginning of the experiments, and at the end of the experiment, invertebrate samples were collected by lifting these bags, resulting in five subsamples from each stream. Subsamples were then washed through the hardware cloth and into a sieve as in 1993. Invertebrates were preserved immediately in 10% formalin. The majority of invertebrates were insects, thus, our analysis focused on this taxonomic group. Samples were sorted under 12x magnification, all insects identified to genus whenever possible (taxonomic references included Merritt and Cummins (1984), and Stewart and Stark (1993), and enumerated. In the 1994 experiments, the contents of three randomly chosen sampling bags were sorted from each stream. Due to the large numbers of immature animals, many individuals were identified only to Family. Growth of numerically dominant taxa (excluding Chironomidae) was estimated by measuring thorax length (Ephemeroptera and Plecoptera) or pronotum width (Trichoptera) with the aid of a *camera lucida* and a digitizing pad system. Biomass (as dry mass) was measured by drying individual animals at 60 °C for 48 h and then weighing them on a CAHN C-31 Series microbalance. If large numbers of animals were present, a randomly chosen subsample of animals were weighed and used to construct length-weight regressions. These regressions were then used to predict the weights for all animals.

The chlorophyll a (Chl*a*) content and AFDM of periphyton on rocks and tiles at the end of each experiment was compared with a one-way ANOVA or t-test (spring 1994) after the data were checked for heteroscedasticity and normality. Transformations were applied if necessary. Means comparisons were done on the autumn data using Fisher's protected Least Significant Difference (LSD) test with α=0.05.

Insect community composition was compared by pooling all genera up to the level of family, removing all taxa present in only one stream, then analyzing the reduced data set using Principal Components Analysis (PCA) on the correlation matrix. Taxa were pooled to family level after it was observed that relatively unimportant genera (i.e., found in low abundance and in a few streams) were very influential in the genera-level PCA's. Thorax lengths of the insects were compared by one-way ANOVA (autumn 1993 and 1994) or t-test (spring 1994) after checking for heteroscedasticity and normality, and applying transformations if necessary. Means comparisons were done using Fisher's protected LSD with α=0.05.

4.0 RESULTS

4.1 AUTUMN 1993

4.1.1 Effluent and Water Chemistry

SRP, NO_2+NO_3, and NH_4^+ were measured in the effluent each day throughout the 28-d experiment (Fig. 5). With the exception of one observation for NO_2+NO_3, effluent values were always greater than those in the Athabasca River upstream of the effluent discharge. A striking observation from these samples was the wide fluctuation of all three variables throughout the experiment. The value of a particular variable sometimes changed 1.5-10 times over a 24-h period. For example, the greatest 24 h change was 153 to 282 µg/L for SRP (7-8 October), 187 to 18 µg/L for NO_2+NO_3 (7-8 October), and 2211 to 588 µg/L for NH_4^+ (24-25 October). Over the course of the experiment, effluent concentrations of SRP, NO_2+NO_3, and NH_4^+ ranged between 122-515 µg/L, 18-430 µg/L, and 315-2365 µg/L, respectively. No other water quality variables were measured in the effluent during autumn 1993.

Most of the total dissolved phosphorus (TDP) in all three treatments was in the form of SRP (Fig. 6). Because TDP concentration never exceeded 23% of the total phosphorus (TP) in any treatment, > 75% of the TP was in the particulate form. Although NP and 1% BKME treatments received continuous inputs of 1-5 µg/L of phosphorus, uptake in the streams was rapid enough that SRP concentrations were similar among treatments. TP in effluent streams was generally higher than in NP and control streams. Additionally, NO_2+NO_3 and NH_4^+ concentrations were similar across treatments despite the fact that the NP and BKME treatments were enriched with nitrogen (Fig. 7). As expected, nutrient concentration in the streams had a more narrow range relative to the effluent samples (Figs. 6 and 7), changing < 3.6 times in magnitude over each 5-d sampling period. The largest proportionate change was observed for the 1% BKME treatment between 15-20 October: 0.5 to 1.4 µg/L for SRP, 15 to 40 µg/L for NO_2+NO_3 , and 7 to 25 µg/L for NH_4^+. For all treatments, NO_2+NO_3, and NH_4^+ were in higher concentration at the end of the experiment compared with initial values (Fig. 7). Conductivity, alkalinity, bicarbonate and pH (Table 3) were similar among treatments except for the higher conductivity in effluent streams.

Table 3. Mean (±SE) value of Conductivity (µmhos/cm), Alkalinity (mg/L as $CaCO_3$), Bicarbonate (mg/L) and pH in the Control, Nitrogen-Phosphorus (NP), and 1% BKME Treatments During the Autumn 1993 Experiments.

VARIABLE	CONTROL	NP	1% BKME
Conductivity	315±12	314±13	328±13
Alkalinity	130±2	129±1	131±2
Bicarbonate	159±3	152±3	155±4
pH	8.1±0.1	8.2±0.1	8.2±0.1

Figure 5. Concentrations (µg/L) of SRP, NO_2+NO_3, and NH_4^+ in Weldwood BKME and the Athabasca River Upstream of the Effluent Discharge During the Autumn 1993 Experiment.

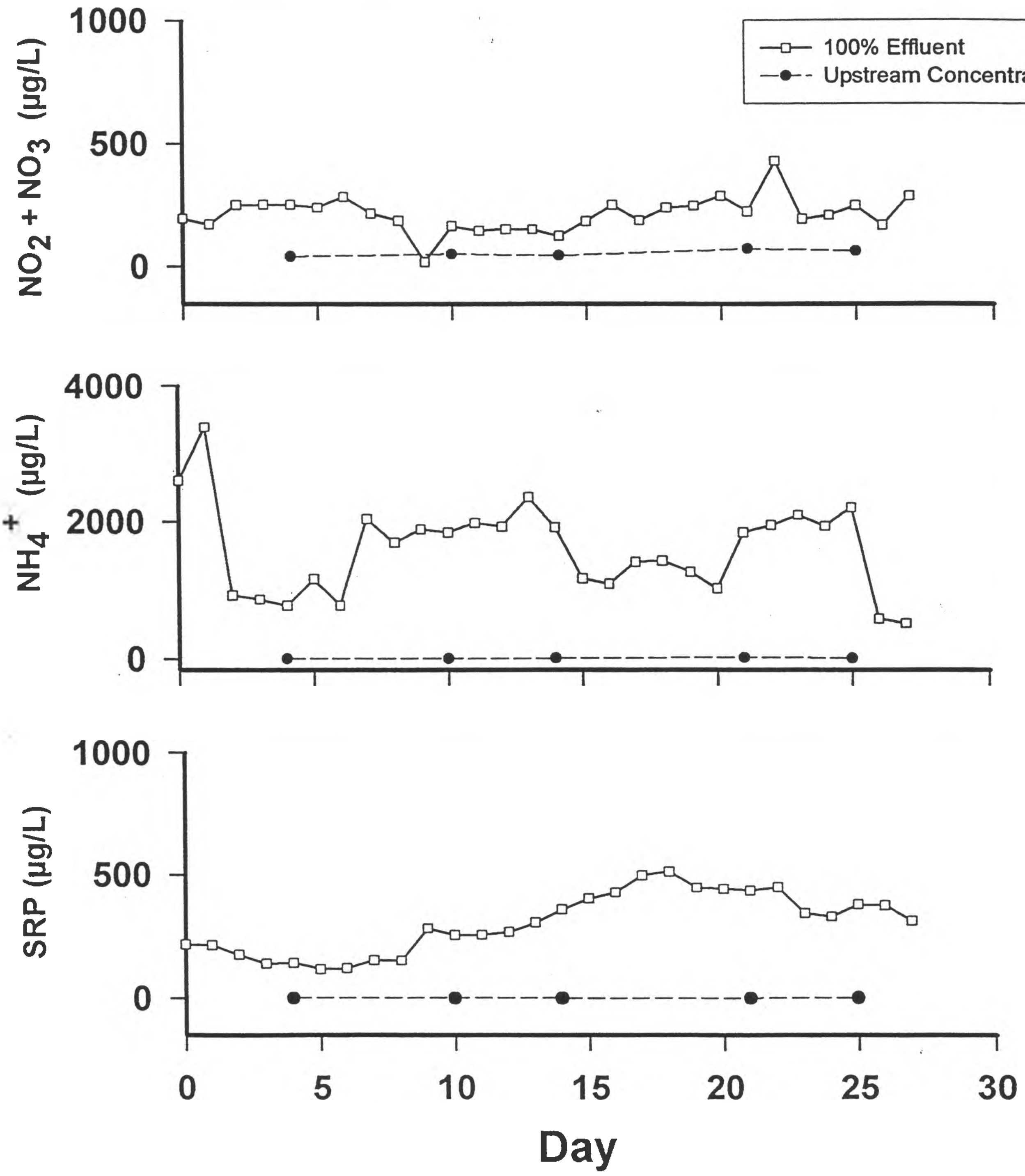


Figure 6. Mean Concentrations (±SE µg/L) of SRP, TDP, and TP in the Control, NP and 1% BKME Treatments During the Autumn 1993 Experiment.

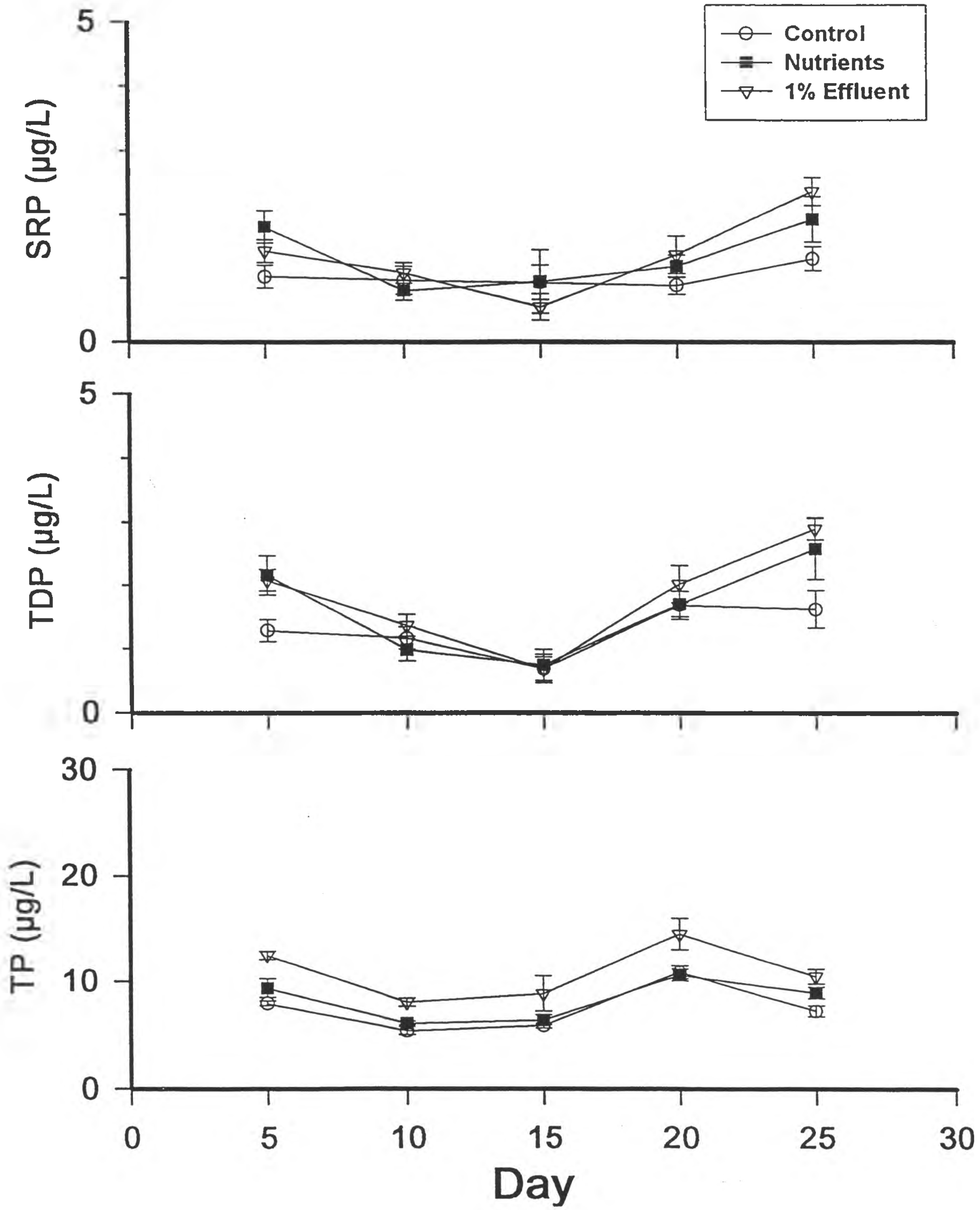
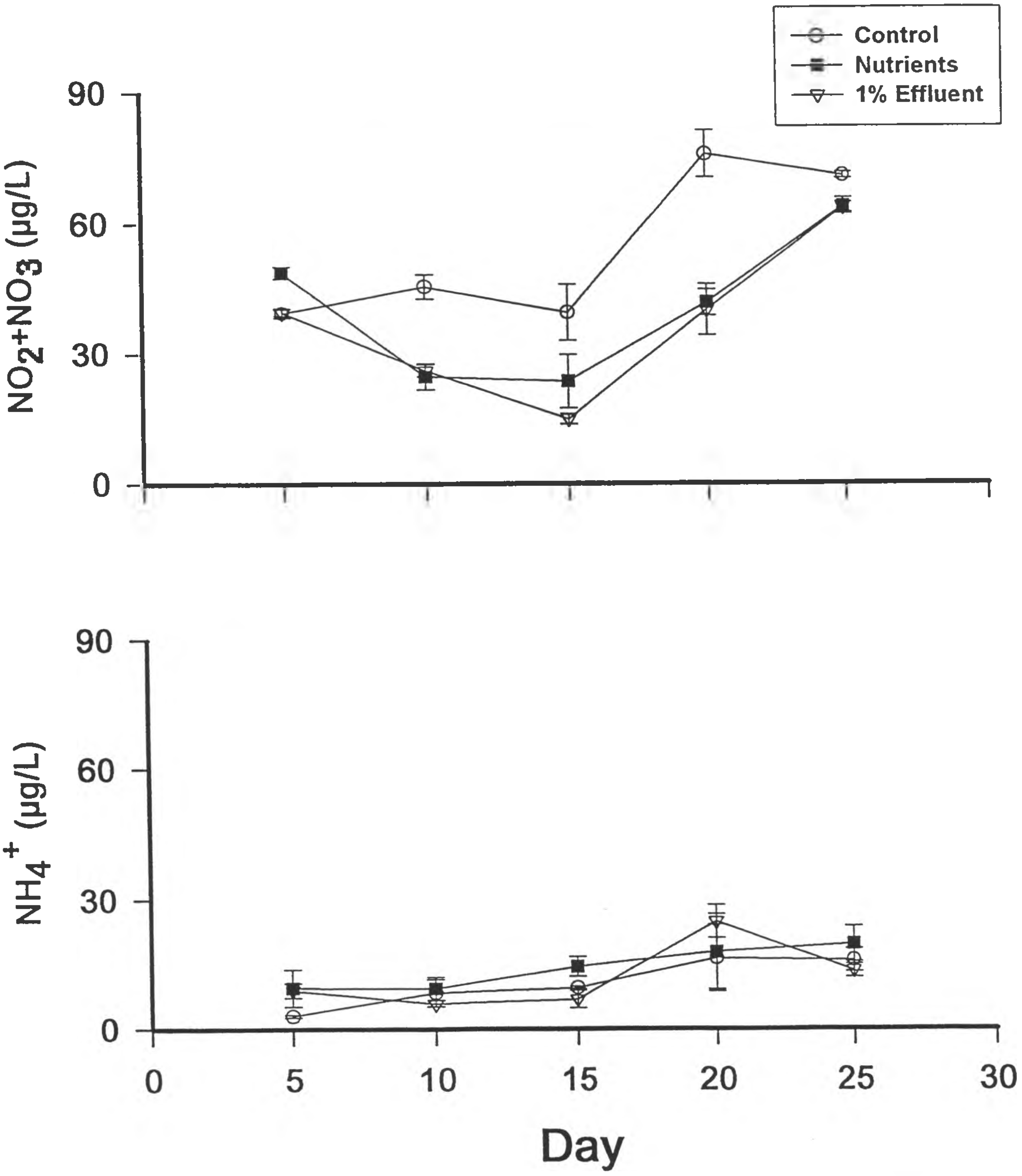


Figure 7. Mean Concentrations (±SE µg/L) of NO_2+NO_3 and NH_4^+ in the Control, NP and 1% BKME Treatments During the Autumn 1993 Experiment.



4.1.2 Periphyton Biomass in Artificial Streams

Periphyton Chl*a* content on rocks and tiles increased in streams receiving either 1% BKME or NP additions compared to control streams (Figs. 8 and 9). After 25 d, periphyton biomass on tiles in effluent- and nutrient-treated streams was 33 times greater than in control streams, and the treatments differed significantly ($F_{2,12}$=242.77, $p<0.001$). While there was no difference in the Chl*a* content of effluent and nutrient streams ($p>0.38$), both treatments were significantly different from the controls ($p<0.05$). Significant differences among treatments were also found in the Chl*a* content of periphyton on rocks ($F_{2,12}$= 7.14, $p< 0.009$). Again, Chl*a* in control streams was significantly lower than that of the effluent treatment ($p<0.05$), and the Chl*a* content of periphyton in the effluent and NP streams did not differ ($p>0.48$).

As observed for Chl*a*, there was a significant treatment effect on the log-transformed AFDM of periphyton on tiles (Day 25 $F_{2,12}$=8.53, $p<0.05$) (Figs. 8 and 9). Biomass levels were significantly higher in streams receiving effluent or nutrient additions compared with control streams ($p<0.05$), and AFDM in the effluent and NP streams were similar ($p>0.12$). Measurements of AFDM on tiles were not available during the first 14 d of the experiment because the amount of material on tiles was insufficient for measurements of both Chl*a* and AFDM. Unlike the Chl*a* findings, periphyton AFDM on rocks was similar among treatments ($F_{2,12}$=0.87, $p>0.445$) (Figs. 8 and 9).

Figure 8. Mean Chlorophyll *a* Biomass (±SE µg/cm²) and Mean Ash-Free-Dry Mass (±SE mg/cm²) of Periphyton on Rocks in Control, Nitrogen-Phosphorus (NP), and 1% BKME Treatments During the Autumn 1993 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

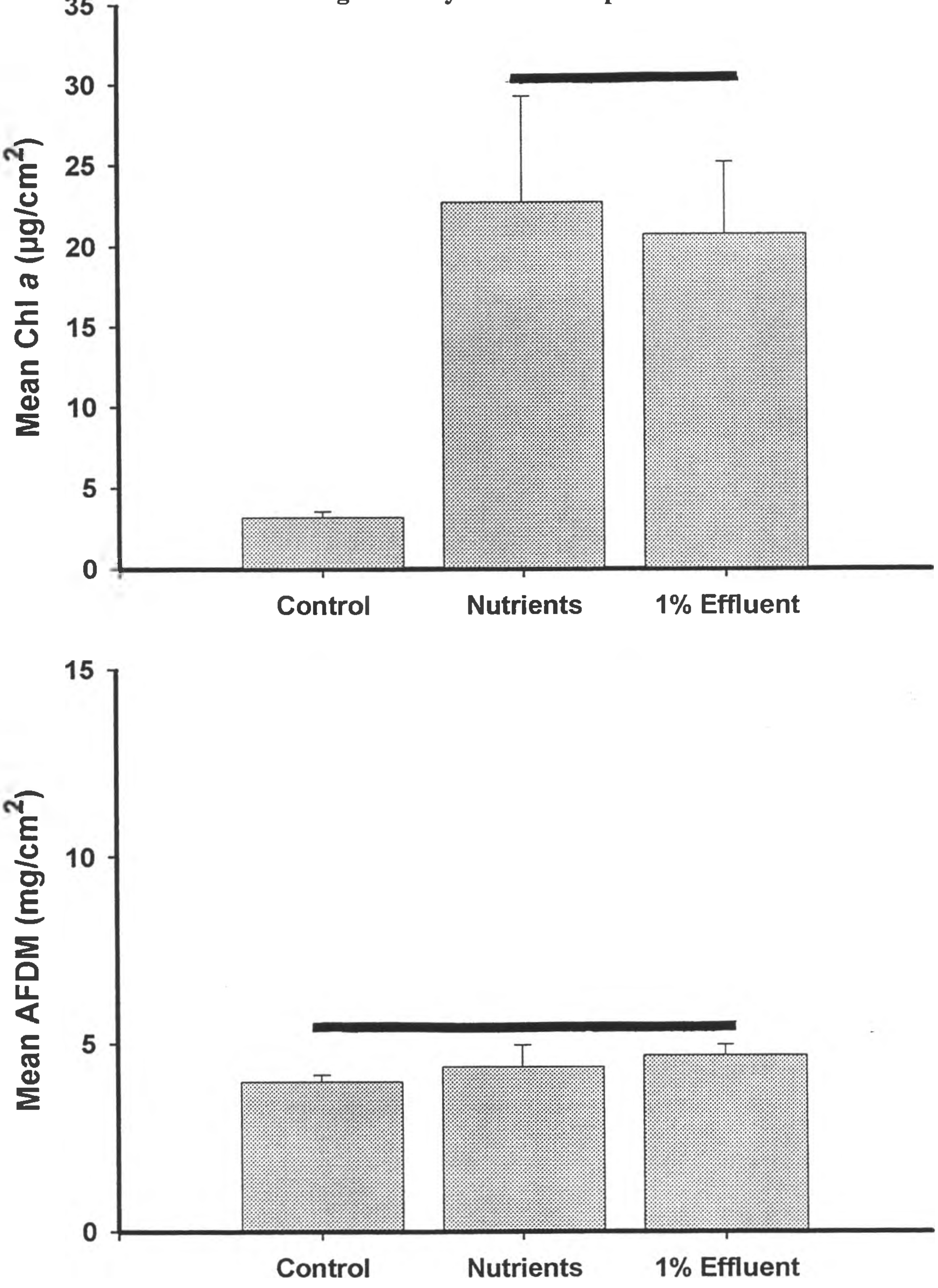


Figure 9. Mean Chlorophyll *a* Biomass (±SE μg/cm^2) and Mean Ash-Free-Dry Mass (±SE mg/cm^2) of Periphyton on Tiles in Control Nitrogen-Phosphorus (NP), and 1% BKME Treatments During the Autumn 1993 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

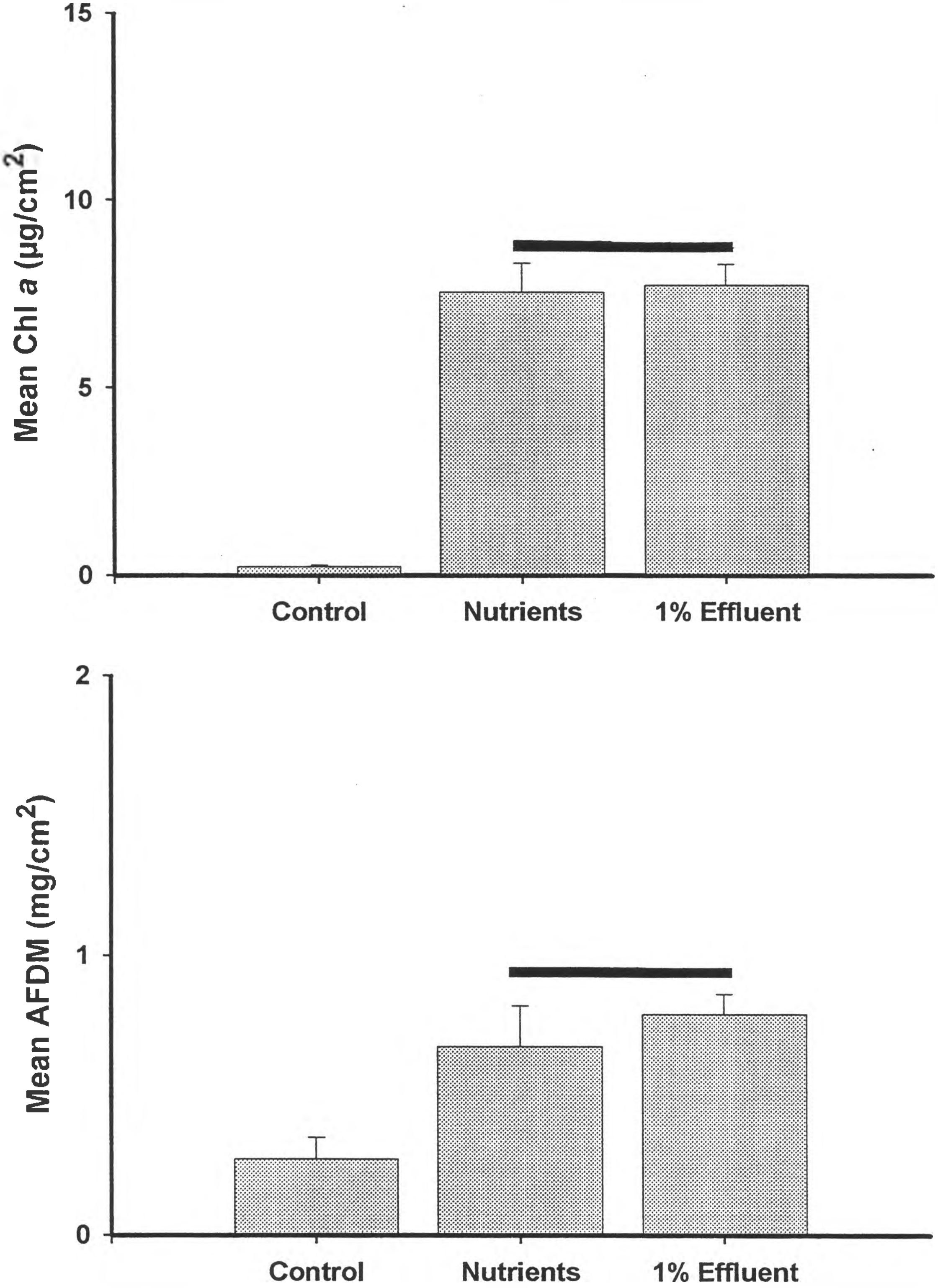
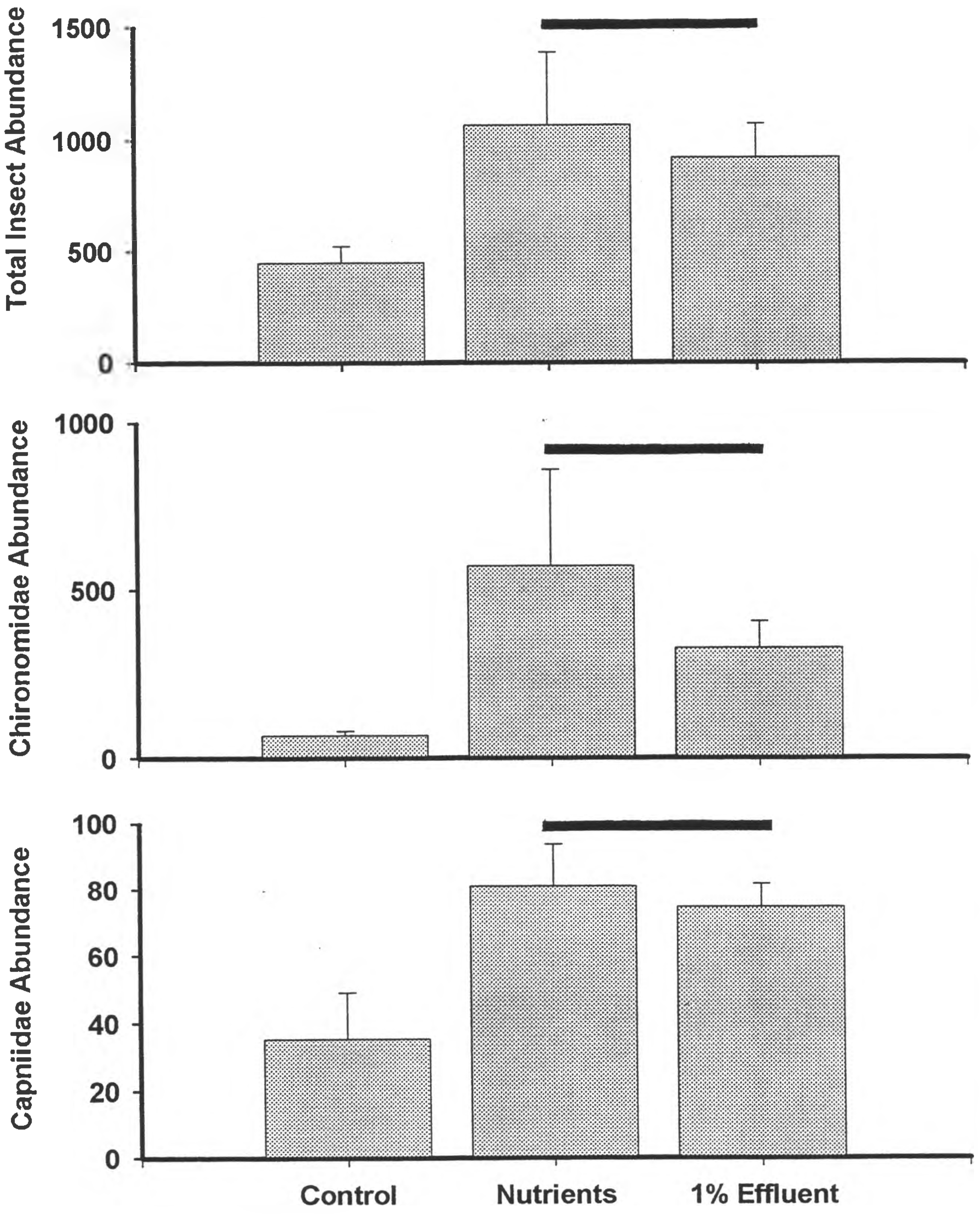


Figure 10. Total Number of Insects, Chironomidae and Capniidae Present in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments At the End of the Autumn 1993 Experiment; Bars Connect Means That Are Not Significantly Different at p=.05.



4.1.3 Insect Density, Community Composition and Body Size in Artificial Streams

Total insect abundance was significantly increased by the 1% BKME and NP treatments ($F_{2,12}$=5.15, $p<0.024$) in autumn 1993 (Fig. 10). Five taxa, including the mayflies Baetidae and *Ameletus*, the stoneflies Capniidae and Nemouridae, and the Chironomidae, comprised ≥ 85% of insect total numbers in the treatments. Of these, the chironomids ($F_{2,12}$=11.09, $p<0.002$) and the capnids ($F_{2,12}$=4.68, $p<0.031$) had significantly higher abundances in the 1% BKME and NP treatments relative to the controls (Fig. 10). Abundances of the remaining three taxa were similar among all treatments.

The first axis of a principal components analysis ordination (PCA) separated 25.9% of the variation in community structure among replicate samples of the treatments (Fig. 11). This axis indicates that replicates from the NP and 1% BKME treatments tended to have higher abundances of baetid and ephemerellid mayflies, capniid and chloroperlid stoneflies, and chironomids. In contrast, the abundances of brachycentrid caddisflies were higher in control streams. The nutrient and effluent treatments changed insect community composition as replicates of these treatments overlapped with one another more than with control replicates. The second axis explained an additional 19.7% of the variance but did not provide a further separation of the three treatments. Despite these treatment-related shifts in community composition, family richness was similar among treatments and ranged between 12-13 ($F_{2,12}$=0.53, $p>0.600$) (Fig. 12).

Final size (growth) in mean thorax length of several taxa was significantly different among the control, NP and 1% BKME treatments (Fig. 13): *Ameletus* ($F_{2,12}$= 5.57, $p< 0.021$), Capniidae ($F_{2,12}$=7.53, $p<0.008$) and Nemouridae nymphs ($F_{2,12}$= 8.41, $p<0.005$). For the capniid and nemourid stoneflies, and *Ameletus* mayflies, individuals in streams receiving effluent or nutrient additions were longer when compared to nymphs from control streams ($p<0.05$). Furthermore, mean thorax length in the effluent and nutrient addition streams were similar ($p>0.05$). Finally, insect weight was significantly affected by experimental treatment in these three taxonomic groups: *Ameletus* ($F_{2,12}$= 5.57, $p<0.021$), Capniidae ($F_{2,12}$=12.55, $p< 0.001$) and Nemouridae nymphs ($F_{2,12}$= 16.76, $p<0.001$) (Table 4).

Table 4. **Mean Weight (±SE µg/individual) of the Stoneflies, Capniidae and Nemouridae, and the Mayflies Baetidae and *Ameletus*, in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments at the End of the Autumn 1993 Experiment.**

TAXA	CONTROL	NP	1% BKME
Capniidae	97±13	159±10	210±35
Nemouridae	94±17	179±10	224±20
Baetidae	66±3	70±31	78±3
Ameletus	1079±143	1523±681	1376±615

Figure 11. Principal Components Analysis Ordination of Insect Communities in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments of the Autumn 1993 Experiment. Taxa With High Loading Values (Eigenvalues) On Axis 1 Are Indicated.

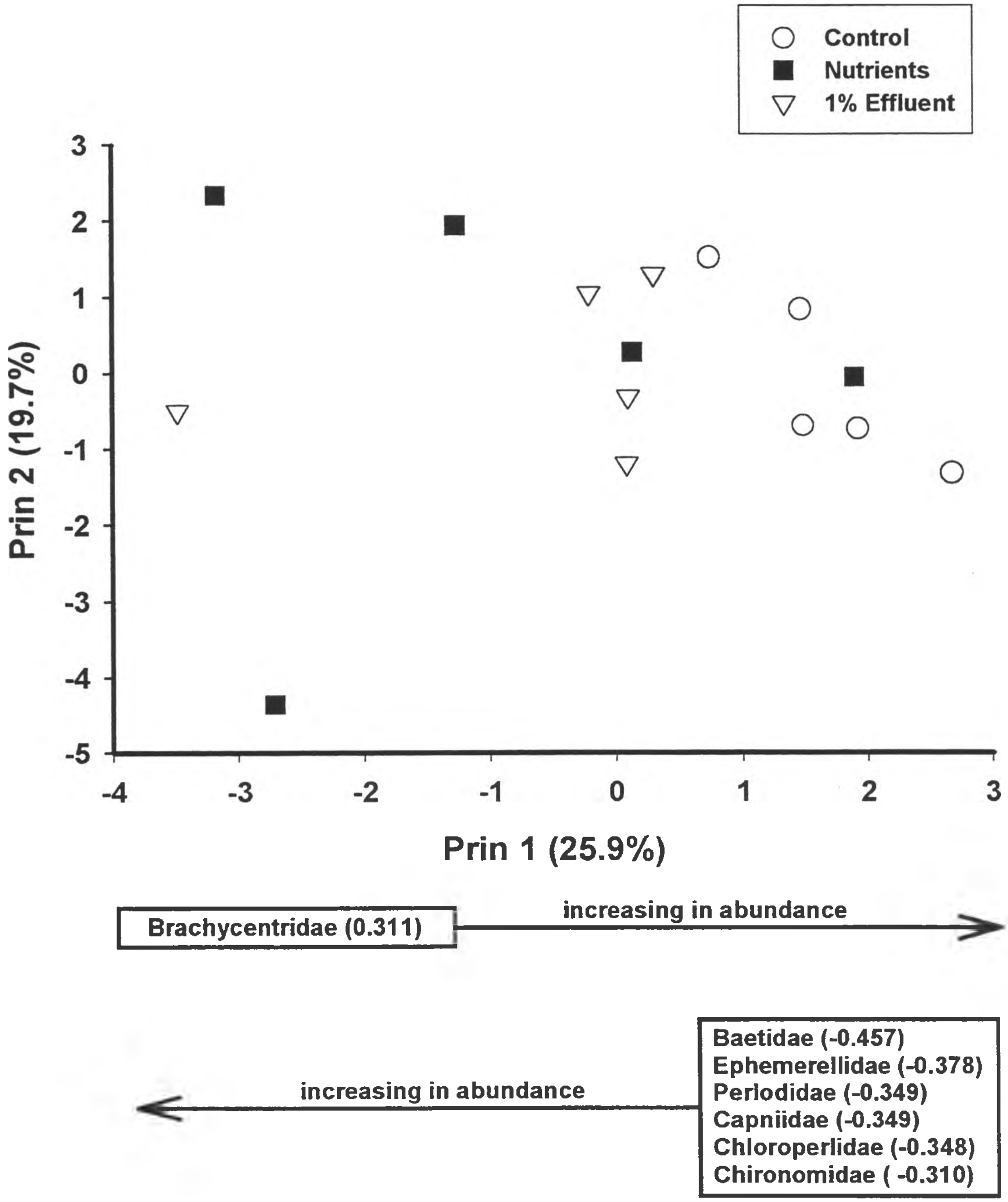


Figure 12. Mean Number of Insect Families Present in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1993 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

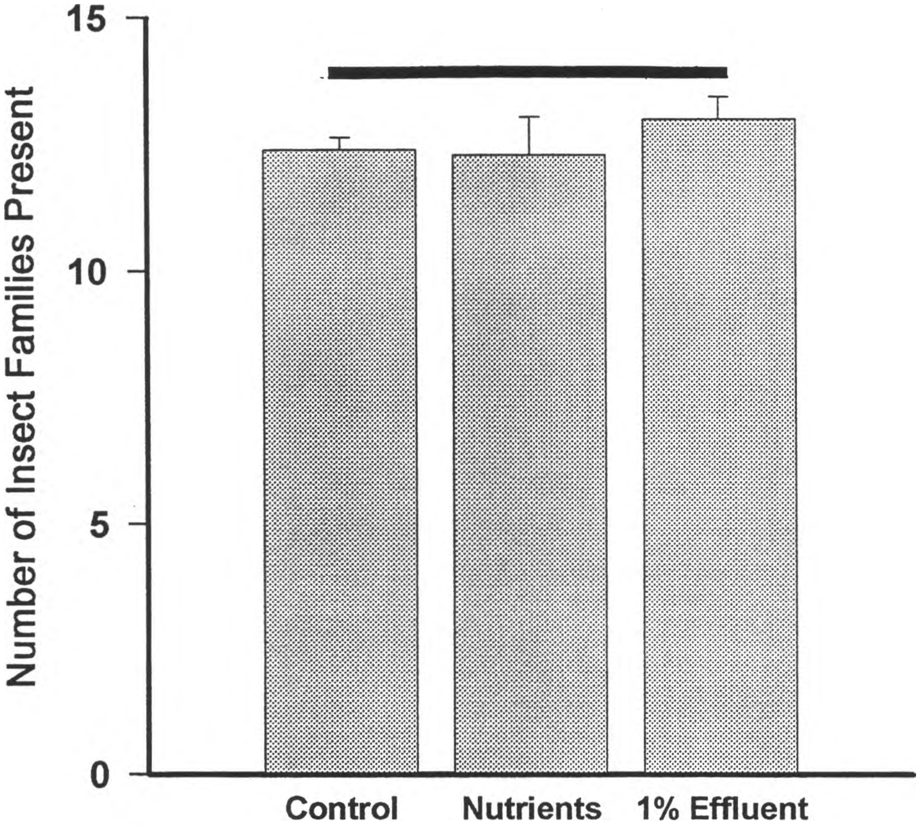
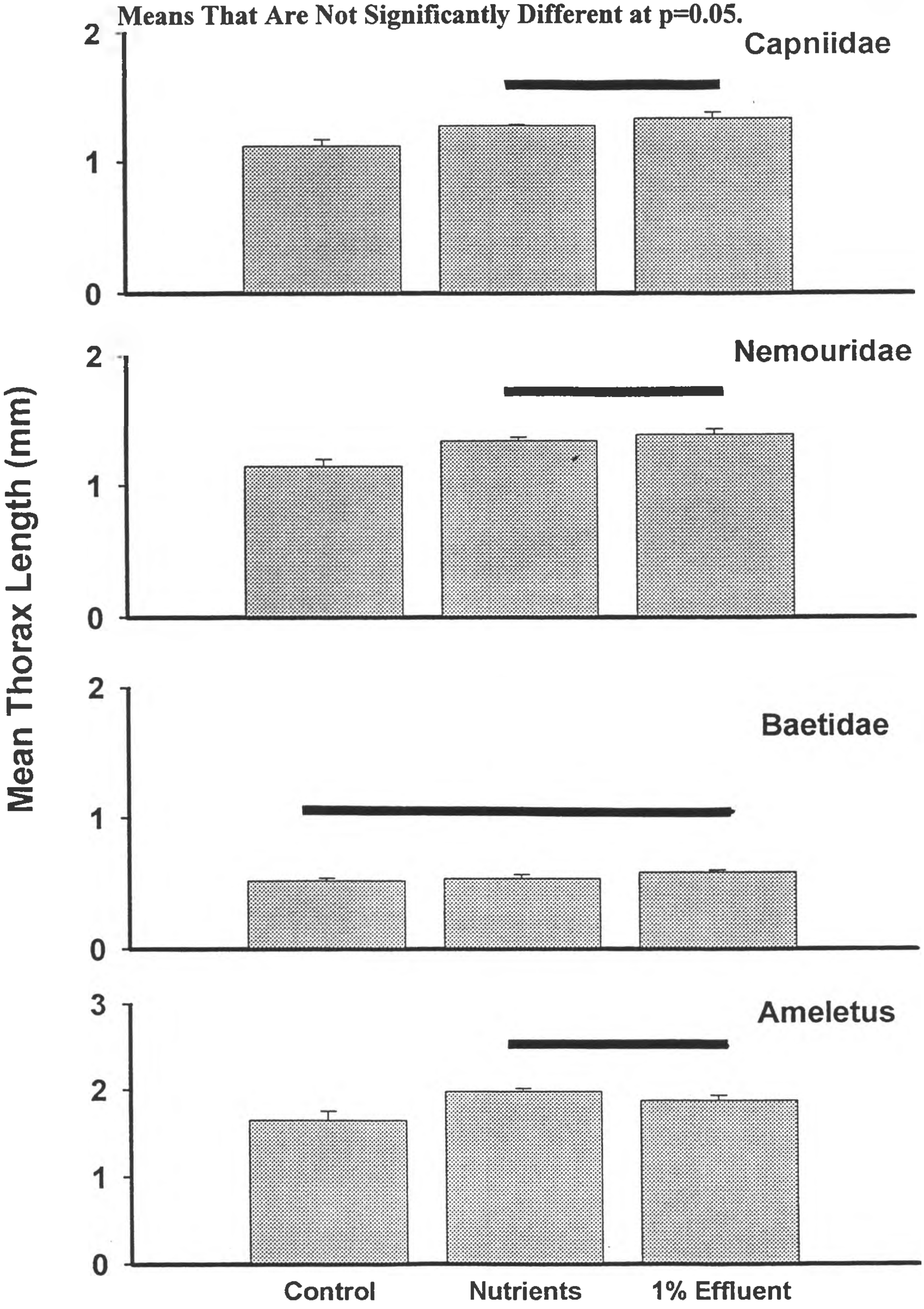


Figure 13. Mean Thorax Length (mm) of the Stoneflies, Capniidae and Nemouridae, the Mayflies Baetidae and *Ameletus*, in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments at the End of the Autumn 1993 Experiment; Bars Connect Means That Are Not Significantly Different at p=0.05.



4.1.4 Riverine Measures of Periphyton and Insect Biomass

In autumn 1993, periphyton biomass (expressed as Chl*a*) on Athabasca River rocks was twice as high 0.8 km downstream of the effluent discharge compared to rocks at the upstream reference site ($t_{5,\ 0.05/2}$=25.89, p<0.001) (Fig. 14). AFDM of the periphyton showed a similar downstream trend, but the increase was not significant ($t_{5,\ 0.05/2}$=0.9, p>0.372; Fig. 14).

Benthic insect data collected upstream and downstream of the effluent discharge during autumn 1992 (TAEM 1993) were compared to the artificial stream data because river data for autumn 1993 data were not available. Total insect density was significantly increased 1 km below the effluent discharge relative to the reference site ($t_{6,\ 0.05/2}$=-4.41, p<0.005) (Fig. 15), as were the densities of the Chironomidae ($t_{5,\ 0.05/2}$=-3.44, p<0.019), Capniidae ($t_{4,\ 0.05/2}$=-2.89, p<0.045) and the Baetidae ($t_{7,\ 0.05/2}$=-2.60, p<0.036) (Fig. 15). The first axis of the autumn 1992 PCA explained 50.8% of the variation in benthic insect community composition at sites above and below the discharge (Fig. 16). BKME discharge appeared to change benthic insect composition as samples below the discharge had higher abundances of several families of stoneflies (Capniidae, Chloroperlidae, Perlodidae and Taeniopterygidae), mayflies (Baetidae, Heptageniidae, and Ephemerellidae), and dipterans (Chironomidae), while the upstream samples had higher abundances of the caddisflies, Hydropsychidae and Hydroptilidae. Axis 2 explained an additional 19.5% of the variance but added no further separation of the sites (Fig. 16). These in-river changes in benthic insect communities are remarkably similar to community shifts attributed to the effects of the NP and 1% BKME treatments in the autumn 1993 experiment (Fig. 11). For example, the PCA ordinations indicate that compositional changes in downstream river samples, and in the nutrient and BKME treatments, were mostly the result of abundance changes in stonefly, mayfly and dipteran families (i.e., Capniidae, Chloroperlidae, Baetidae, Ephemerellidae, Chironomidae). As in the artificial stream experiments, family richness above and below the discharge was not significantly different ($t_{8,\ 0.05/2}$=1.72, p>0.12; Fig. 17).

Figure 14. Mean Chlorophyll *a* Biomass (±SE μg/cm^2) and Ash-Free Dry Mass (AFDM) (±SE mg/cm^2) of Periphyton on Rocks in the Athabasca River Upstream and Downstream of the BKME Discharge at Hinton, Alberta, in October 1993. Bars Connect Means That Are Not Significantly Different at p=0.05.

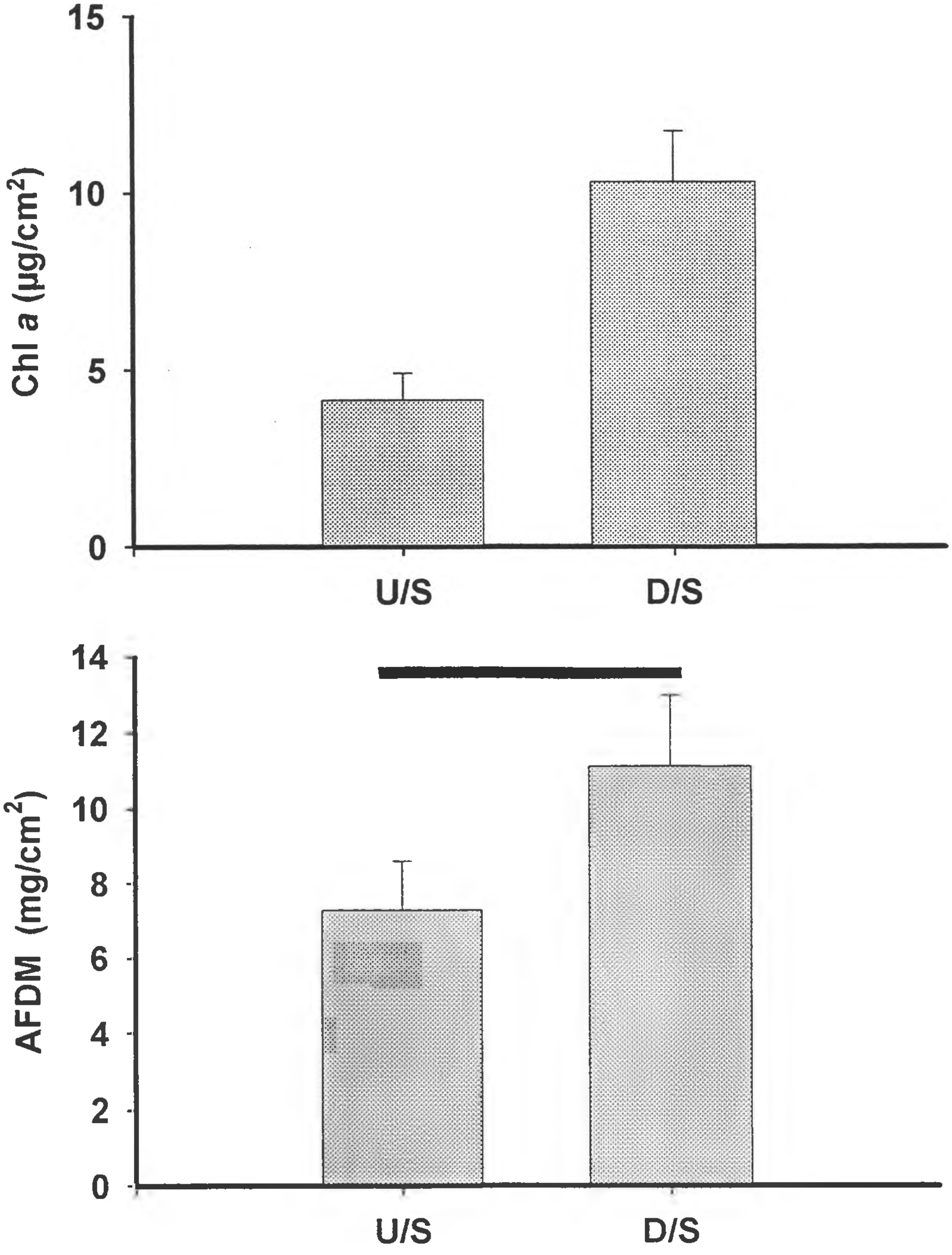


Figure 15. **Mean Density (±SE number/m^2) of Total Insects, Chironomidae, Capniidae and *Baetis* Larvae During Autumn 1992 At Sites 0.8 km Downstream and 2.1 km Upstream of the Pulp Mill Effluent Discharge to the Athabasca River At Hinton, Alberta (Data From TAEM 1993). Bars Connect Means That Are Not Significantly Different at p=0.05.**

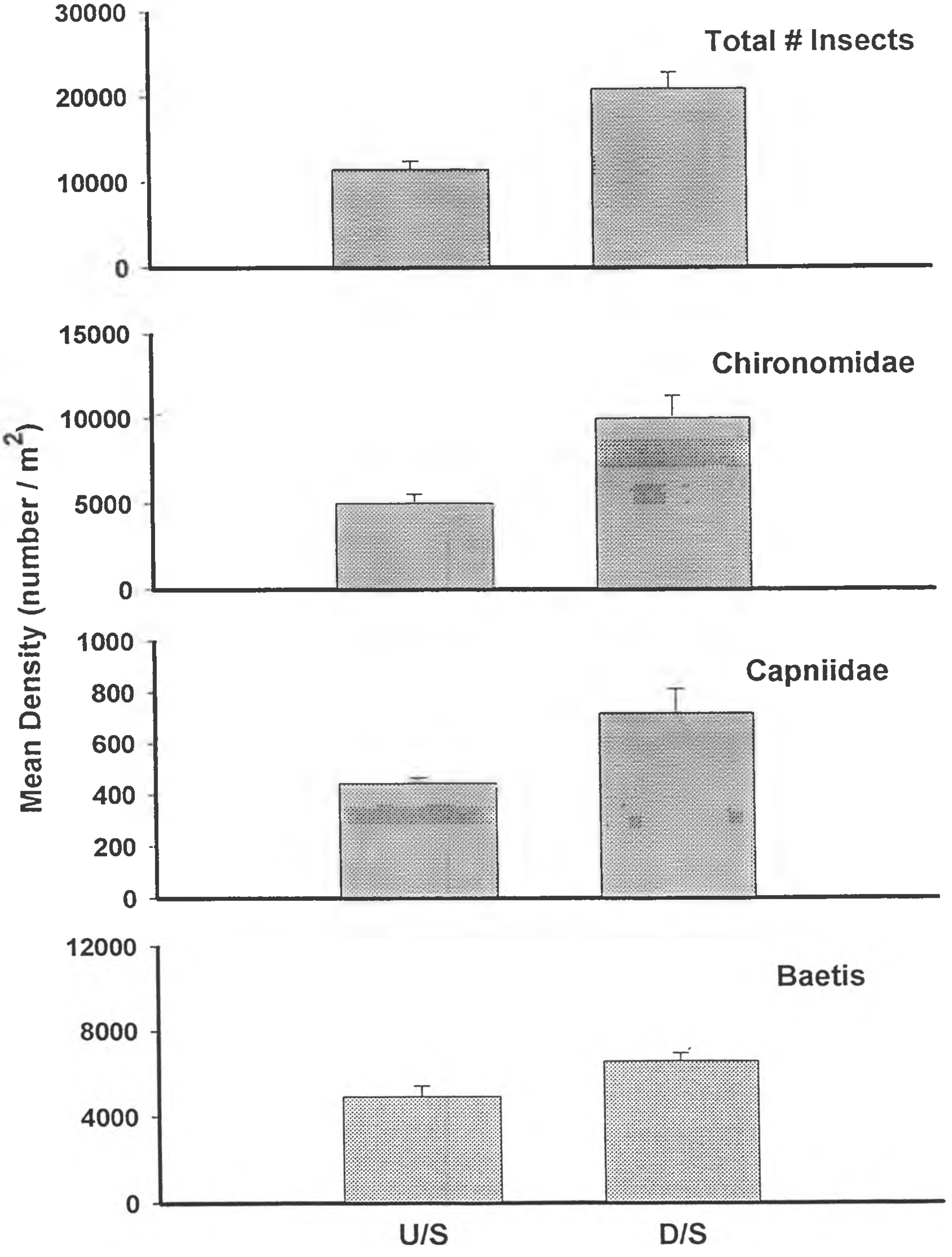


Figure 16. Principal Components Analysis Ordination of the Autumn 1992 Insect Communities at the Sites Above and Below the BKME Discharge at Hinton, Alberta. Taxa With High Loading Values (Eigenvalues) On Axis 1 Are Indicated (Data From TAEM 1993).

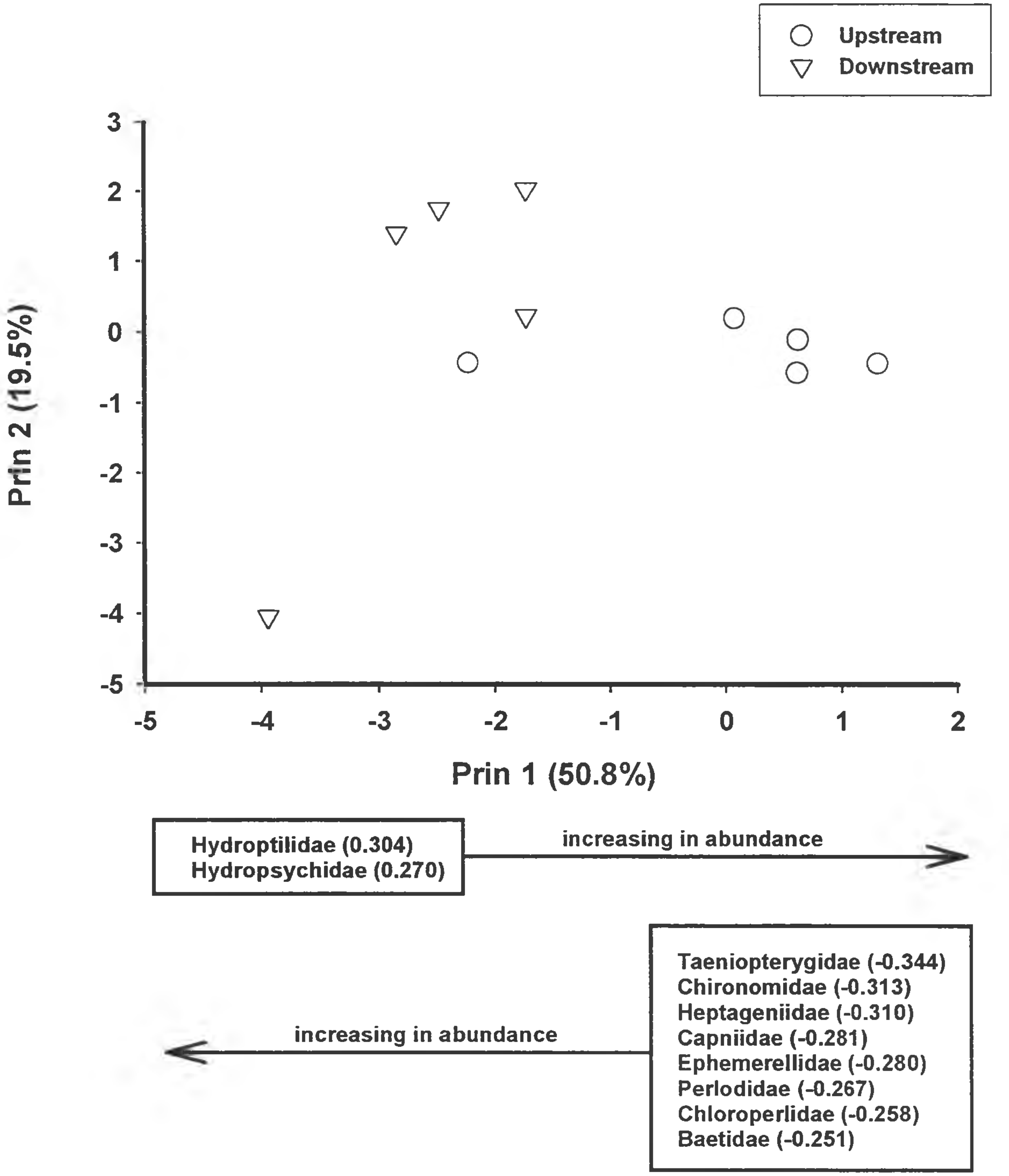
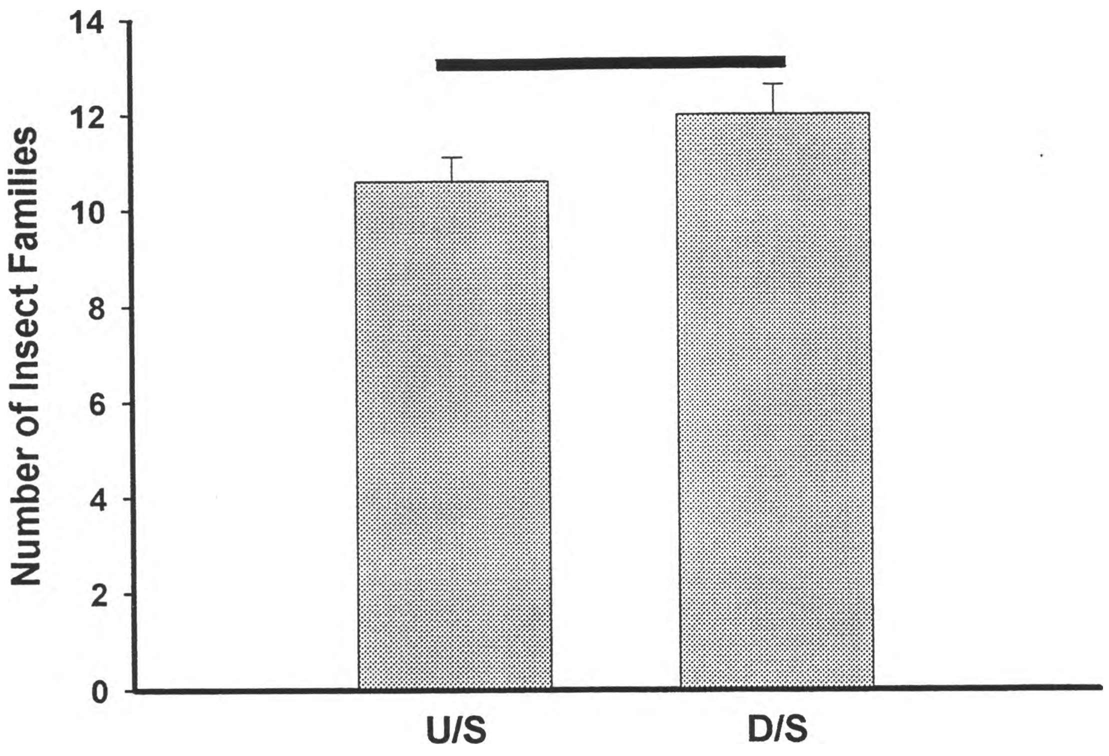


Figure 17. Mean (±SE) Number of Insect Families Present at the Sites Above and Below the BKME Discharge at Hinton, Alberta. Bars Connect Means That Are Not Significantly Different at p=0.05.



4.2 SPRING 1994

4.2.1 Effluent and Water Chemistry

Effluent SRP concentrations in the spring were lower than during the previous autumn and similar to upstream river concentrations for the first 14 d of the experiment (Fig 18). Because SRP concentration in the effluent was low, the 1% BKME treatments always received < 1.6 μg/L of additional phosphorus. The effluent concentration of SRP, NO_2+NO_3 and NH_4^+ during spring 1994 ranged between 0.8 to 161 μg/L, 36-375 μg/L and 1-892 μg/L, respectively. With the exception of the SRP value for 21 May, all of the daily fluctuations were ≤ 3.3 times the value for the previous day. The range for NO_2+NO_3 was similar to the autumn 1993 observations, but NH_4^+ concentrations were higher in the spring.

The NP treatment was compromised by intractable technical difficulties, leaving only the control and BKME treatments for data analysis during spring 1994. Control and 1% BKME treatments had similar concentrations of SRP, NO_2+NO_3 and NH_4^+ during the spring experiment indicating rapid nutrient uptake in the BKME treatment (Fig. 19). As observed in autumn 1993, most of the TDP was in the biologically available SRP form, and most of the total phosphorus was particulate P. Furthermore, SRP values in the control streams, which reflect ambient conditions in the river, were always < 2 μg/L. Concentrations of NO_2+NO_3 and NH_4^+ at the end of the experiment were similar to autumn 1993 values (Fig. 20). Alkalinity, bicarbonate and pH where similar among treatments, while conductivity was highest in the 1% BKME treatment (Table 5).

Table 5. Mean (±SE) value of Conductivity (μmhos/cm), Alkalinity (mg/L as $CaCO_3$), Bicarbonate (mg/L) and pH in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Spring 1994 Experiments.

VARIABLE	CONTROL	NP	1% BKME
Conductivity	300±25	305±21	333±8
Alkalinity	105±6	105±5	105±5
Bicarbonate	128±7	128±7	128±6
pH	7.8±0.1	7.8±0.1	7.8±0.1

Figure 18. Concentrations (µg/L) of SRP, NO_2+NO_3, and NH_4^+ in Weldwood BKME and the Athabasca River Upstream of the Effluent Discharge During the Spring 1994 Experiment.

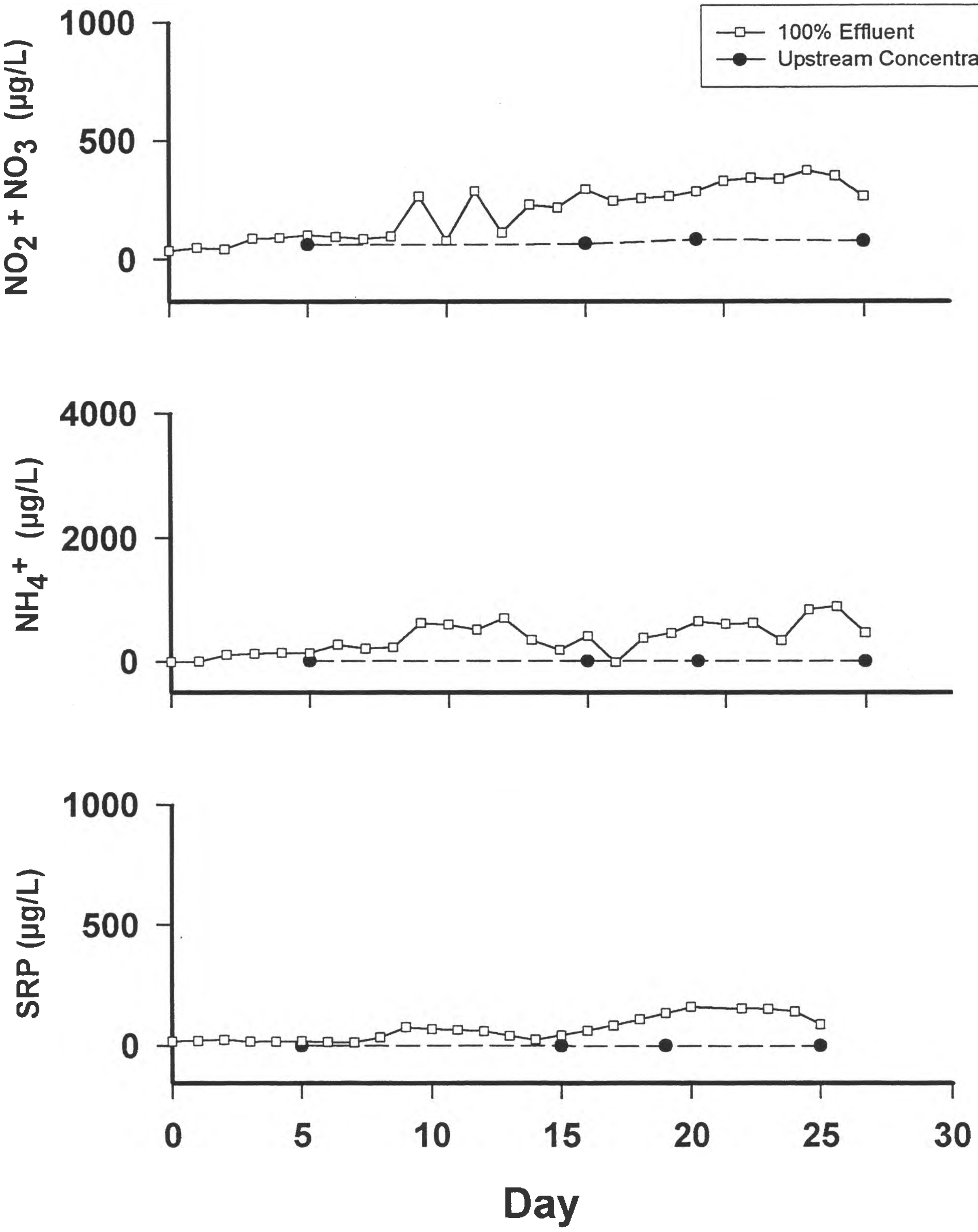


Figure 19. Mean Concentrations (±SE µg/L) of SRP, TDP, and TP in the Control and 1% BKME Treatments During the Spring 1994 Experiment. Note That the Y Axis Scale for TP Is Different Than Figs. 6 and 29.

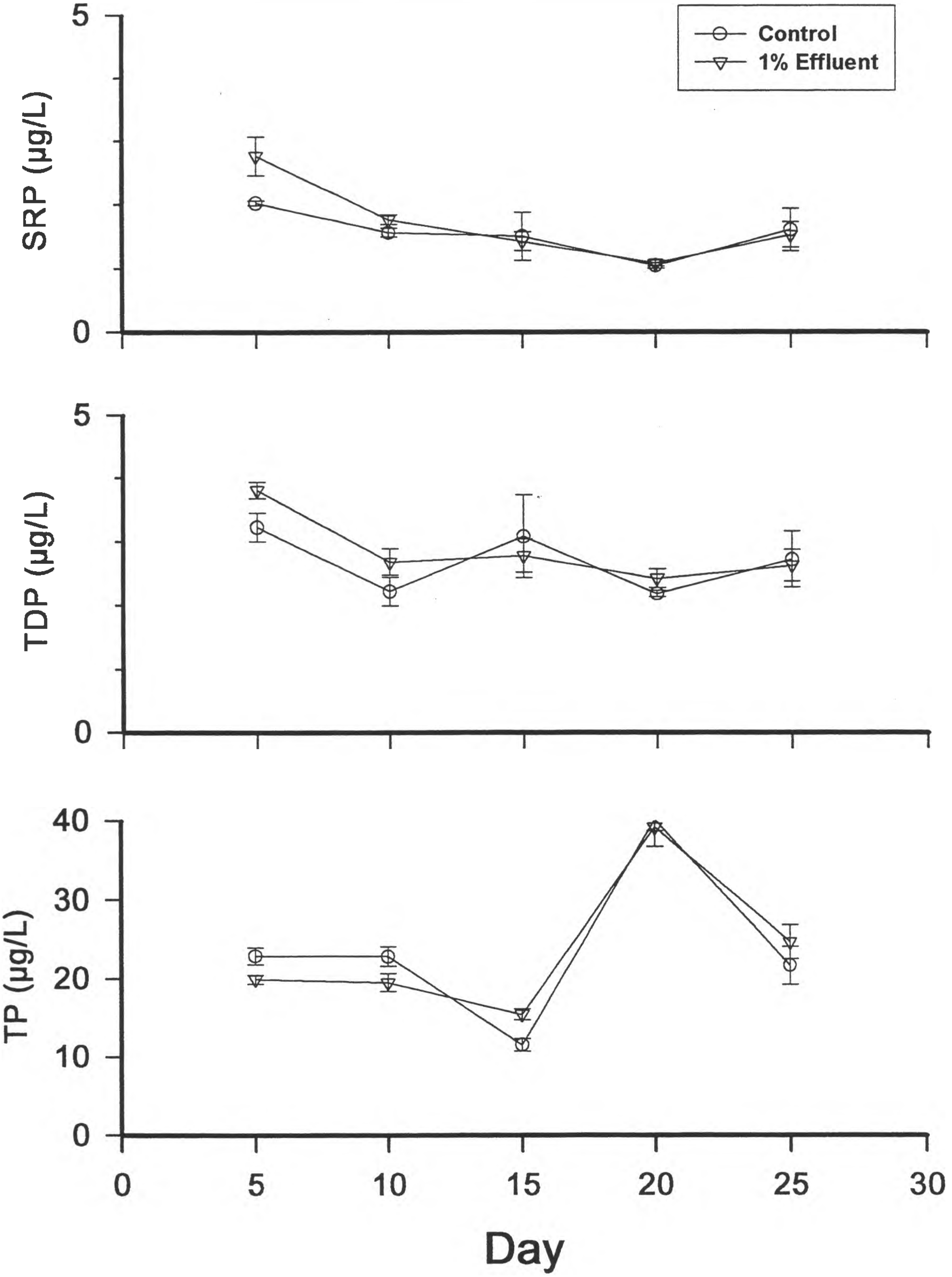
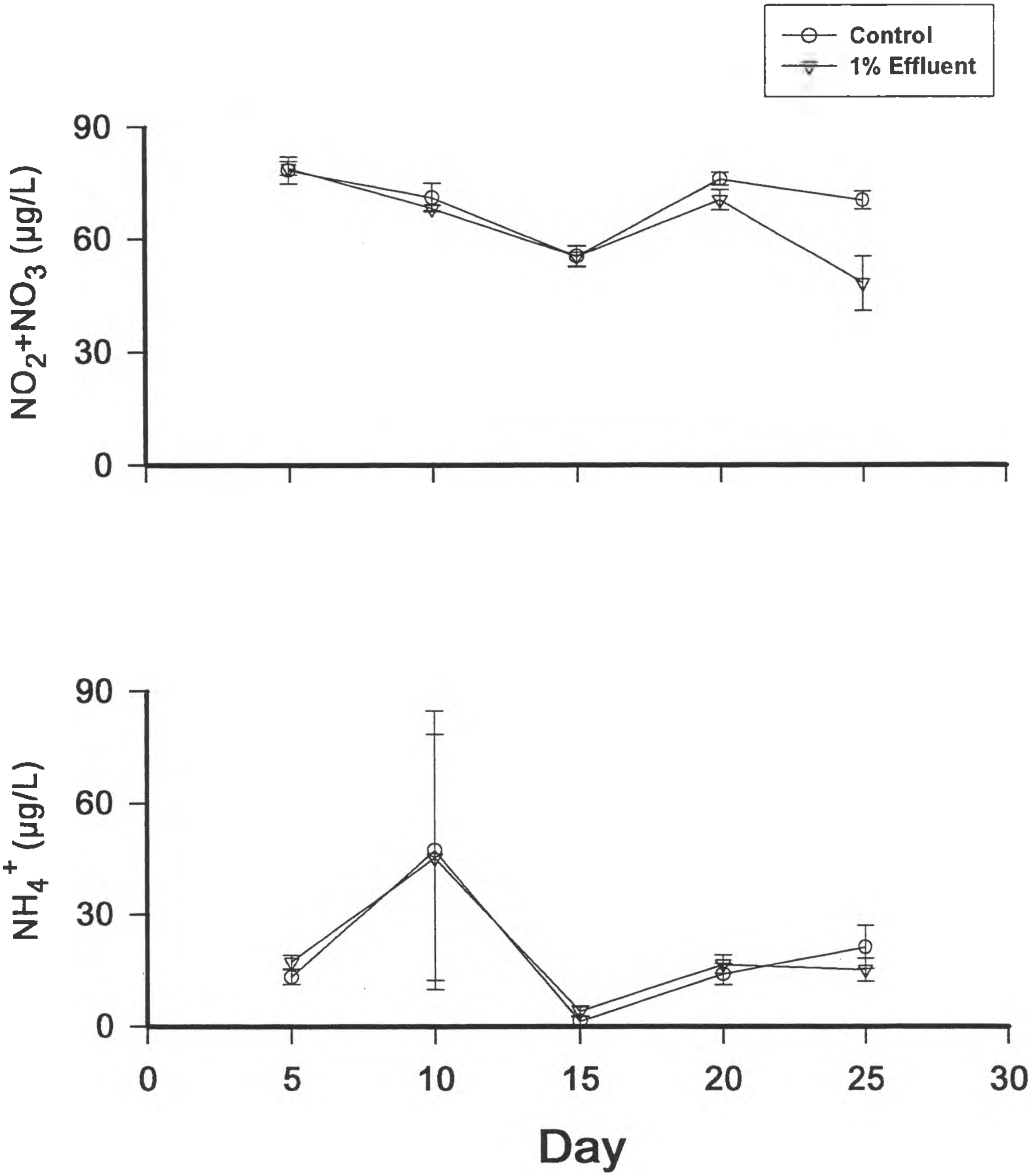


Figure 20. Mean Concentrations (±SE µg/L) of NO_2+NO_3 and NH_4^+ in the Control and 1% BKME Treatments During the Spring 1994 Experiment.



4.2.2 Periphyton Biomass in Artificial Streams and the Athabasca River

The response of the spring periphyton community to the 1% BKME treatment (Figs. 21 and 22) was not as marked as the previous autumn (Figs. 8 and 9). Although periphyton AFDM and Chl*a* on rocks in the spring tended to be higher under BKME exposure (Fig. 21), this tendency was not significant (AFDM $t_{8,\ 0.05/2}$=-0.91, p>0.39; Chl*a* $t_{8,\ 0.05/2}$=-2.08, p<0.07). Periphyton communities that colonized tiles exhibited a similar trend (Fig. 22) and, while AFDM was not affected by BKME ($t_{7,\ 0.05/2}$=-1.84, p>0.11), Chl*a* on tiles was > 8 times higher in the 1% BKME treatment ($t_{4,\ 0.05/2}$=-6.92, p<0.002). Riverine biomass of spring periphyton communities also diverged from trends noted in autumn 1993, as the AFDM and Chl*a* measured on rocks at sites upstream and downstream of the BKME discharge were not significantly different (AFDM $t_{18,\ 0.05/2}$=1.61, p>0.12, Chl*a* $t_{18,\ 0.05/2}$=-0.81, p>0.43) (Fig. 23).

4.2.3 Insect Density, Community Composition and Body Size in Artificial Streams

Although total insect densities of both the control and 1% BKME treatments were much higher than during the previous autumn (Fig. 10), spring insect abundance was not affected by the addition of BKME ($t_{8,\ 0.05/2}$=-1.93, p>0.089; Fig. 24). In fact, densities of all insect families were similar in the control and 1% BKME treatments as illustrated for the Ephemerellidae ($t_{8,\ 0.05/2}$=-1.68, p>0.13) and Chironomidae ($t_{8,\ 0.05/2}$=-1.92, p>0.091), the dominant taxa which comprised > 90 % of the total abundance (Fig. 24). Not surprisingly, insect community composition was similar in the control and effluent treatments during spring 1994 (Fig. 25). Although PCA axes 1 and 2 cumulatively describe 62.6% of the community variance among the replicates, positioning of replicates from the two treatments broadly overlapped. For example, replicates from the control are plotted on either end of axis 2, while effluent replicates span much of the gradient in community composition along axis 1. In addition, there was no significant difference in family richness between treatments ($t_{8,\ 0.05/2}$=0.60, p>0.600), and the 12-13 families recorded was similar to the richness observed during autumn 1993 (Figs. 12 and 26).

Mean thorax length of the mayfly *Baetis* (p<0.01) was significantly increased in the 1% BKME treatments relative to the control and NP treatments (Fig. 27; $t_{8,\ 0.05/2}$=-3.58, p<0.001). Furthermore, BKME-exposed *Baetis* were heavier than in control streams (Table 6; $t_{8,\ 0.05/2}$=-3.95, p<0.004). Growth in thorax length or weight was not significantly affected by the treatments for any other taxa (Fig. 27, Table 6).

Figure 21. **Mean Chlorophyll *a* Biomass (±SE µg/cm^2) and Ash-Free Dry Mass (AFDM) (±SE mg/cm^2) of Periphyton on Rocks in Control and 1% BKME Treatments During the Spring 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05. Note That the Y Axis Scale Is Different Than Figs. 8 and 32.**

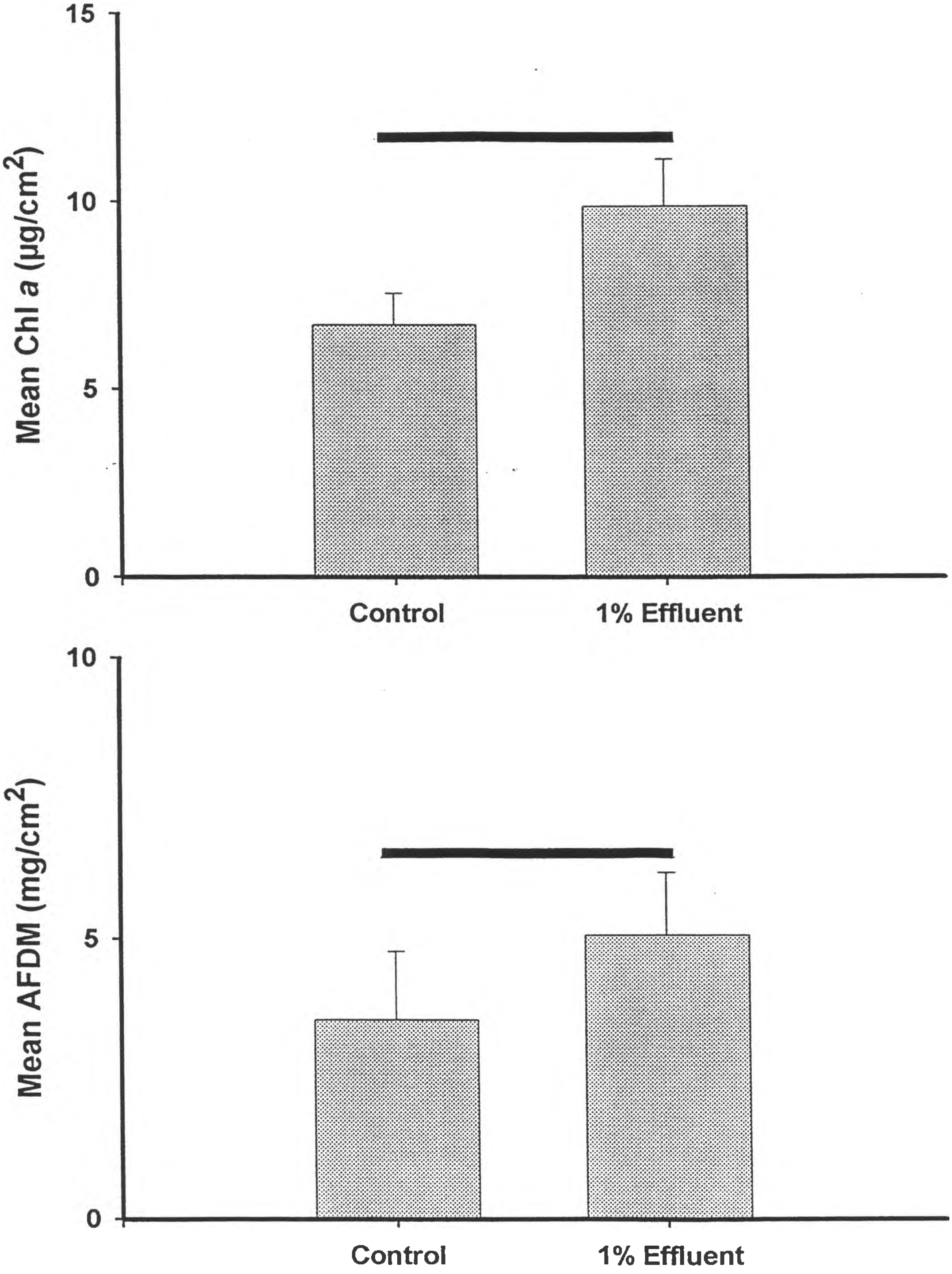


Figure 22. Mean (±SE) Chlorophyll *a* Biomass (±SE $\mu g/cm^2$) and Ash-Free Dry Mass (AFDM) (±SE mg/cm^2) of Periphyton on Tiles in Control and 1% BKME Treatments During the Spring 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05. Note That the Y Axis Scale For Chlorophyll *a* Is Different Than Fig. 9.

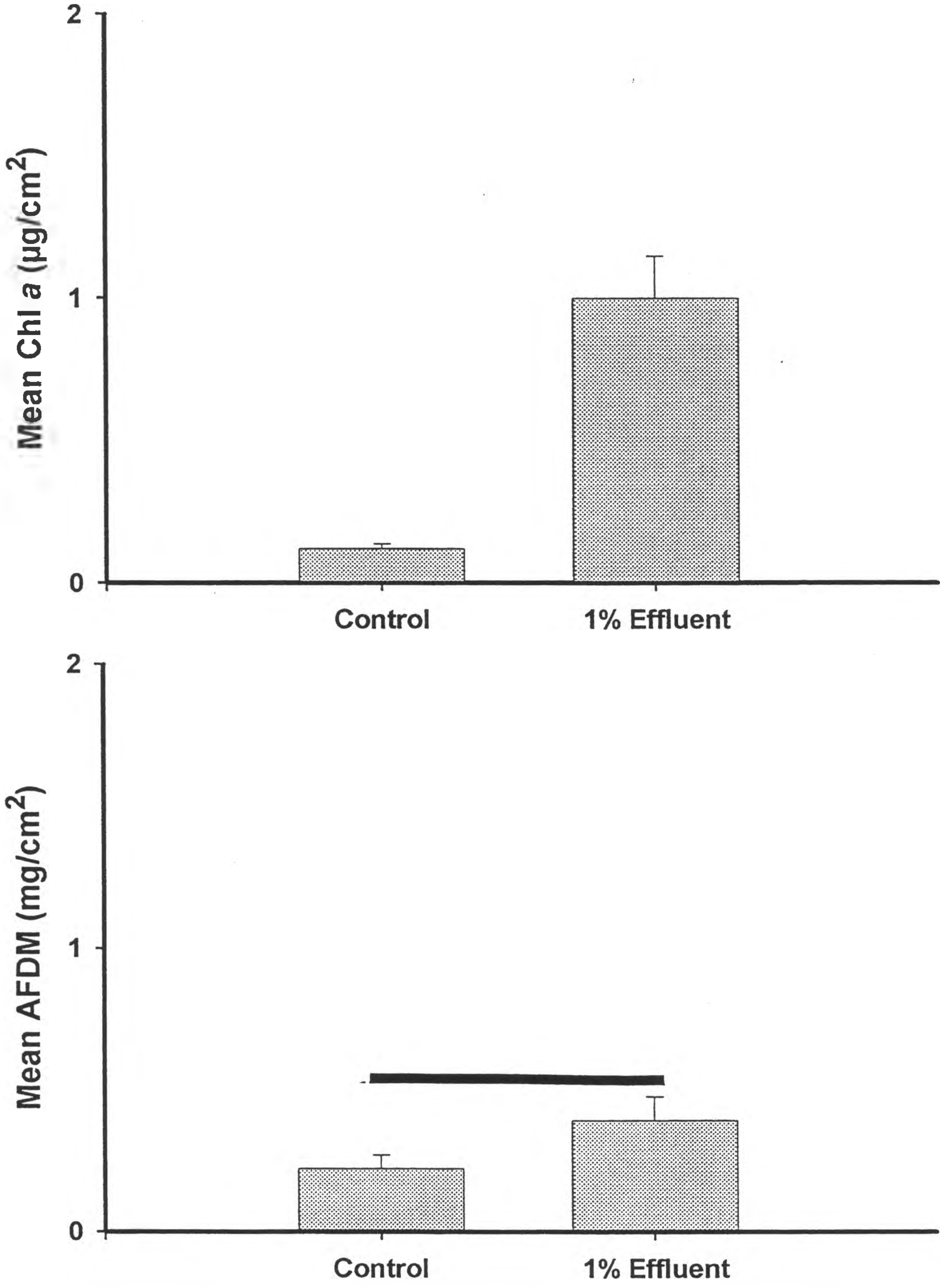


Figure 23. **Mean Chlorophyll *a* Biomass (±SE µg/cm²) and Ash-Free Dry Mass (AFDM) (mg/cm²) of Periphyton on Rocks in the Athabasca River Upstream and Downstream of the BKME Discharge at Hinton, Alberta in May 1994. Bars Connect Means That Are Not Significantly Different at p=0.05. Note That the Y Axis Scale For AFDM Is Different Than Figs. 14 and 34.**

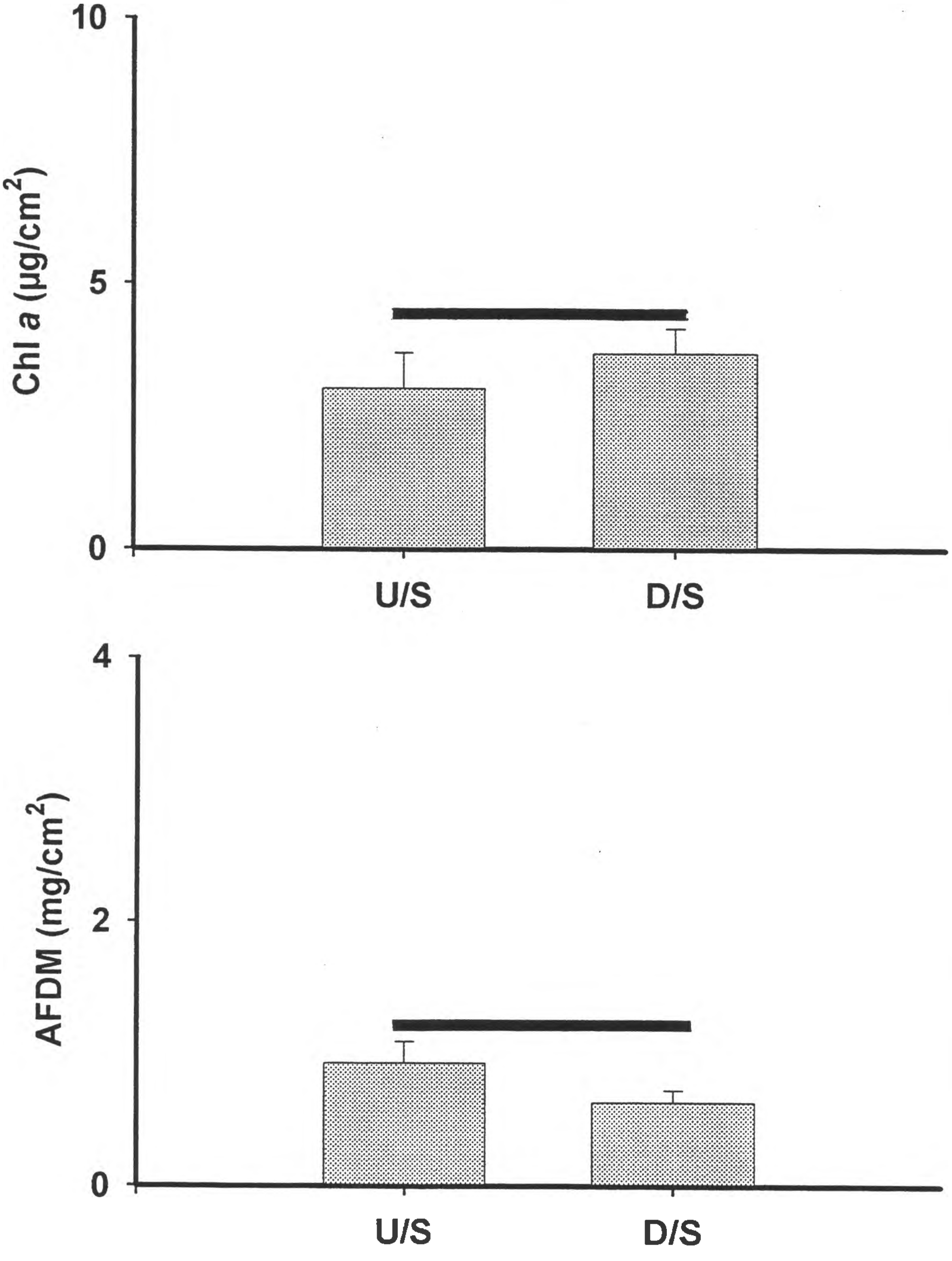


Figure 24. Total Number of Insect, Chironomidae and Ephemerellidae in Control and 1% BKME Treatments At the End of the Spring 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

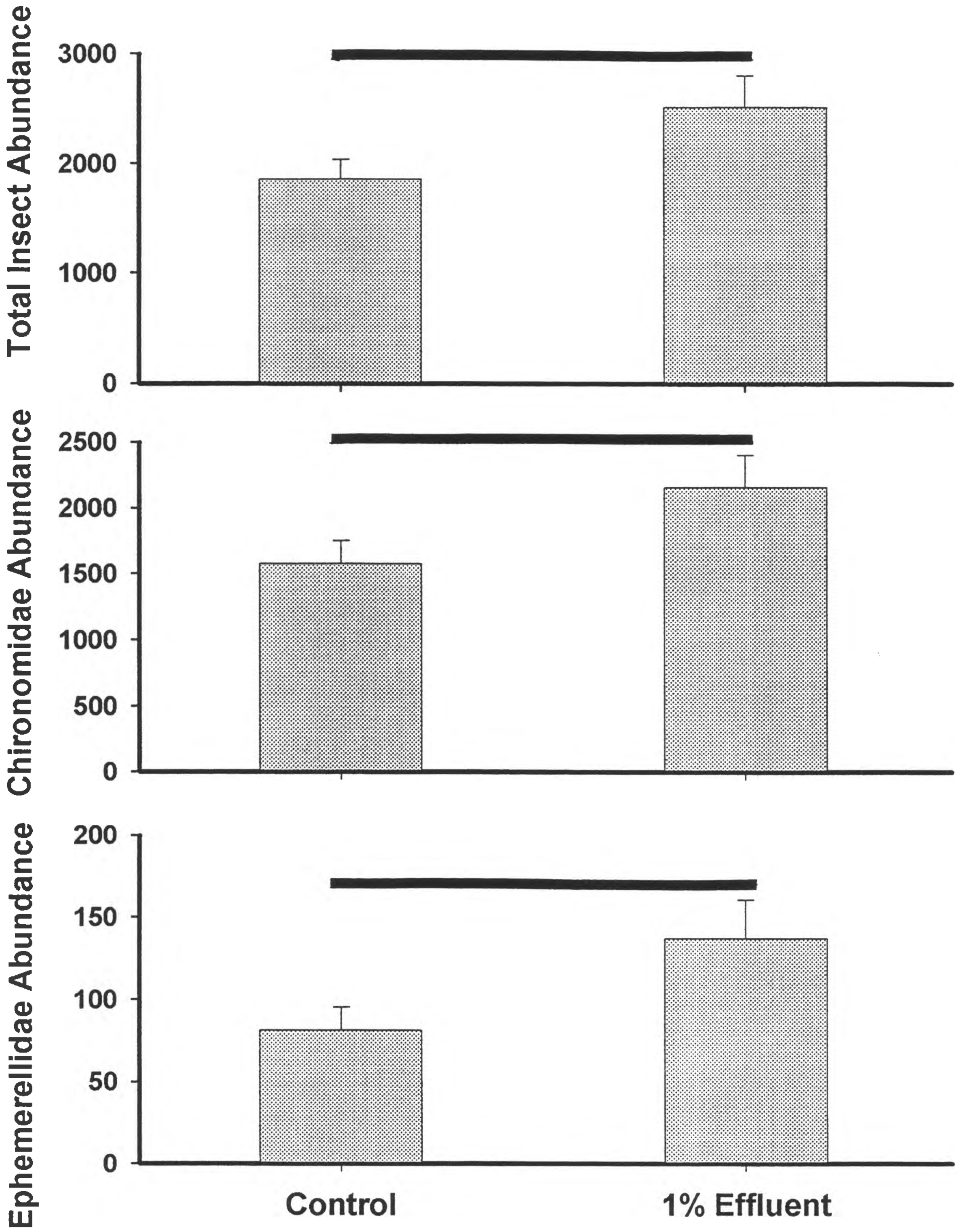


Figure 25. Principal Components Analysis Ordination of Insect Communities in the Control and 1% BKME Treatments of the Spring 1994 Experiment. Taxa With High Loading Values (Eigenvalues) on Axis 1 Are Indicated.

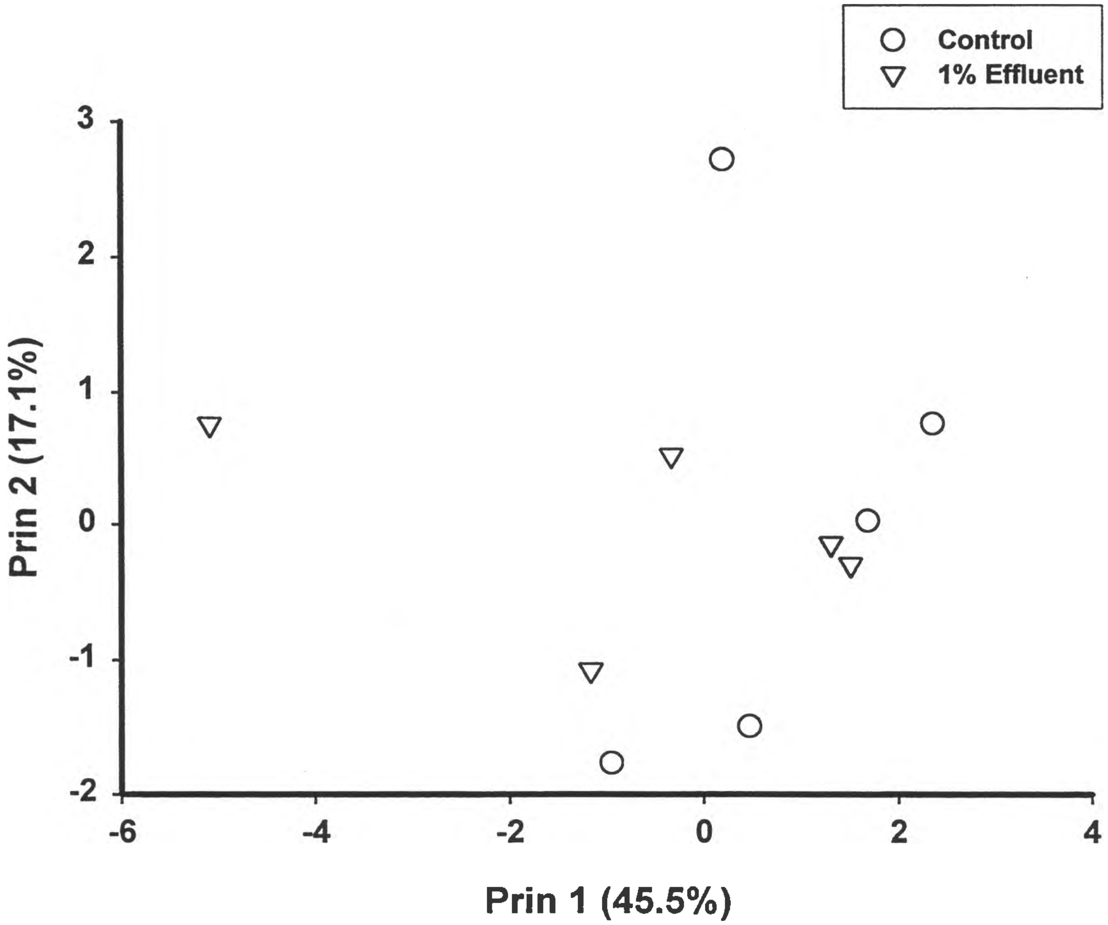


Figure 26. Number of Insect Families in Control and 1% BKME Treatments During the Spring 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

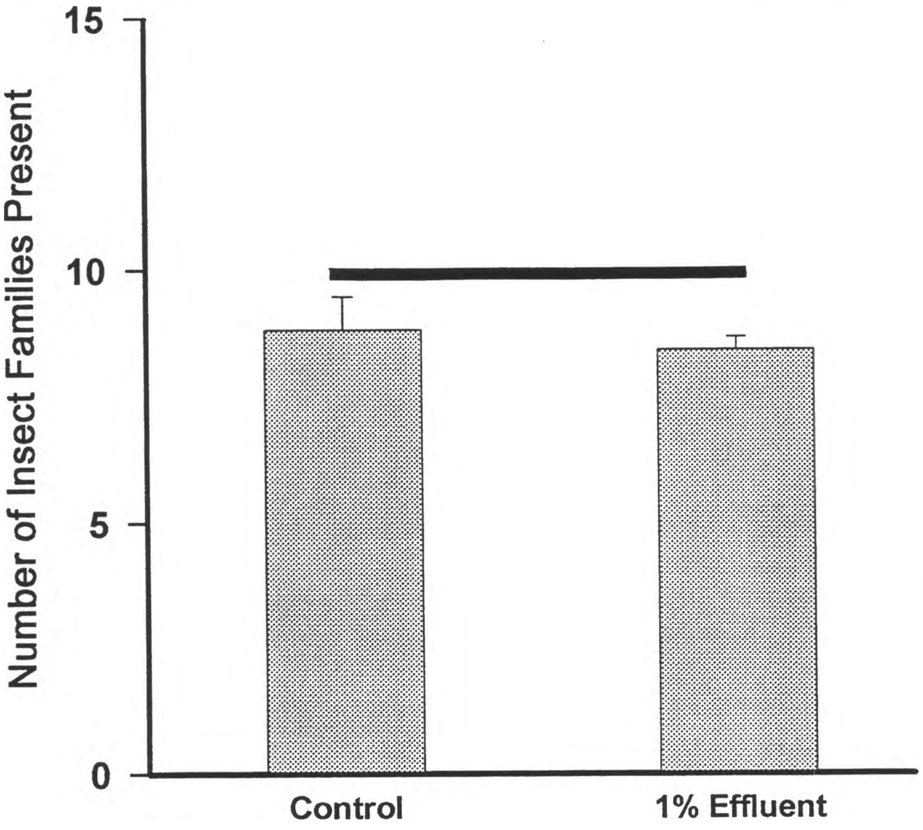


Figure 27. Mean Thorax Length (±SE mm) of the Mayflies, *Baetis*, *Ephemerella* and *Rhithrogena*, and Mean Pronotum Width (±SE mm) of the caddisfly, *Hydropsyche*, in the Control and 1% BKME Treatments at the End of the Spring 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

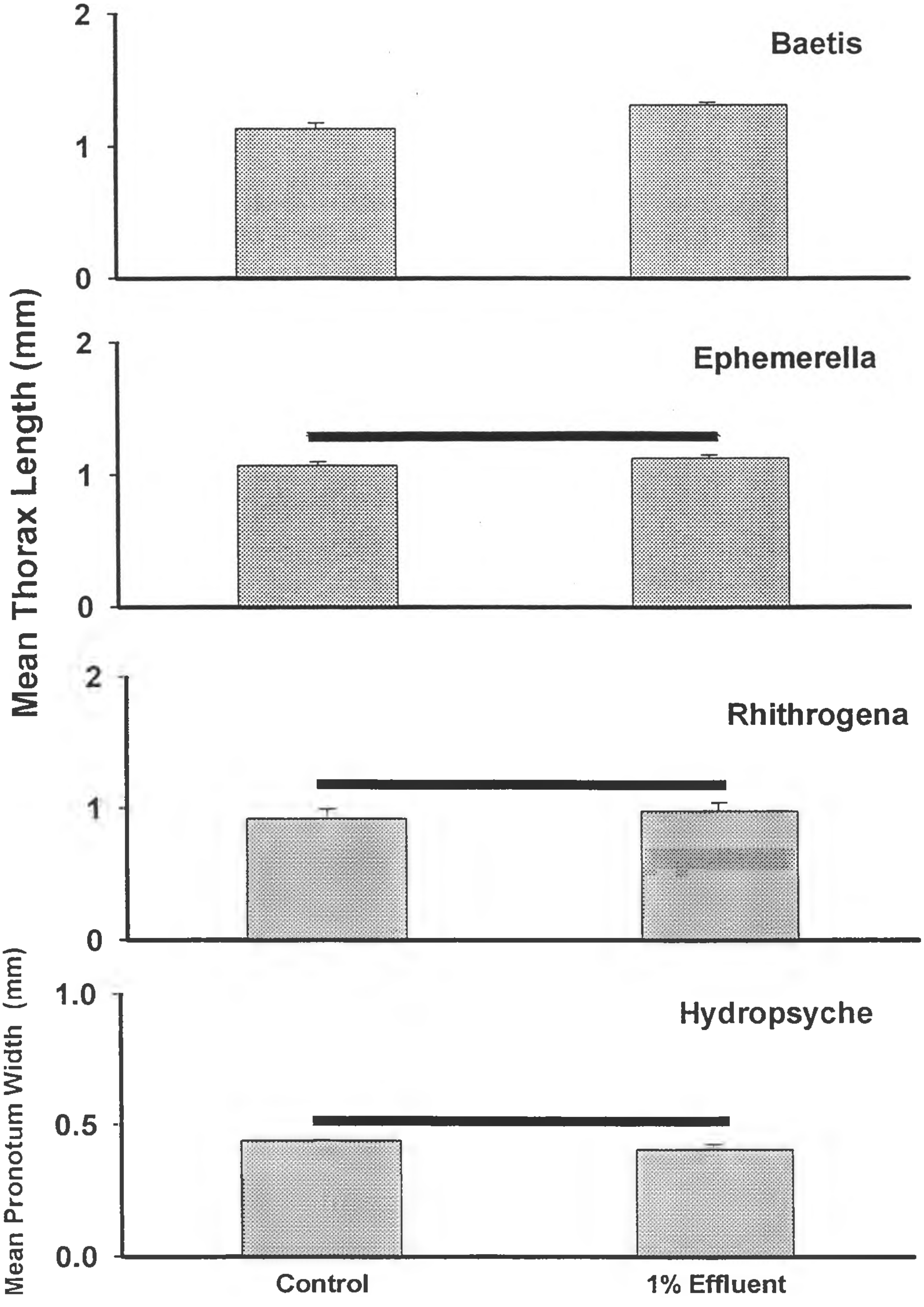


Table 6. **Mean Weight (± SE g/individual) of the Mayflies, *Baetis*, *Ephemerella* and *Rhithrogena*, and the caddisfly, *Hydropsyche*, in the Control and 1% BKME Treatments at the End of the Spring 1994 Experiment.**

TAXA	CONTROL	1% BKME
Baetis	261±20	353±12
Ephemerella	171±27	191±15
Rhithrogena	447±86	510±94
Hydropsyche	841±120	549±140

4.3 AUTUMN 1994

4.3.1 Effluent and Water Chemistry

Daily fluctuations in effluent chemistry during autumn 1994 (Fig. 28) were smaller than in autumn 1993 (Fig. 5) and always were ≤ 2.2 times the previous day's value. For example, in autumn 1994 the largest single-day proportionate change in these variables was 182 to 399 µg/L for SRP (5-6 October), 457 to 892 µg/L for NO_2+NO_3 (4-5 October), and 1650 to 1008 for NH_4^+ (8-9 September) (Fig. 28). During the experiment, effluent concentrations of SRP, NO_2+NO_3, and NH_4^+ ranged between 182-883 µg/L, 457-892 µg/L, and 448-1679 µg/L, respectively. Thus, NH_4^+ levels tended to be lower in 1994 compared to 1993; concentrations of SRP and NO_2+NO_3 were higher. Concentrations of SRP, NO_2+NO_3 and NH_4^+ in the effluent were always greater than those in the Athabasca River upstream of the discharge.

During autumn 1994, SRP, NO_2+NO_3 and NH_4^+ concentrations followed similar temporal patterns in the control, NP and 1% BKME treatments (Figs. 29 and 30). Most of the TDP in BKME was biologically available SRP, but TP was largely composed of particulate phosphorus. Phosphorus additions to the NP and 1% BKME treatments were between 1.8 and 8.8 µg/L, and biological uptake was rapid because mean SRP concentrations were similar in all treatments (Fig. 29). NO_2+NO_3 concentration tended to rise throughout the experiment, but NH_4^+concentration was higher during the midpoint than the beginning or end of the experiment (Fig. 30). Alkalinity, bicarbonate and pH were similar among treatments, while conductivity and dissolved organic carbon (DOC) were higher in the BKME treatment (Table 7).

Table 7. **Mean (±SE) value of Conductivity (µmhos/cm), Alkalinity (mg/L as $CaCO_3$), Bicarbonate (mg/L), pH and dissolved organic carbon (DOC mg/L) in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1994 Experiments.**

VARIABLE	CONTROL	NP	1% BKME
Conductivity	269±7	268±7	283±8
Alkalinity	84±1	84±1	85±8
Bicarbonate	103±2	100±4	104±2
pH	7.9±0.1	7.9±0.1	7.9±0.1
DOC	0.48±0.09	0.52±0.07	0.97±0.10

Because sodium is a conservative ion that can indicate the dilution of BKME in receiving waters, it was measured in the river and the treatments. Sodium concentration was higher in the BKME streams relative to control and NP streams (Fig. 31) and, over the course of the experiment, mean sodium concentration in the BKME treatment was similar to that expected in a 1% dilution of concentrated BKME ($t_5 > 0.57$). Additionally, the sodium concentration of the 1% BKME treatment and the Athabasca River values 2.1 km downstream of the effluent discharge were not significantly different ($t_{5,\ 0.05/2} > 0.67$), thus, providing verification that a 1% dilution was representative of the in-river environment (Fig. 31).

Figure 28. Concentrations (µg/L) of SRP, NO_2+NO_3, and NH_4^+ in Weldwood BKME and the Athabasca River Upstream of the Effluent Discharge During the Autumn 1994 Experiment.

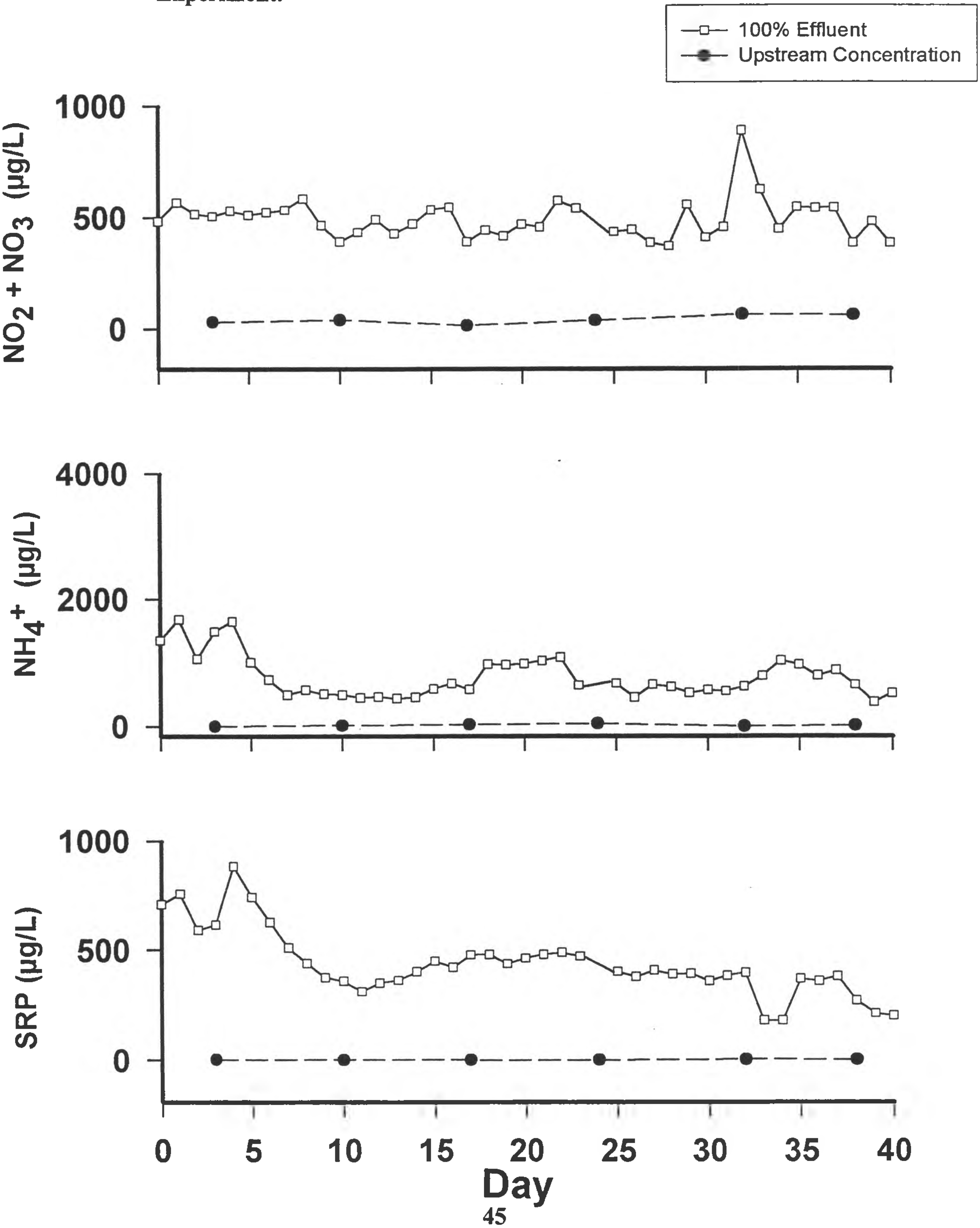


Figure 29. Mean Concentrations (±SE µg/L) of SRP, TDP, and TP in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1994 Experiment. Note That the Y Axis Scales for SRP and TP Are Different Than Figs. 6 and 19.

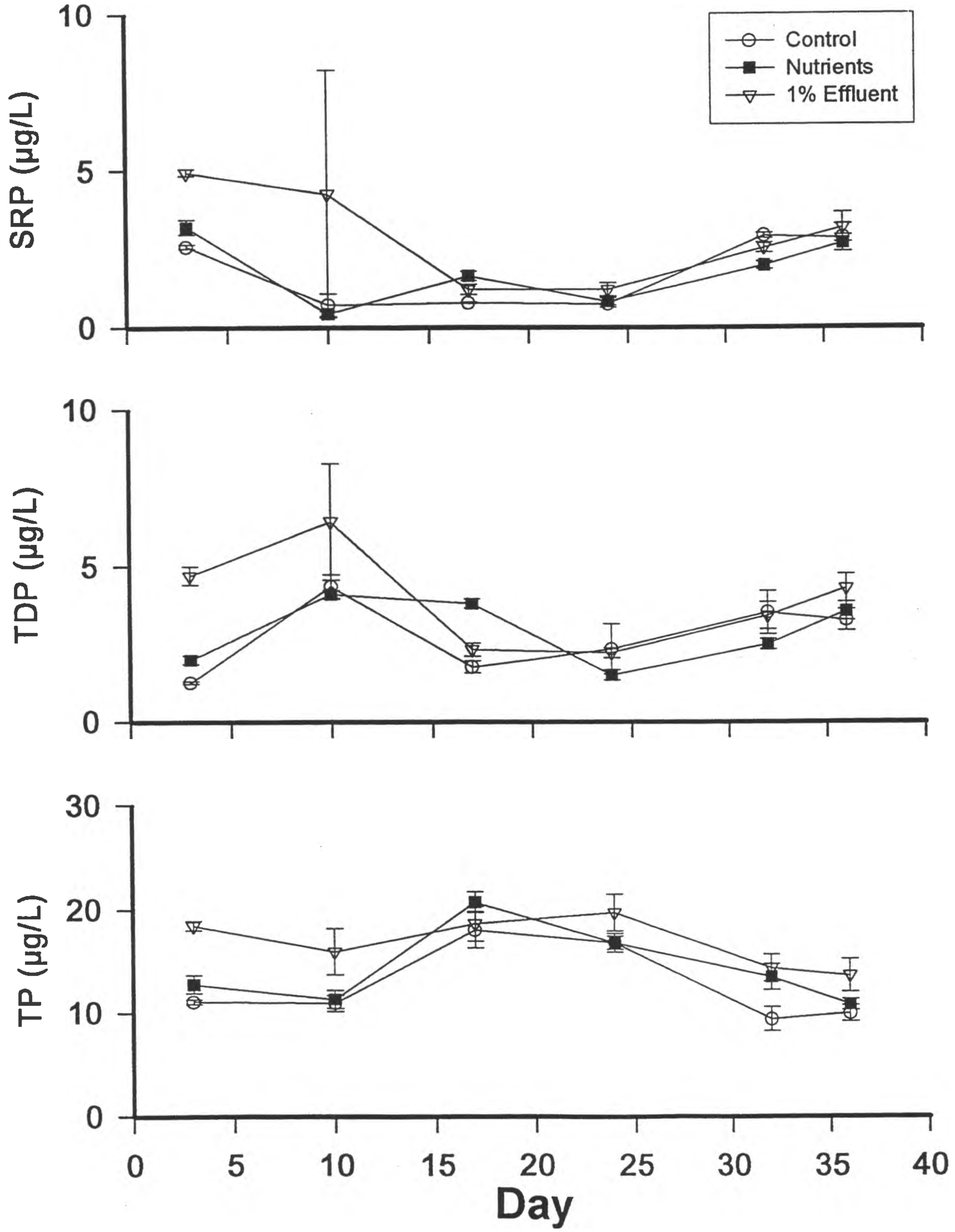


Figure 30. Mean Concentrations (±SE µg/L) of NO_2+NO_3 and NH_4^+ in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1994 Experiment.

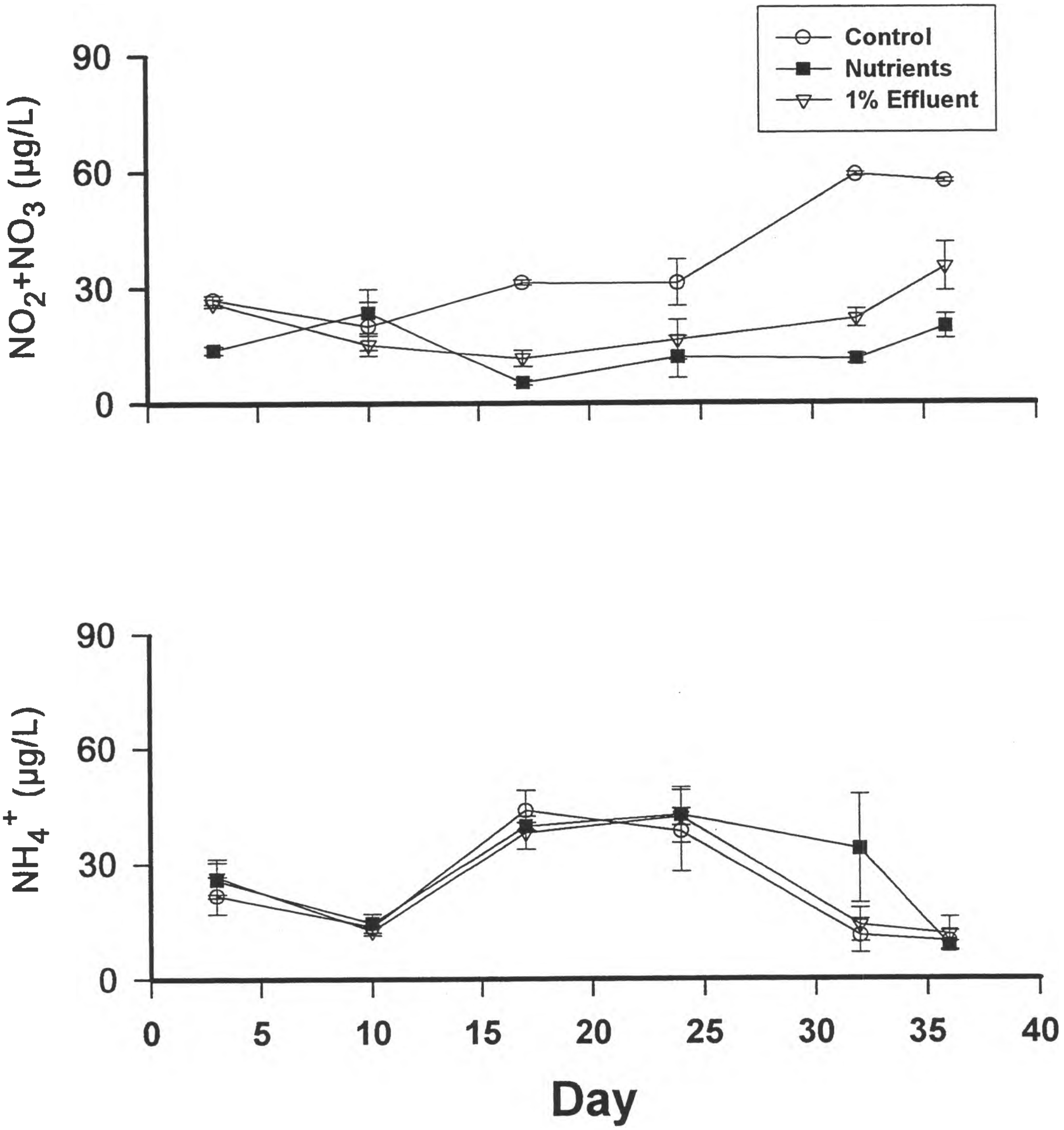
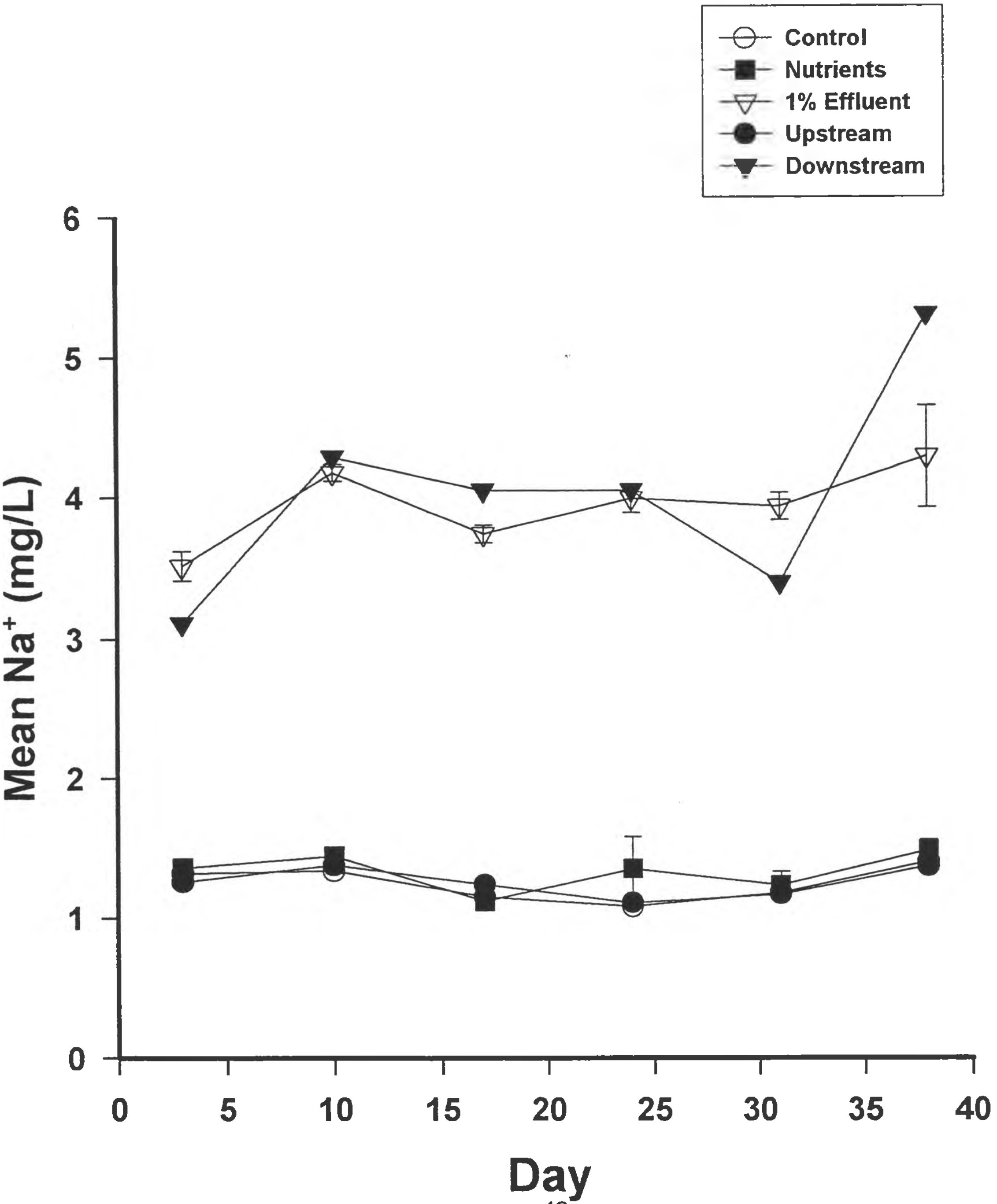


Figure 31. **Autumn 1994 Concentration of Sodium (µg/L) in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments, and 2.1 km Downstream of the Effluent Discharge.**



4.3.2 Periphyton Biomass in Artificial Streams and the Athabasca River

Chla biomass of periphyton on rocks and tiles was increased in autumn 1994 by exposure to NP or 1% BKME treatments (Figs. 32 and 33). Relative to control streams, Chla biomass was > 30 times higher on tiles in effluent- and nutrient-treated streams ($F_{2,12}$=19.86, p<0.001) and 10 times as high on rocks ($F_{2,12}$=15.21, p<0.001). Periphyton AFDM on tiles differed significantly across treatments ($F_{2,12}$=19.58, p<0.001), but rock AFDM did not ($F_{2,12}$=1.15, p>0.35). Trends of periphyton biomass in the river upstream and downstream of the effluent discharge were similar to these experimental findings, such that Chla was higher on downstream rocks and AFDM was similar at both sites (Chla $t_{17,0.05/2}$=13.47, p<0.002, rocks $t_{17,0.05/2}$=3.82, p>0.07; Fig. 34).

4.3.3 Insect Density, Community Composition and Body Size in Artificial Streams

Insect abundance in autumn 1994 was lower than the previous spring or autumn, and the Chironomidae, Capniidae and Baetidae comprised > 75 % of the individuals in the three treatments (Fig. 35). The low starting abundances in autumn 1994 were most likely the result of the experiments being initiated earlier than in 1993 when the relatively lower discharge and reduced wetted-area of streambed yielded more concentrated in-river abundances. Total abundance and chironomid densities tended to be higher in the nutrient and effluent treatments, but these trends were not significant (total $F_{2,12}$=3.98, p>0.08; chironomids $F_{2,12}$=4.34, p>0.07). In contrast, densities of capniid stoneflies were significantly higher in the control streams ($F_{2,12}$=6.61, p<0.03).

The PCA for the autumn 1994 insect communities was similar to that of autumn 1993 in that the NP and 1% BKME treatments were more similar to one another than to the control (Fig. 36). Although the first two axes explained 59.3% of the variation in community composition, axis 1 contributed most to treatment separation. Nutrient and effluent streams tended to have higher abundances of Hydropsychidae and Chironomidae, but lower numbers of Brachycentridae; the same trends were also evident in autumn 1993. In contrast to autumn 1993, control replicates had higher abundances of Ephemerellidae, Capniidae, Chloroperlidae, and Perlodidae. Thus, autumn community composition was affected by nutrient and effluent treatments in 1993 and 1994, but only the Chironomidae, Brachycentridae and Hydropsychidae exhibited consistent responses to the treatments. Family richness in autumn 1994 ranged between 11-12 families and was not significantly different among control, NP and 1% BKME treatments ($F_{2,12}$=0.86, p>0.471) (Fig. 37).

Insects in the autumn 1994 nutrient and effluent treatments tended to be larger than those in the control streams, as was observed in autumn 1993 (Fig. 38, Table 8). However, only the trends for *Hydropsyche* were significant ($F_{2,12}$=6.52, p<0.04). It is notable that the autumn 1994 results are based on fewer replicates (n=3) relative to 1993 (n=5), therefore, the power to detect differences was lower in 1994.

Table 8. **Mean Weight (±SE g/individual) of the Stoneflies, Capniidae and Nemouridae, the Mayflies, Baetidae and *Rhithrogena*, in Control, NP and 1% BKME Treatments at the End of the Autumn 1994 Experiment.**

TAXA	CONTROL	NP	1% BKME
Capniidae	217±41	309±28	308±12
Nemouridae	228±47	275±49	280±31
Baetidae	39±3	25±4	25±5
Rhithrogena	345±86	407±46	219±42

Figure 32. Mean Chlorophyll *a* Biomass (±SE µg/cm^2) and Ash-Free-Dry-Mass (AFDM) (±SE mg/cm^2) of Periphyton on Rocks in Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05.

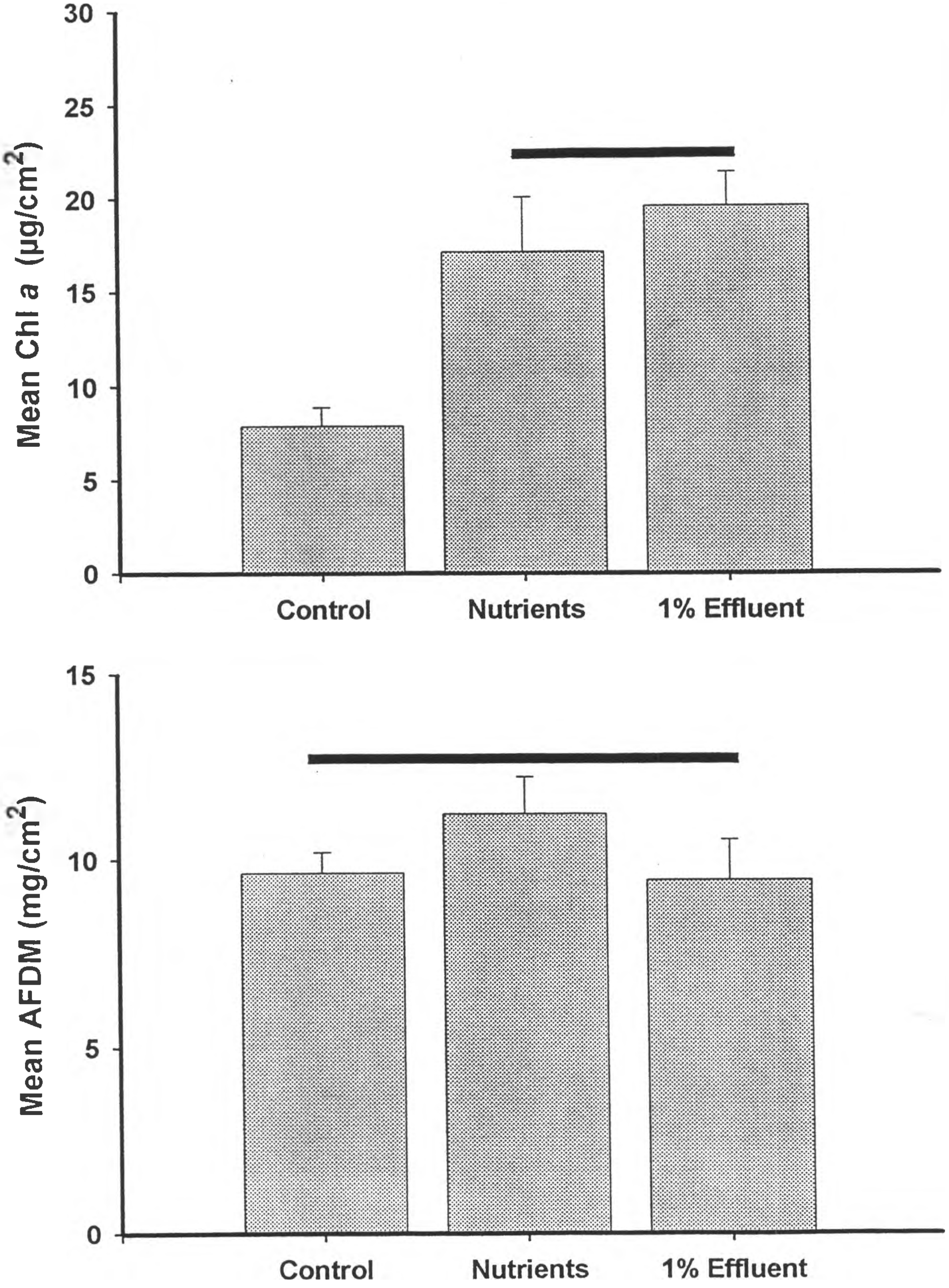


Figure 33. Mean Chlorophyll *a* Biomass (±SE µg/cm^2) and Ash-Free Dry Mass (AFDM) (±SE mg/cm^2) of Periphyton on Tiles in Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1994 Experiment. Bars Connect Means That Are Not Significantly Different at p=0.05. Note That the Y Axis Scale For AFDM Is Different Than That in Fig. 9 and 22.

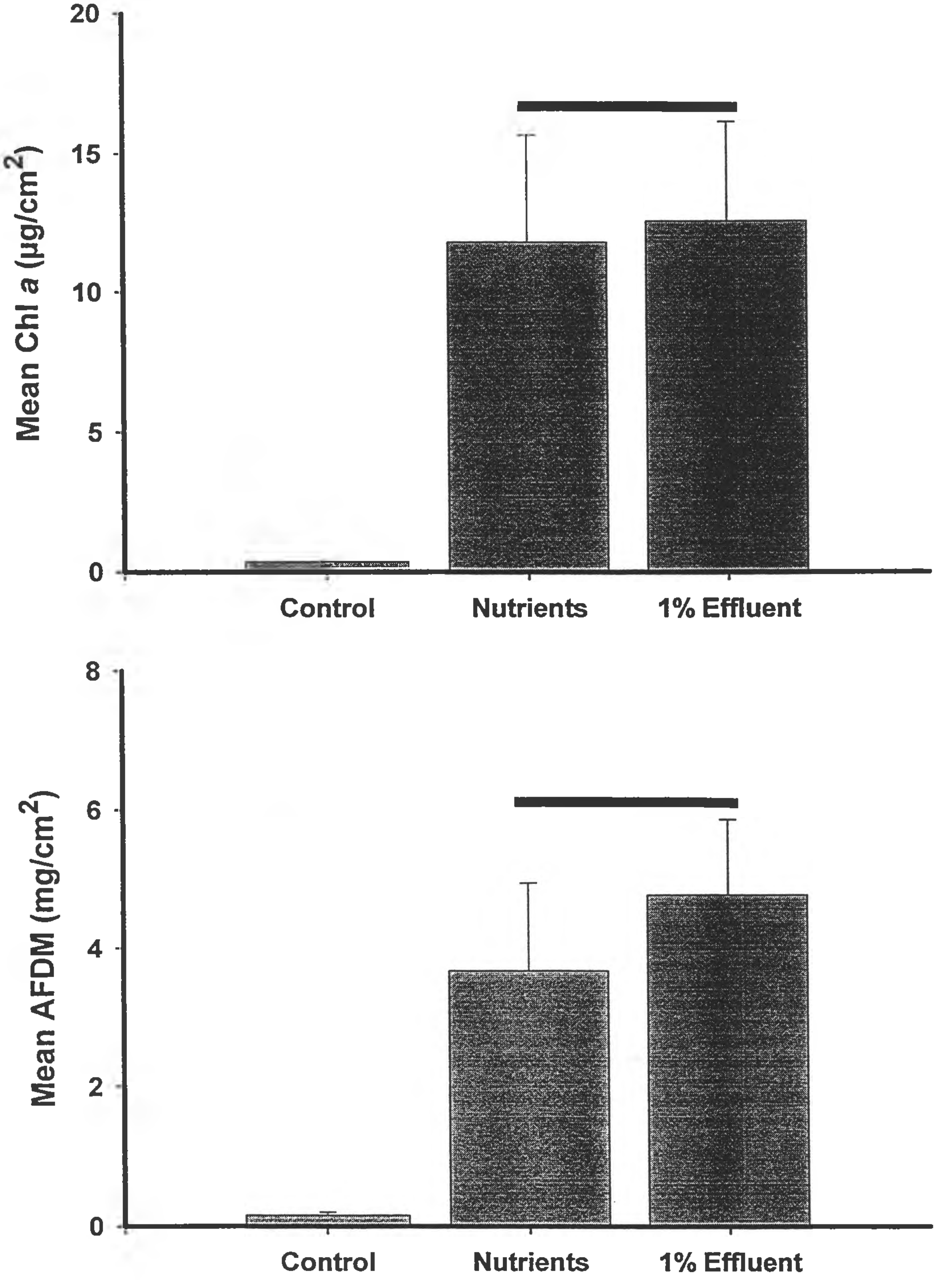


Figure 34. **Mean Chlorophyll *a* Biomass (±SE µg/cm^2) and Ash-Free Dry Mass (±SE AFDM) (mg/cm^2) of Periphyton on Rocks in the Athabasca River Upstream and Downstream of the BKME Discharge at Hinton, Alberta in October 1994. Bars Connect Means That Are Not Significantly Different at p=0.05. Note That Y Axis Scale For Chlorophyll *a* Is Different Than That in Figs. 14 and 23.**

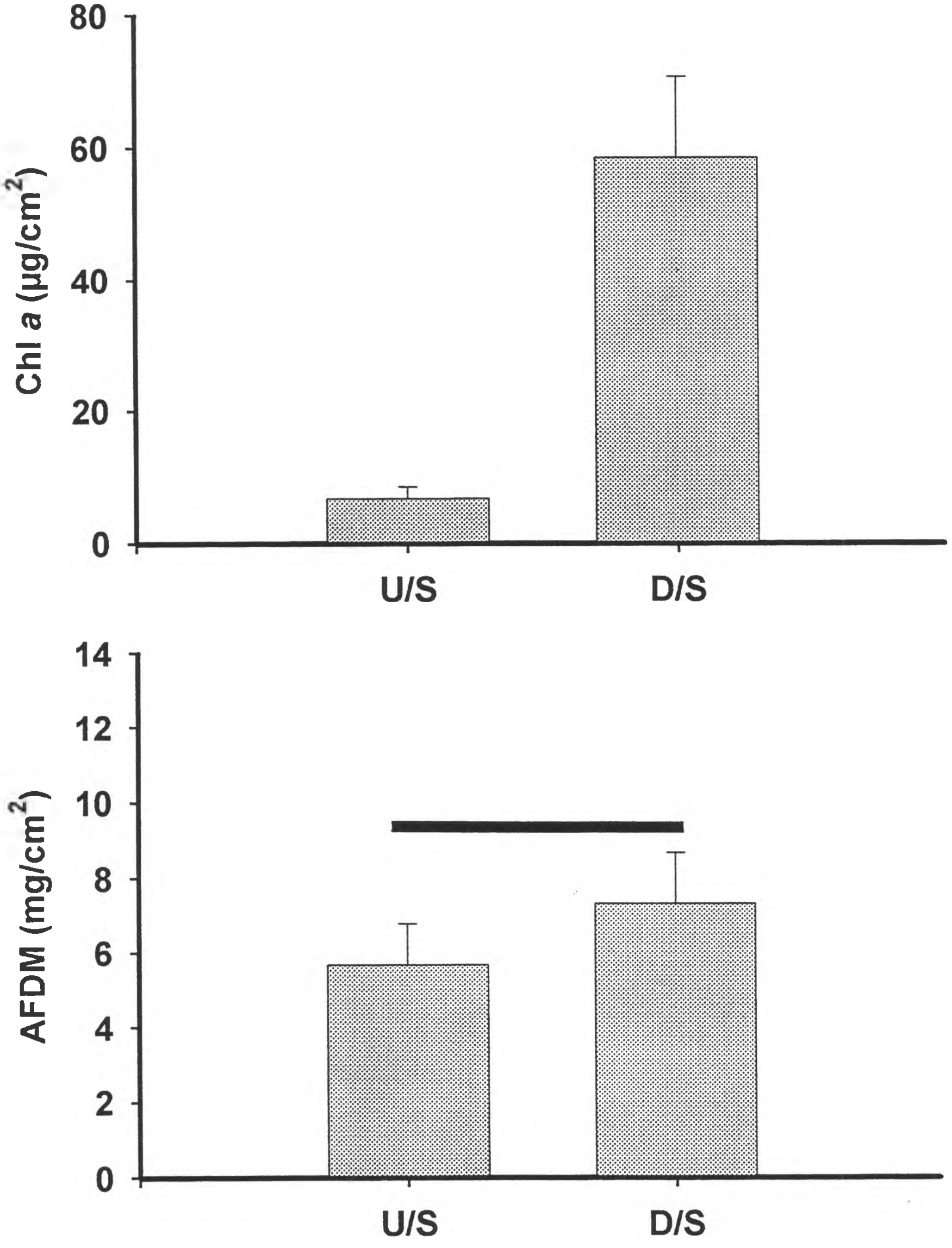


Figure 35. Total Number of Insects, Chironomidae and Capniidae Present in Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments At the End of the Autumn 1994 Experiment; Bars Connect Means That Are Not Significantly Different at p=0.05.

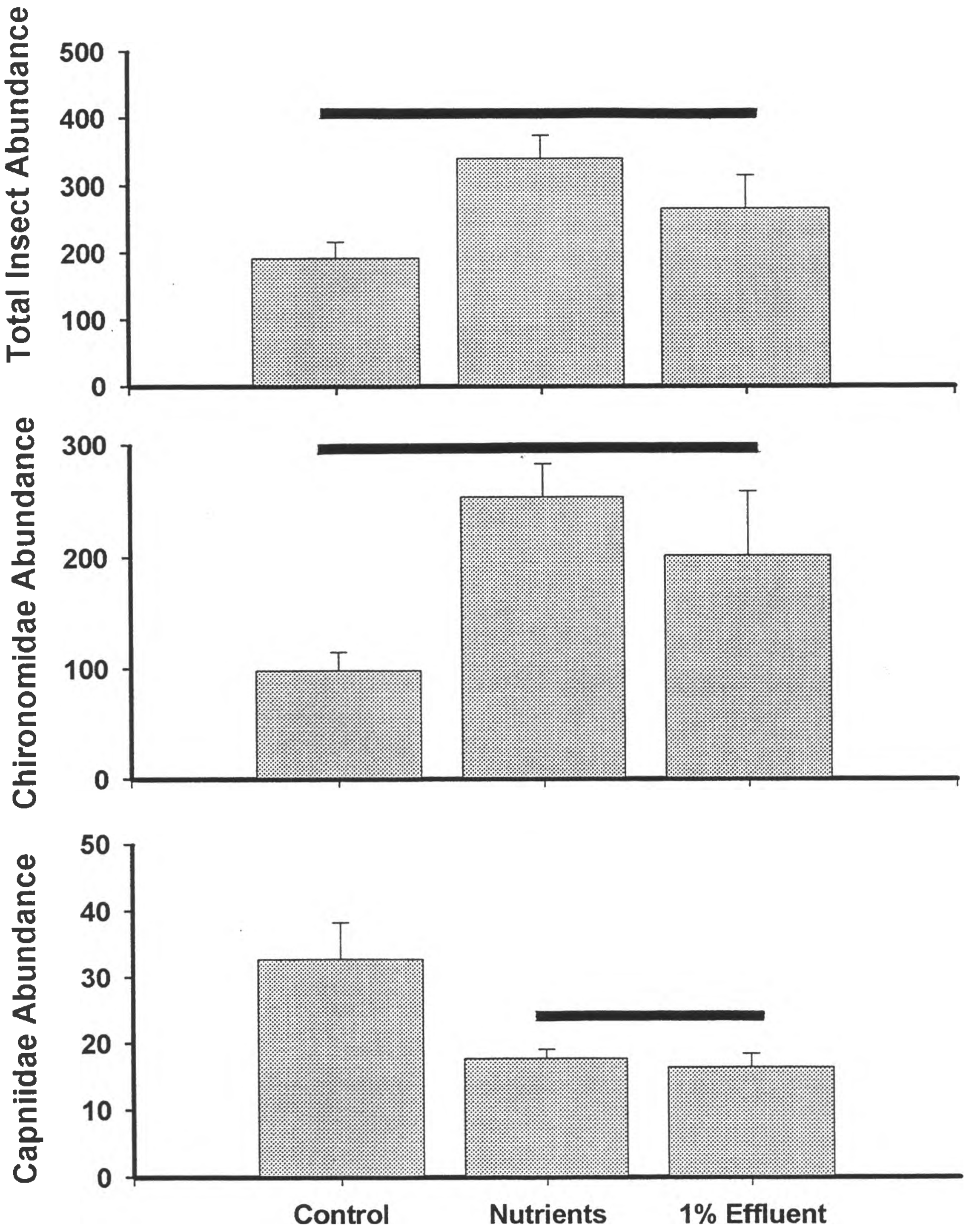


Figure 36. Principal Components Analysis Ordination of Insect Communities in Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments of the Autumn 1994 Experiment. Taxa With High Loading Values (Eigenvalues) On Axis 1 Are Indicated.

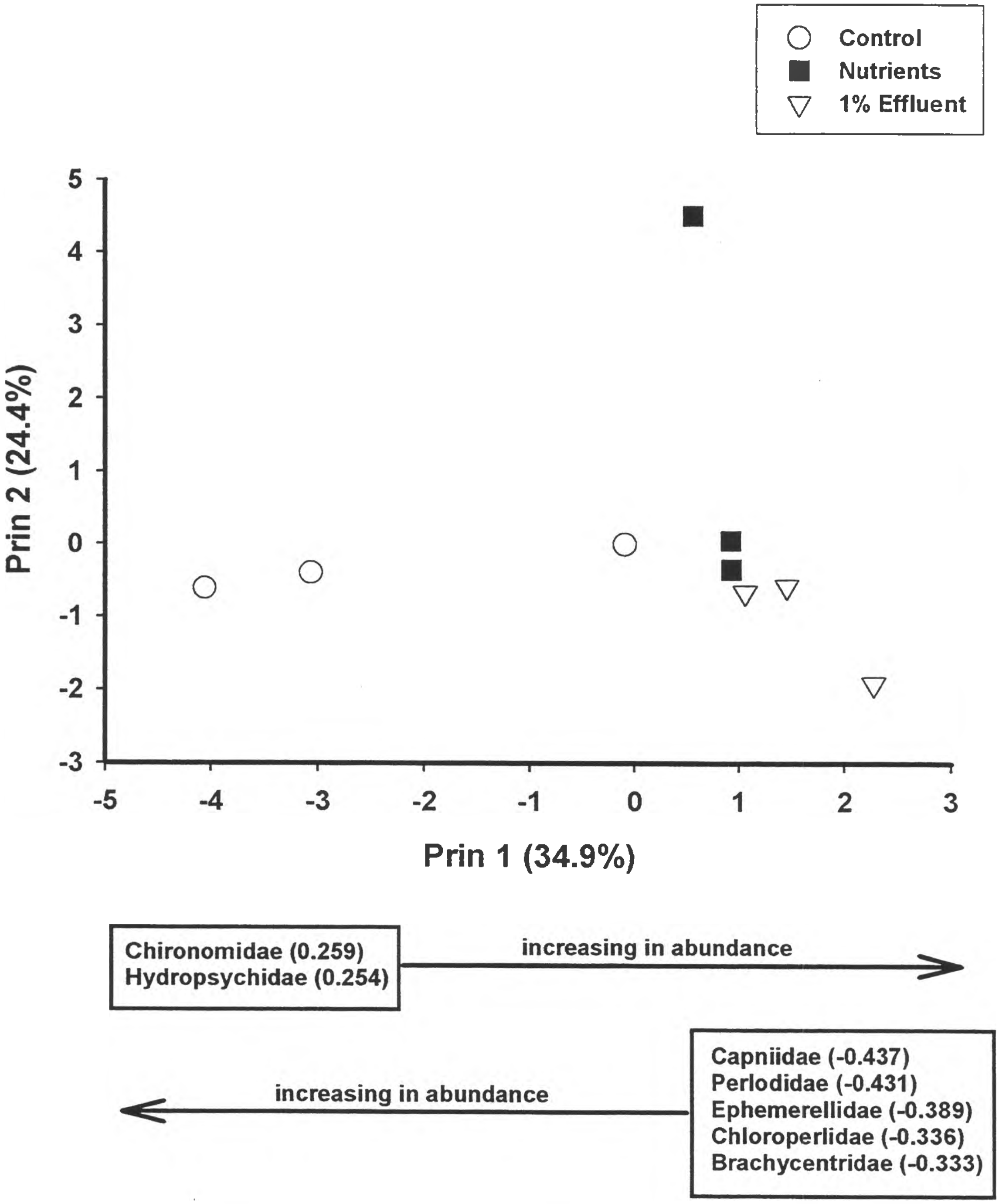


Figure 37. Number of Insect Families Present in Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments During the Autumn 1994. Bars Connect Means That Are Not Significantly Different at p=0.05.

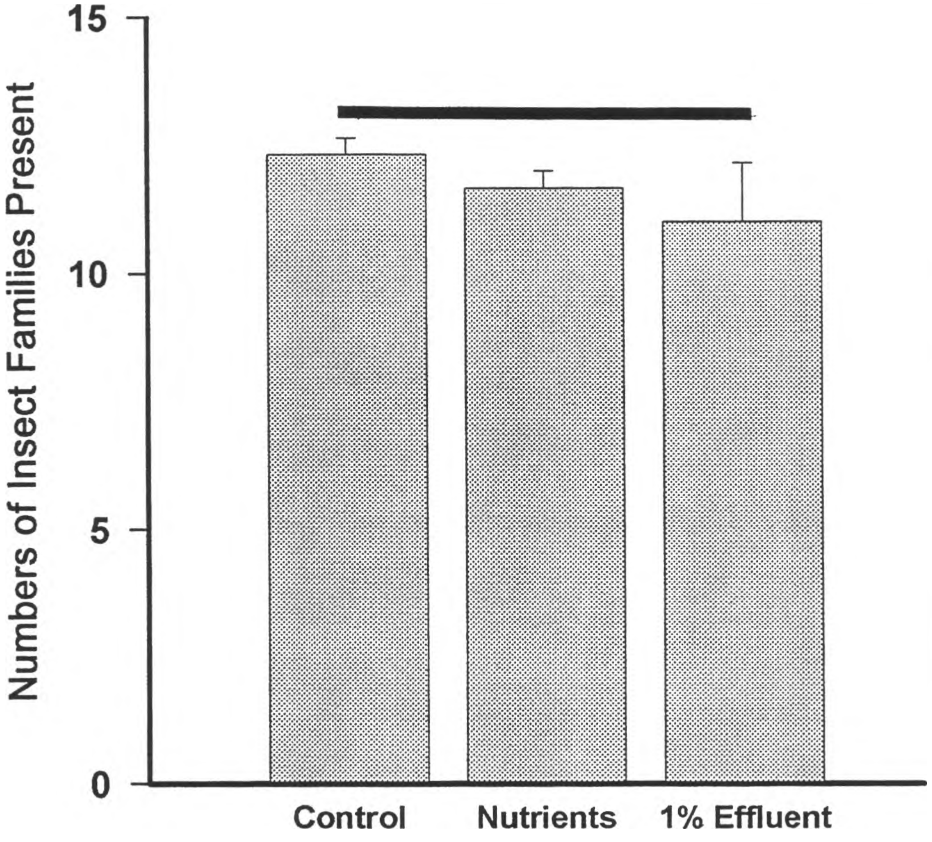
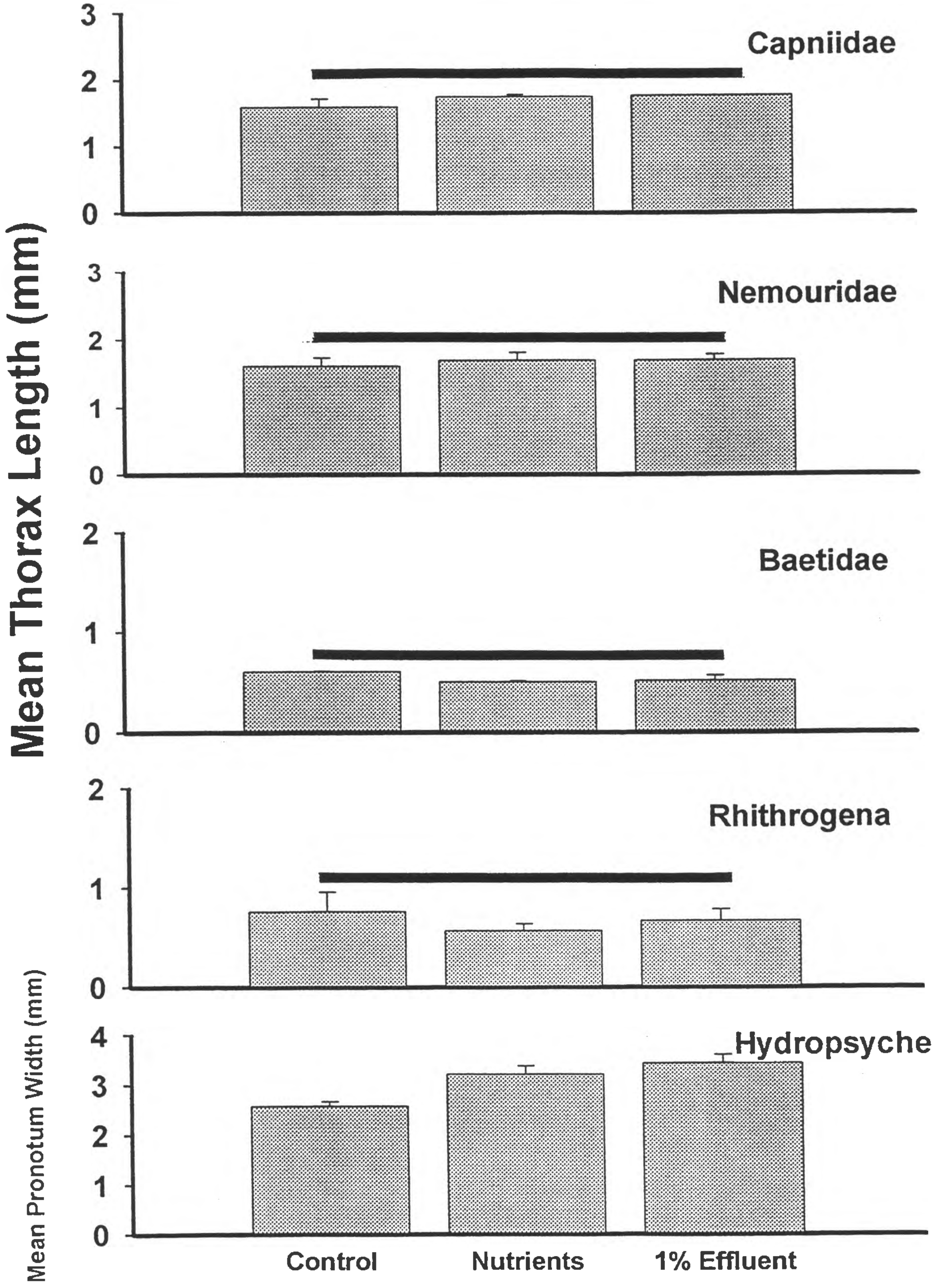


Figure 38. Mean Thorax Length (mm) of the Stoneflies, Capniidae and Nemouridae, the Mayflies, Baetidae and *Rhithrogena*, and Pronotum Width (mm) of the Caddisfly, *Hydropsyche*, in the Control, Nitrogen-Phosphorus (NP) and 1% BKME Treatments at the End of the Autumn 1994 Experiment; Bars Connect Means That Are Not Significantly Different at p=0.05.



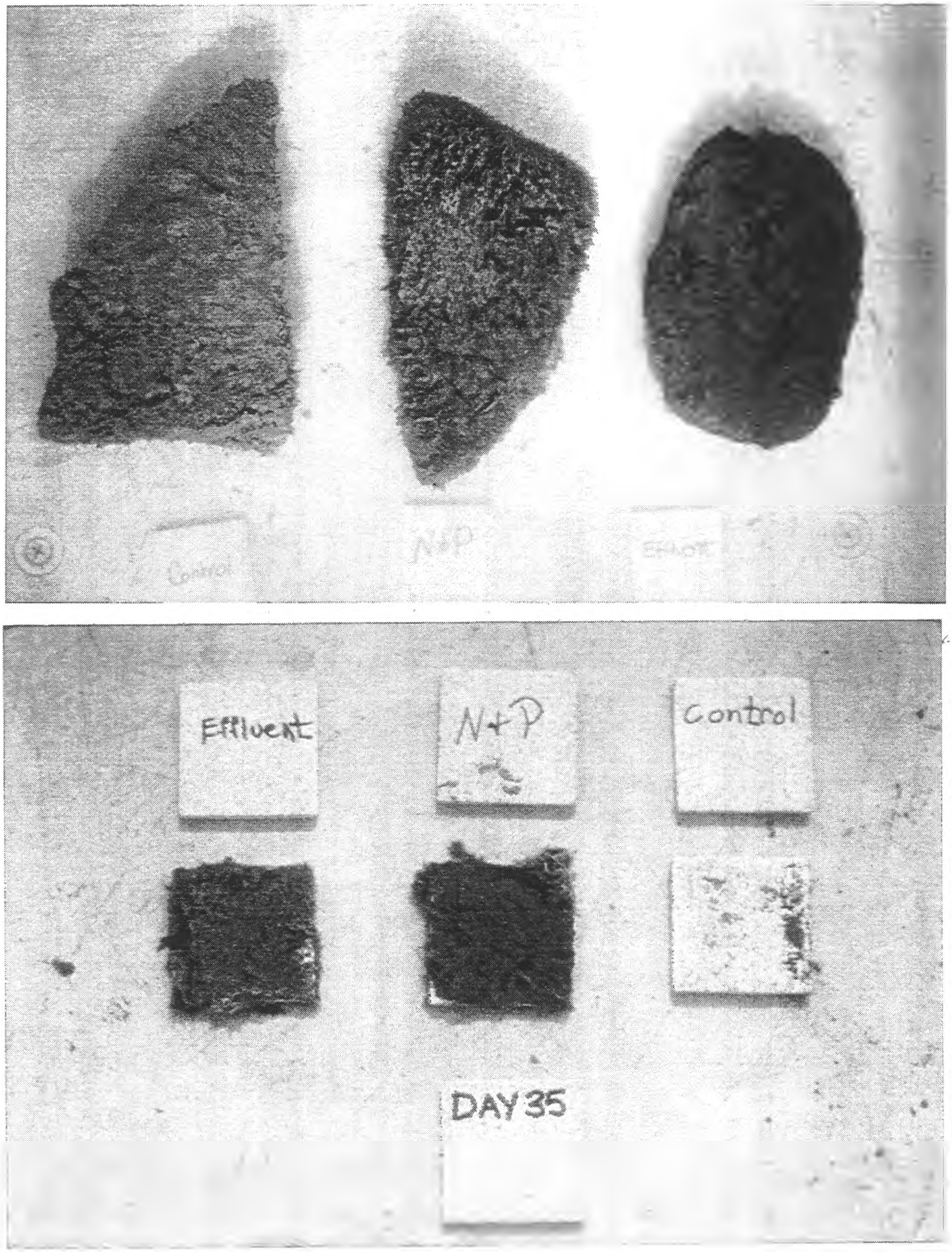
5.0 DISCUSSION

Pulp mill effluents contain a complex variety of compounds that have differing effects on aquatic organisms and communities in receiving waters (McLeay 1987, Lowell et al. 1995). Even though more than 250 compounds have been identified in pulp mill effluents, their measurable effects on biota can be categorized into compounds that have inhibitory or stimulatory consequences. Because Alberta pulp mills are modern by international standards and discharge effluent that normally passes acute lethality tests, the effluents from these mills are characterized as weakly toxic (McCubbin and Folke 1993). If inhibitory effects in the Athabasca River at Hinton, Alberta occur as a result of the combined BKME and municipal sewage discharge, these would be expected to be of the sublethal chronic variety, and would include changes in community composition or reductions in growth rates of benthic organisms such as aquatic insects. In contrast, pulp mill effluents often contain high levels of the algal nutrients, phosphorus and nitrogen. Previous research in marine and freshwater ecosystems suggests that these nutrients can lead to an enrichment effect in aquatic systems (Hansson 1987, Feder and Pearson 1988, Hall et al. 1991, Bothwell 1992). In particular, nutrients in BKME discharges can cause changes in benthic algal and insect community composition (Hall et al. 1991) and affect insect growth (Dubé 1995, Lowell et al. 1995). Our artificial stream experiments were designed to decouple the effects on the riverine food web of the potentially confounding effects of nutrients and contaminants within BKME (Podemski and Culp 1995). This goal required that we design a novel riverside artificial stream system (Culp et al. 1994, Culp and Podemski 1995), and establish experimental treatments consisting of a control that received raw river water, a 1% dilution of treated BKME, and a 1% dilution of the nitrogen and phosphorus contained in the concentrated effluent. By comparing the effects of the NP and BKME treatments on different trophic components of the food web, we were able to provide a mechanistic understanding of the stimulatory and/or inhibitory effects of pulp mill effluents on the benthic food webs of the Athabasca River.

Our experiments and in-river observations provide strong evidence that the dominant effect of the fully-mixed BKME discharge (i.e., 1% dilution) to the upper Athabasca River at Hinton was that of nutrient enrichment and stimulation of food web productivity. In autumn (1993 and 1994), both the NP and BKME treatments stimulated primary production of the largely diatom algal community relative to the control treatment, which was representative of conditions upstream of the effluent discharge. These experimental findings corresponded to in-river trends where periphyton biomass increased at sites downstream of the effluent relative to upstream reference sites. The increased accumulation in the artificial streams occurred both on rocks with existing periphytic communities and on tiles which experienced rapid accumulation of algae over the experiment's course (Fig. 39). The marked difference between BKME- or NP-exposed tiles and control tiles after only 14 d suggests that algal community biomass downstream of the effluent discharge can recover rapidly after scouring disturbance events (e.g., annual spring flood). In addition, the higher primary production in the NP and BKME treatments represents increased levels of food for the benthic insects dependent on this periphytic periphyton.

The effect of BKME on periphyton communities in the upper Athabasca River changed seasonally,

Figure 39. Photograph of Rocks and Tiles At the End of the Autumn 1994 Experiment Illustrating the Higher Algal Biomass on Nitrogen-Phosphorus and 1% BKME Treatments Relative to the Control Treatment.



such that the responses of algal communities both in the river and artificial streams were less marked during spring than in autumn. For example, whereas algal biomass on rocks upstream and downstream of the effluent outfall was similar in the spring, in autumn 1993 Chl*a* was eight times higher than downstream. Periphyton growth rate in the upper Athabasca River is reduced when water temperature is very low (Scrimgeour et al. 1995; Dale and Chambers 1996). However, temperature was not the cause for the seasonal differences we observed since the increase in periphyton in the NP and BKME streams was greater in autumn 1993 than spring 1994, yet temperatures were lower in autumn 1993. Furthermore, the range in mean water temperature over the three experiments was only 3 °C.

The seasonal differences in periphytic algal biomass was due to the much lower concentration of SRP in spring 1994. In spring, the SRP addition from the fully-mixed BKME was ≤1 µg/L. In contrast, SRP enrichment from the fully-mixed effluent during the autumn usually exceeded 2-3 µg/L with excursions to 5 µg/L on several days. Relative specific growth rates of algal communities in the upper Athabasca River saturate in both spring and autumn at 2-5 µg/L (Dale and Chambers 1996). On average, background SRP levels in the river upstream of the effluent outfall were 1.5 µg/L in the spring, and autumn concentrations were 1 µg/L (1993) and 1.7 µg/L (1994). When the fully-diluted effluent concentrations of SRP are added to the ambient SRP in the river, spring values were between 2-3 µg/L, while autumn SRP concentrations ranged between 2-6 µg/L (1993) and 4-10 µg/L (1994). Thus, the weak response of the spring periphyton communities to BKME compared to the enrichment response of the autumn periphyton communities is consistent with Dale and Chambers' (1996) hypothesis that substantial increases in periphytic algae can occur at low P concentrations (2-5 µg/L). Additionally, our observations suggest that periphyton biomass in the downstream community can be reduced when the fully-mixed BKME concentration of SRP is below 2-3 µg/L. It cannot be over-emphasized that the specific conclusions on potential thresholds of BKME-related enrichment with SRP were possible solely because artificial streams (with riverside deployment) used in this and Dale and Chambers' (1996) study provided the tools for conducting controlled experiments to separate nutrient and contaminant effects.

The effluent-induced nutrient enrichment, and subsequent increase in periphyton biomass during autumn 1993 and 1994, elevated food availability for secondary producers inhabiting the upper Athabasca River. Abundances of total insects and several dominant taxa (stoneflies, mayflies and midges) increased in the NP and BKME treatments, a trend that corresponds to the autumn 1992 in-river samples, when abundance increased downstream of the effluent discharge. Anderson's (1989, 1991) field studies also noted an increase in mayflies and midges downstream of the effluent outfall. Our evidence was stronger for autumn 1993, probably because starting densities for the experiments were lower in 1994, fewer replicates were analyzed, and statistical power was reduced. Insect communities in the NP and BKME treatments were more similar to one another than to the control biota and were dominated by mayflies, stoneflies and midges. Communities in the Athabasca River downstream of the BKME discharge exhibited a similar shift and were likewise dominated by mayflies, stoneflies and midges. Although these community shifts are clearly effluent-induced, the BKME-exposed communities included many taxa (mayflies and stoneflies) that are considered to be sensitive to pollution (Rosenberg and Resh 1993), suggesting that composition shifts were a response to enrichment rather than toxicity. However, because the Athabasca River upstream of the BKME discharge is phosphorus-limited and oligotrophic, the current effluent-loads to the upper river provide levels of nutrient enrichment that increase benthic riverine productivity without the biotic changes associated with severe eutrophication. Our artificial stream studies provide experimental evidence that substantiates speculation from earlier field studies which attributed the increased insect abundance downstream of the Hinton discharge to BKME-induced nutrient enrichment (Anderson 1989, 1991). Finally, although spring insect communities were not changed by effluent exposure, this result does not reflect seasonal differences in the mechanisms by which BKME affects the stream biota. Rather, the difference is related to mill operations which produced effluent with low SRP concentration during the spring investigations, thereby resulting in only mild nutrient enrichment effects on the periphytic food base of the insects.

Studies of other mills indicate that biotreated BKME can enhance the growth of fish and insects through nutrient enrichment as the resultant increase in food availability (as carbon fixed by primary producers) is transferred to consumer trophic levels (McLeay 1987, Hall et al. 1991, Dubé 1995, Lowell et al. 1995). Our study yielded the first evidence that BKME-induced increases of periphyton biomass had growth-enhancing effects on benthic insects (mayfly, stonefly and caddisfly taxa) in the upper Athabasca River. Treatment-related differences in autumnal growth rates were better detected in 1993 when statistical power was higher. Interestingly, the growth rates of both herbivores and detritivores were augmented, suggesting the nutrient enrichment response is not restricted to algae and their grazers. Periphyton in BKME and NP treatments often had higher AFDM than the control, a response that suggests an increased production of detritus and microbial biomass available to secondary consumers such as insect detritivores. Although experiments conducted in the Thompson River, B.C., indicate that BKME exposure can stimulate mayfly growth beyond the increase attributable to nutrient-induced availability of food (Lowell et al. 1995), this was not apparent in our autumn experiments because insect growth in the NP and BKME treatments was similar. Results from the experimental streams agreed with the results of our unpublished in-river sampling which found that capnid stoneflies and the mayflies, *Ameletus* and *Ephemerella*, were longer and heavier downstream of the effluent discharge. In the spring, fewer taxa exhibited increased growth, nevertheless, the mayfly *Baetis* grew faster when exposed to BKME even though no differences in periphyton were detected during this season. Because the NP treatment was confounded in the spring, it cannot be determined whether the spring results represent growth stimulation similar to that observed by Lowell et al. (1995).

If the BKME treatments had deleterious effects on the benthic biota, these would be expected to be manifested through reductions in insect growth and biodiversity, and through distinct changes in community composition where pollution tolerant taxa become dominant. Exposure to pulp mill effluent has the potential to reduce insect growth because effluents contain low concentrations of compounds known to act as antifeedants or growth inhibitors (Rosenthal and Jansen 1979). However, our experiments do not support the growth inhibition hypothesis because insect growth in the control streams was always lower than the NP or BKME treatments, and growth was never lower in the effluent streams than in NP streams. In addition, the fact that growth was similarly increased in the NP and BKME treatments indicates that nutrient enrichment effects where not masked by deleterious contaminant effects. BKME exposure also produced no measurable effect of contaminants at the community level. Biodiversity, as measured by family richness, was similar among all treatments and seasons. Furthermore, shifts in community composition in the NP and BKME treatments were similar and largely reflect the abundance of taxa responding to increased periphytic resources. McCubbin and Folke (1993) indicate that effluents to the Athabasca River have low toxicity but may have the potential to produce chronic, sublethal effects. Our insect studies provide evidence that loadings to the upper Athabasca River in autumn (1993 and 1994) and spring (1994) produced no measurable sublethal toxicity effects at the population or community level. We caution that our experiments did not examine the bioaccumulation of lipophilic contaminants that may not affect insects, but could be concentrated by higher consumers including fish and birds. Furthermore, metabolic changes caused by the effluent could negatively affect successful reproduction through decreased fecundity or less viable eggs (Lowell et al. 1995). Additional research is required to answer these questions.

Other NRBS research indicates that phosphorus availability upstream of Hinton limits periphyton abundance (Scrimgeour et al. 1995) and that phosphorus requirements for maximum periphytic growth are exceeded downstream of Hinton (Dale and Chambers 1996). Previous studies also showed that periphyton abundance and insect density increase below this BKME outfall (Anderson 1989, 1991). Our evidence extends this information by demonstrating that nutrients in the effluent have a bottom up (enrichment) effect on the river food web. Indeed, this is the first study to provide unequivocal evidence of this ecological mechanism. This achievement was possible only because we employed controlled experiments in artificial streams to demonstrate that benthic communities responded similarly to the enrichment effects of the nutrient and effluent treatments.

6.0 ASSESSMENT AND CONCLUSIONS

The dominant effect of BKME discharge to the upper Athabasca River at Hinton, Alberta, was that of nutrient enrichment and stimulation of food web productivity. Despite the common perception that pulp mill effluents may induce sublethal toxicity in the benthic biota, our studies do not provide evidence that this is occurring in the Athabasca River. For example, BKME concentrations equivalent to present loadings to the upper Athabasca River in autumn (1993 and 1994) and spring (1994) produced no measurable sublethal toxicity effects as measured by insect growth, familial biodiversity or community structure. Indeed, the effects of BKME and NP treatments were similar. We do provide the caveat that our experiments did not examine bioaccumulation of lipophilic contaminants that could act as stressors in the ecosystem if concentrated by higher consumers. Other components of the NRBS have assessed bioaccumulation of contaminants in insects and fish.

Pulp mill effluents are well known to contain high levels of nutrients that can induce increased levels of primary production when the receiving waters are oligotrophic (McLeay 1987, Bothwell 1992, Lowell et al. 1995), and in-river sampling programs at Hinton have suggested that effluent discharges increased periphyton biomass and insect abundance downstream (Anderson 1989, 1991). In the upper Athabasca River, where NRBS studies established that phosphorus limits primary production (Scrimgeour and Chambers 1996), BKME-associated increases in SRP were responsible for the enrichment effects which increased periphytic algal biomass and, thus, provided increased food availability to consumer trophic levels. Although the enrichment effect was less marked in spring, this result was due to reduced SRP levels in the BKME rather than a seasonal shift in the functional relationship between nutrient addition and primary producer response. In fact, our seasonal comparison supports the hypothesis that periphyton biomass in the downstream community can be reduced by maintaining the fully-mixed BKME concentration of SRP below 2-3 µg/L. Elevated amounts of periphyton in BKME-exposed treatments had direct affects on the abundance and growth of aquatic insects in the upper trophic levels. Because insects are an important food source for many northern river fish, the nutrient-induced stimulation of secondary producers in the benthos is probably transferred upward through the food web (Gibbons et al. 1995). Under the present open-water nutrient regime in the upper Athabasca River, effluent loading provides levels of nutrient enrichment that increase benthic riverine productivity and not biotic changes normally associated with severe eutrophication. Future studies should attempt to establish whether these conclusions also apply to the winter period of ice cover.

The development of a unique, field-based artificial stream system for NRBS provided the means by which a mechanistic understanding of the effects of BKME-related nutrient and contaminant stressors on the benthic biota of the upper Athabasca River could be obtained. Because artificial streams provided the ability to conduct a properly replicated experimental design with a high degree of environmental realism, we could quantitatively address questions that could not be examined using field observations alone. Our approach also differs from other nutrient-periphyton studies within the NRBS (Scrimgeour et al. 1995, Dale and Chambers 1996) because it allowed the separation of nutrient and contaminant effects. Causality could be assigned definitively in a field application where inferential hypothesis testing was very limited, such that, for the first time, the nutrient enrichment and contaminant effects of BKME on the riverine biota could be unequivocally determined. Essentially, the use of artificial streams allowed the ecological effects of BKME on riverine benthos to be investigated at a more manageable scale than that of whole ecosystem manipulation (Kimball and Levin 1985). By combining artificial stream results with field observation, we were also able to link this mechanistic understanding of stressor effects directly to *in situ* situations in the upper Athabasca River ecosystem. Future applications of artificial streams to northern rivers could include the linkage of artificial stream experiments with water quality models in order to contribute directly to the development, parameterization, and testing of models for predicting ecosystem-level responses to nutrient and contaminant addition. They would also be valuable tools for assessing the potential for additional mills along the river to raise overall contaminant and nutrient concentrations to levels that could degrade the ecosystem. Artificial stream research is also a promising technique for consideration in aquatic environmental effects monitoring (EEM) programs for the pulp and paper industry because cause and effect scenarios can be investigated, and ecological indicators for riverine biota developed under experimentally controlled dose-response regimes.

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8.0 APPENDIX: NRBS TERMS OF REFERENCE

NORTHERN RIVER BASINS STUDY

ASSIGNMENT NO. 11 - TERMS OF REFERENCE

Project 2615-D1: Impacts of Nutrients and Contaminants from Pulp Mill Effluents on Benthic Invertebrate Communities

I. Background and Objectives

Sustainable development of the Peace and Athabasca rivers may be affected by environmental stresses from pulp mill sewage effluents. For example, increases in nutrients and contaminants below effluent inputs may cause deterioration in water quality and, subsequently, negatively affect the composition and abundances of benthic invertebrates, a major source of food for riverine fish. Presently, we cannot predict the effect on ecosystem health of further loadings to the system because the effluents contain: (1) nutrients which may stimulate the algal and microbial food supplies of invertebrates; and (2) contaminant stressors which may reduce invertebrate growth and production. The importance of nutrient and contaminant interaction in setting the production of key trophic linkages in riverine receiving waters can only be revealed through research designs that are experimentally-based.

The achievement of sustainable development in these rivers requires answers to the following questions: (1) What is the effect of contaminant stressors on the production of benthic invertebrate communities?; (2) What is the role of the benthic biofilm (i.e., algae and microbes) in stimulating invertebrate production?; (3) Is the inhibitory effect of contaminant stressors on invertebrate communities lessened by increases in biofilm biomass?; and (4) Do pulp mill effluents act as a stressor to cause changes in the riverine food chain?

In August and September 1993 partial flow-through mesocosms were established and tested at Hinton. In September and October 1993, an experiment was conducted using the mesocosms to measure the interactive effects of effluent stressors and nutrient enrichment on benthic invertebrate communities (NRBS project #2615-C1). The three treatments consisted of (i) a control receiving raw river water, (ii) a 1% dilution of treated pulp mill effluent, and (iii) a N + P addition equal to the nutrients contained in the 1% effluent dilution. Preliminary results indicate that, during autumn, the combined sewage and pulp mill effluent loading primarily acts on riverine benthos to increase primary and secondary production. This increased production appears to be caused by the P and N loading from effluent.

The purpose of this project is to conduct similar experiments in the artificial streams at the Hinton site in Spring and Fall 1994. The spring experiments will be carried out to examine the importance of seasonal changes in water temperature in moderating the nutrient and contaminant effects on food web interactions. The fall experiments will be used to document inter-annual variability in the relationships between effluent additions and increased primary and secondary productivity.

This research will provide information relating to Question 5. "Are the substances added to the rivers by man-made discharges likely to cause deterioration of the water quality" . and Question 1A. "How has the aquatic ecosystem. including fish and/or other aquatic organisms. been affected by exposure to organochlorines or other toxic compounds?"

II. Requirements

Repeat the mesocosm experiments carried out in September and October 1993 to measure the effects of effluent stressors and nutrient enrichment on benthic invertebrate communities. The experiments are to be repeated in both the spring and fall of 1994. Treatments are to consist of (i) a control receiving raw river water from upstream of the Hinton Combined Effluent (combined treated effluent from the Weldwood of Canada Ltd. bleached craft pulp mill and sewage from the town of Hinton). (ii) a 1% dilution of treated Hinton Combined Effluent and (iii) a nitrogen + phosphorus addition equivalent to the nutrients contained in the 1% effluent dilution. in mesocosm experiments where contaminant and nutrient concentrations will be manipulated. The response variables to be measured will include algal biomass and the density. growth rate and biomass of river invertebrates. Invertebrate identification and measurement of biomass will be completed in the laboratory during the winter on 1994-95.

III. Reporting Requirements

1. Prepare a comprehensive report outlining the results of the 1994 artificial stream experiments and discussing the results in relation to the effects of effluent stressors and nutrient enrichment on benthic invertebrate communities. The report is also (i) to compare the results from the Fall 1993 and the Fall 1994 to document inter-annual variability in the relationships between effluent additions and primary and secondary productivity and (ii) to compare the results from Spring 1994 with those from Fall 1993 and fall 1994 to determine the importance of seasonal changes in water temperature in moderating the nutrient/contaminant and food web interactions in benthic invertebrate communities.

2. Ten copies of the draft report are to be submitted to the Component Coordinator by March 31st. 1995.

3. 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 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. The style and format of the final report is to conform with that outlined in the NRBS Style Manual. A copy of the Style Manual will be supplied to the contractor by the NRBS.

IV. Project Administration

The Scientific Authority for this project is:

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