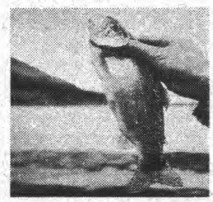
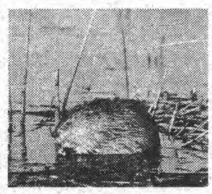
Canada Alberta Northwest Territories

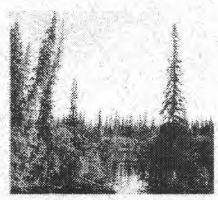
Northern River Basins Study





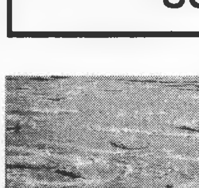





NORTHERN RIVER BASINS STUDY PROJECT REPORT NO. 98

COMBINED EFFECTS OF DISSOLVED OXYGEN LEVEL AND BLEACHED KRAFT PULP MILL EFFLUENT AND MUNICIPAL SEWAGE ON A MAYFLY (*Baetis tricaudata*): ASSESSMENTS USING ARTIFICIAL STREAMS






DATE DUE

Prepared for the
Northern River Basins Study
under Project 2618-D1

by

Richard B. Lowell and Joseph M. Culp
National Hydrology Research Institute
Environment Canada

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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;

this publication be subjected to proper and responsible review and be considered for release to the public.

14 Feb 96

(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",

IT IS HERE ADVISED BY THE SCIENCE ADVISORY COMMITTEE THAT;

this publication has been reviewed for scientific content and that the scientific practices represented in the report are acceptable given the specific purposes of the project and subject to the field conditions encountered.

SUPPLEMENTAL COMMENTARY HAS BEEN ADDED TO THIS PUBLICATION: [ ] Yes [ ] No

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,

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this publication be released to the public, and that this publication be designated for: **[ ] STANDARD AVAILABILITY [ ] EXPANDED AVAILABILITY**

23 Feb /96

(Lucille Partington, Co-chair) (Date)

23 Feb /96.

(Robert McLeod, Co-chair) (Date)

COMBINED EFFECTS OF DISSOLVED OXYGEN LEVEL AND BLEACHED KRAFT PULP MILL EFFLUENT AND MUNICIPAL SEWAGE ON A MAYFLY (Baetis tricaudata): ASSESSMENTS USING ARTIFICIAL STREAMS

STUDY PERSPECTIVE

An important initiative of the Northern River Basins Study is to assess the effects of industrial effluents on the health and integrity of the aquatic ecosystem. Effluents from pulp mills, for example, contain a wide array of compounds, and their effects on river biota can be stimulatory (in the case of nutrients) or inhibitory (in the case of some natural tree compounds and man-made chemicals). Nutrients contained in pulp mill effluents have been shown to result in significant changes in the primary productivity of rivers, but it is also possible for these enhancement effects to mask the toxic effects of contaminants on riverine biota. Another environmental variable most likely to modify the effects of pulp mill effluent is the concentration of dissolved oxygen (DO) in the river, particularly during periods of ice cover when levels are naturally lower. Because of the complex interaction between effluent compounds and the receiving environment, difficulties arise when biomonitoring studies attempt to predict the impacts of effluents on the aquatic food chain under natural conditions.

> ***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?*
>
> *7) What concentrations of dissolved oxygen are required seasonally to protect the various life stages of fish, and what factors control dissolved oxygen in the rivers?*
>
> *13b) What are the cumulative effects of man-made discharges on the water and aquatic environment?*

The objective of this project was to measure the response of a common northern river invertebrate (mayfly) to the combined effects of pulp mill effluent and low winter DO levels. Experiments were conducted under laboratory conditions using artificial streams and the combined bleached kraft pulp mill and municipal sewage effluent from Hinton. Mayflies were exposed to two different concentrations of effluent (control river water and 1% effluent) and two levels of DO (5 mg/L and 11 mg/L). The measured responses of mayflies included feeding rate, survival, and positioning of individuals within the streams.

The low DO treatment in both control water and 1% effluent had notable negative effects on the mayflies, including reduced feeding rate and survival. DO level also had a significant effect on mayfly positioning in the streams, with a greater proportion of mayflies moving into regions of higher current velocity when exposed to low levels of DO. In comparison to control water, mayfly survival over the two week course of the experiment increased with the addition of 1% effluent. This increased survival may be due in part to stimulation of increased mayfly feeding rates by the effluent.

The results of this project suggest that DO level in the Athabasca River downstream of Hinton has a greater influence on mayfly behaviour and survival than contaminants in 1% effluent, in the short term. Future laboratory experiments using different invertebrate species and higher effluent concentrations may help provide a more complete picture of effluent effects. Ultimately, these results will contribute information needed to determine DO guidelines for the protection of fish and other organisms in the Athabasca River.

REPORT SUMMARY

Effluents produced by pulp mills and sewage plants on northern Alberta rivers have the potential for a variety of effects on downstream aquatic biota via increased levels of toxicants and nutrients, and decreased levels of dissolved oxygen (DO). Low DO levels are particularly evident during the winter when ice cover restricts reaeration of oxygen-depleted waters. To determine how these impacts may interact, we experimentally measured the combined effects of low winter DO levels and pulp mill/municipal sewage effluent on a common northern river invertebrate, the mayfly *Baetis tricaudatus* Dodds. This report briefly summarizes research funded by the National Hydrology Research Institute and is being contributed as a companion study to the Nutrients/Dissolved Oxygen component of the Northern Rivers Basin Study.

The experiments were run in artificial streams using pulp mill/sewage effluent from the Weldwood bleached kraft pulp mill on the Athabasca River in Hinton, Alberta. Using a 2x2 factorial design, mayflies were exposed to one of two levels of DO (low - 5 mg/L vs. high - 11 mg/L) at each of two effluent concentrations (control river water vs. 1% effluent). The low DO and 1% effluent treatments were typical of what benthic invertebrates are likely to experience in some benthic microhabitats downstream of Hinton during the winter.

The low DO treatment had several negative effects on the mayflies. Feeding rate was greatly reduced and, after two weeks exposure, survival was also significantly reduced. Furthermore, mayflies in the low DO treatment moved up into regions of greater current velocity close to the surface of the artificial streams. Previous studies suggest that this behavior would make them more susceptible to fish predation. Data in the literature also suggest that some benthic invertebrates in northern Alberta rivers may be exposed to even lower DO levels. On the other hand, the negative effects of low DO levels in the field may be lessened for invertebrates that experience longer acclimation periods and lower temperatures than used in these experiments.

In contrast to the negative low DO effects, the 1% effluent treatment increased mayfly survival, at least over the two week course of the experiment. This may have been partially due to stimulation of increased mayfly feeding rates by the effluent. Further study is needed to determine whether this short-term effect involves a trade-off against other longer-term fitness measures.

Given the potential importance of the negative impacts of low DO levels downstream of effluent outfalls on benthic invertebrate production, we recommend additional research on these effects. Assessments of future impacts would benefit from measurements of the effects of longer acclimation periods at low temperatures and DO levels. *In situ* measurements of DO levels within the streambed where the invertebrates reside, especially during the winter, would also be helpful. In addition, studies of the combined effects of effluent and low DO levels on invertebrate-fish interactions would further facilitate predictions of future impacts of pulp mill and sewage effluents on northern rivers covered by winter ice.

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1.0 OBJECTIVES

Effluents from pulp mills and municipal sewage plants are the major point sources of toxicant and nutrient loading in the Peace and Athabasca Rivers. Toxicants in the effluents have the potential for negative impacts on benthic invertebrates in these rivers (Servos et al., 1996). In contrast, the nutrients found in the effluents appear to stimulate increased algal and microbial growth which, in turn, may affect the abundance and size distribution of benthic invertebrate herbivores (Chambers, 1996; Culp and Podemski, 1996).

Benthic invertebrates may be further impacted when effluents contribute to lowered levels of dissolved oxygen (DO) in the river. In the Athabasca River, DO levels vary substantially through the year, with the lowest levels being observed during the period of winter ice cover when surface ice restricts reaeration of oxygen depleted waters (Chambers and Mill, 1996). Very little data are available to assess the combined effects of pulp mill effluent and low levels of DO on lotic organisms. Previous work with bleached kraft pulp mill effluent in the United States 15-25 years ago showed that contaminant toxicity to fish increased with decreasing DO levels (Hicks and DeWitt, 1971; Graves et al., 1981). Additional information is required, however, to determine the overall effect on benthic invertebrates of the pulp mill effluent presently being released in northern rivers. This is particularly true during the critical winter period when DO levels are at their lowest.

Benthic invertebrates are a major source of food for northern river fish. Therefore, regulating pulp mill and sewage effluent to manage the fish resource and protect the aquatic ecosystem requires answers to the following questions: 1) What are the effects of pulp mill effluent on these invertebrates? 2) Do the negative toxicant or the growth-related, nutrient enhancement effects predominate? 3) Furthermore, what is the added effect of the low levels of DO experienced by northern river invertebrates during the winter? Answers to these questions are needed to manage development in the northern river basins for sustainable use while preserving the aquatic organisms found there.

These questions were addressed in an experimental study of the combined impacts of low winter DO levels and pulp mill/sewage effluent on a common northern river invertebrate, the mayfly *Baetis tricaudatus* Dodds. This report briefly summarizes research funded by the National Hydrology Research Institute and is being contributed as a companion study to the Nutrients/Dissolved Oxygen component of the Northern Rivers Basin Study.

2.0 MATERIALS AND METHODS

2.1 EXPERIMENTAL DESIGN

Exposure of *B. tricaudatus* to effluent and DO was controlled by conducting experiments in artificial streams arranged in a 2x2 factorial design. The mayflies were exposed to one of two levels of DO (low - 5 mg/L vs. high - 11 mg/L) at each of two effluent concentrations (control river water vs. 1% effluent, v/v). The low DO level was comparable to low levels experienced in the field (see Discussion) and the high level was near saturation. The experiments were run in the laboratory at the National Hydrology Research Institute using water from the South Saskatchewan River collected upstream of the City of Saskatoon. Water from this location has a similar chemistry (e.g., conductivity, alkalinity, pH, nitrate/nitrite, ammonium, soluble reactive phosphorus) to the Athabasca River upstream of Hinton, Alberta (Chambers and Prepas, 1994; Culp and Podemski, 1996; Lowell et al., 1995a). Full-strength treated effluent was collected from the Weldwood bleached kraft pulp mill on the Athabasca River in Hinton and stored frozen at -40° C until dilution to 1% at the beginning of the experiments. Pulp mill effluents are commonly stored deep-frozen before use in toxicity tests (Ahtiainen et al., 1996; Eklund et al., 1996; Priha, 1996; Verta et al., 1996) and, although the full effects of freezing are not completely understood, these experimental results were comparable to previous experiments using fresh effluent (Lowell et al., 1995b). The Weldwood effluent also contains Hinton municipal sewage (7.5% by volume; Culp and Podemski, 1996). The 1% effluent concentration used in these experiments is typical of full-mix levels in the Athabasca River near the onset and end of the period of winter ice cover (Culp and Podemski, 1996).

The streams were circular Plexiglas chambers (diameter = 8.8 cm; Lowell et al., 1995a). Current was provided by small water jets driven by a vacuum chamber/pump system drawing water from control river water or 1% effluent water holding reservoirs. The water jets and pumps were adjusted so that current velocity within 1.5 cm of the bottom substratum was approximately 6 cm/s so as to mimic velocities experienced by these mayflies in the field (Culp et al., 1983; Lowell et al., 1995a). Water returned to the holding reservoirs via a central standpipe drain in each stream, thus comprising a recirculating system. The water supplies in the holding reservoirs were renewed after the first week; freshly-thawed effluent was premixed into the renewed 1% effluent water supply.

DO level in the water supplied to the low DO (5 mg/L) streams was controlled by passing the water through a partial vacuum chamber (Mount, 1961, 1964). For each holding reservoir, water was circulated from the reservoir into an adjacent vacuum chamber utilizing the pressure differential between the reservoir and vacuum chamber. The water entered the partial vacuum as a spray; the water droplets provided a large surface to volume ratio within the vacuum which facilitated rapid removal of DO from the water. DO level was controlled by varying the degree of vacuum. The deoxygenated water was then pumped through the low DO streams at a flow-through rate (approx. 1 L/min) sufficient to ensure a uniform distribution of DO throughout each stream as measured with a small DO probe. Water outflow from all streams was reaerated in the holding reservoirs using air-stone bubblers. Water flow to the high DO streams was provided by pumping water directly from the aerated holding reservoirs. Heat exchange tubing was used in each reservoir to maintain equivalent temperatures between the low and high DO streams.

Larval *B. tricaudatus* were collected 10 days before the start of the experiment from Big Hill Springs Creek, Alberta, a site where several other aspects of the biology of this species have been previously studied (Culp and Scrimgeour, 1993; Lowell et al., 1995a,b; Scrimgeour et al., 1991). Big Hill Springs Creek has a similar chemistry to the South Saskatchewan River water used during the experiments in terms of alkalinity and pH (Lowell et al., 1995a; Walde and Davies, 1984). During this 10 day acclimation period, the mayflies were held in continuously aerated 8 L containers (for both acclimation and experimental periods: temperature = 4.5° C; photoperiod = 16:8 h light:dark) and fed an *ad libitum* supply of algae (the diatom *Navicula*) cultured on 2.4x2.4x0.5 cm ceramic blocks. Identical algae-covered blocks were used to supply food to the mayflies in the streams during the experiment. At the beginning of the experiment, all mayflies in the most abundant size class (approx. 6-7 mm in length) were randomly allocated to the 5 replicate streams per treatment (6-7 mayflies per stream). Mayfly responses were averaged for each stream and the streams were the experimental units used for the statistical analyses.

2.2 RESPONSE VARIABLES

The experiment was run for 14 d, during which several mayfly response variables were measured. The key variables were positioning of animals within the streams, and mayfly feeding rate and survival (measured daily after 8 h light). Effects of the effluent and DO treatments on these response variables were analyzed using factorial analysis of variance (ANOVA). Proportional data (positioning behavior and survival) were arcsine-square root transformed before analysis to improve data normality and variance homogeneity (Sokal and Rohlf, 1981).

Positioning of mayflies within each stream was measured by scoring the number of individuals found on each of the three categories of attachment surfaces available within each stream: a) ceramic blocks, b) water jets, or c) outflow screens. The ceramic blocks were at the bottom of the streams in a region of low current velocity; velocity was lower in this region due to the drag effects imparted by the walls and floor of each stream. The water jets and outflow screens were near the upper surface of the streams in a region of high current velocity.

Feeding rate was measured by visually scoring the areal percent of diatom biofilm removed over the previous 24 h period from the two *Navicula* covered food blocks placed into each stream. As the algae was removed, the blocks were replaced at least every 3 days. In streams where feeding rate was high, blocks were replaced more frequently to ensure that food was always available in all treatments. Twenty five algal-covered food blocks were set aside during the experiment for determination of the average ash-free dry mass (AFDM) of diatoms per block. This average was multiplied by percent algal film removal measured during the experiment to determine the amount of AFDM removed per mayfly per day. The algal removal during mayfly feeding activities was due to a combination of ingestion and incidental (non-ingested) algal detachment and is proportional to feeding rate (Scrimgeour et al., 1991).

3.0 <u>RESULTS</u>

DO level had a highly significant effect on mayfly positioning in the streams, with a greater proportion of mayflies moving into regions of higher current velocity when exposed to low levels of DO (Fig. 1; Table 1, $P<0.001$). Most mayflies exposed to high DO levels remained on the ceramic blocks at the bottom of the streams. In the low DO treatments, however, a greater number of individuals moved up to the water jets and the outflow screens (measured each day as proportion occupying these high-velocity positions, averaged over 2 wk), the only positions within the streams where they could remain attached while exposed to higher current velocity. This upward distribution was confined to submersed regions of greater current velocity and we did not observe any individuals moving all the way up to the air-surface interface. Effluent concentration and the interaction between concentration and DO did not have a significant effect on mayfly positioning in the streams (Table 1, $P>0.34$).

Low levels of DO also had a highly significant negative effect on feeding rate (Fig. 2; Table 2, $P<0.001$). Averaged over the 2-week experimental period, feeding rates when exposed to low DO levels were only approximately 20% of high DO feeding rates, regardless of effluent concentration. Furthermore, although not significant at the 0.05 level, the data suggests that the 1% effluent may have stimulated an increase in feeding rate (Fig. 2; Table 2, $P=0.08$). The concentration-DO interaction effect was not significant (Table 2, $P=0.36$).

By the end of the experiment, survival was also greatly reduced for those mayflies exposed to low DO levels (Fig. 3; Table 3, $P<0.001$). Some mortality occurred in all treatments, likely due to the late developmental stage of the mayflies; mortality typically increases in the laboratory just prior to emergence. One individual emerged during the second week of the experiments (1% effluent/high DO treatment). Interestingly, survival was actually enhanced for mayflies exposed to the 1% effluent (Fig. 3; Table 3, $P=0.002$). Again, the concentration-DO interaction effect was not significant (Table 3, $P=0.87$).

Figure 1: Proportion of Mayflies Positioned in Regions of Greater Current Velocity within the Artificial Streams (Proportion Measured Each Day, Averaged over 2 Weeks, ±1 SE) at Two Concentrations (Control, 1% Effluent) and Two Dissolved Oxygen (DO) Levels. N = 5 replicate streams per treatment.

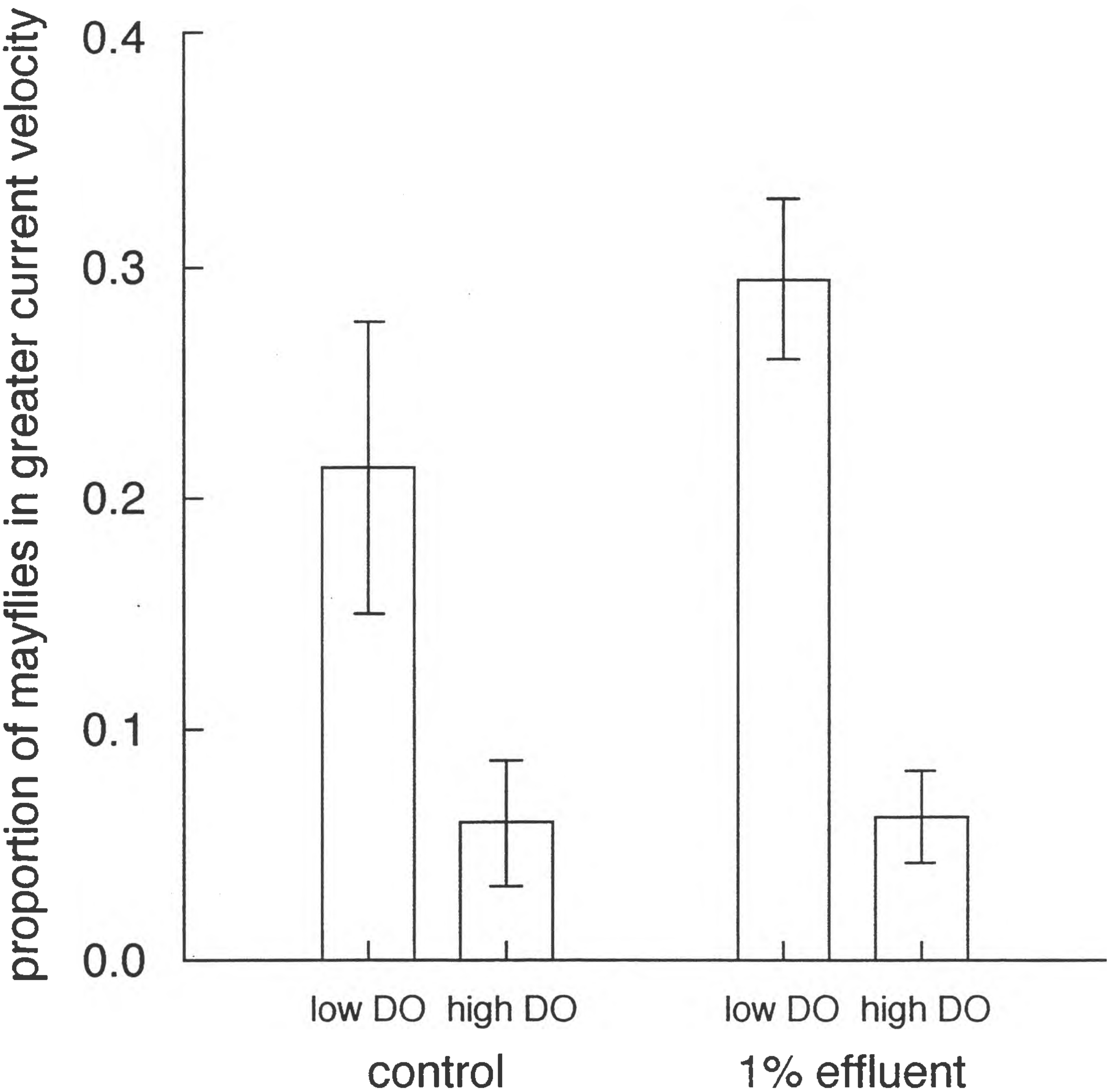

Table 1: Mayfly Position (within the Artificial Streams) Analysis of Variance for Two Concentrations (Control vs. 1% Effluent) and Two Dissolved Oxygen Levels (Low vs. High). Values used in analysis (proportion of mayflies within regions of greater flow each day averaged over 2 weeks) were arcsine-square root transformed.

Source of Variation	SS	df	MS	F	P
Concentration	0.0198	1	0.0198	0.954	0.343
Dissolved Oxygen	0.4518	1	0.4518	21.755	<0.001
Concentration x Dissolved Oxygen	0.0112	1	0.0112	0.540	0.473
Error	0.3323	16	0.0208		

Figure 2: Rate of Food Removal by Mayflies (Daily Rate Averaged over 2 Weeks, ±1 SE) at Two Concentrations (Control, 1% Effluent) and Two Dissolved Oxygen (DO) Levels. AFDM - ash-free dry mass. N = 5 replicate streams per treatment.

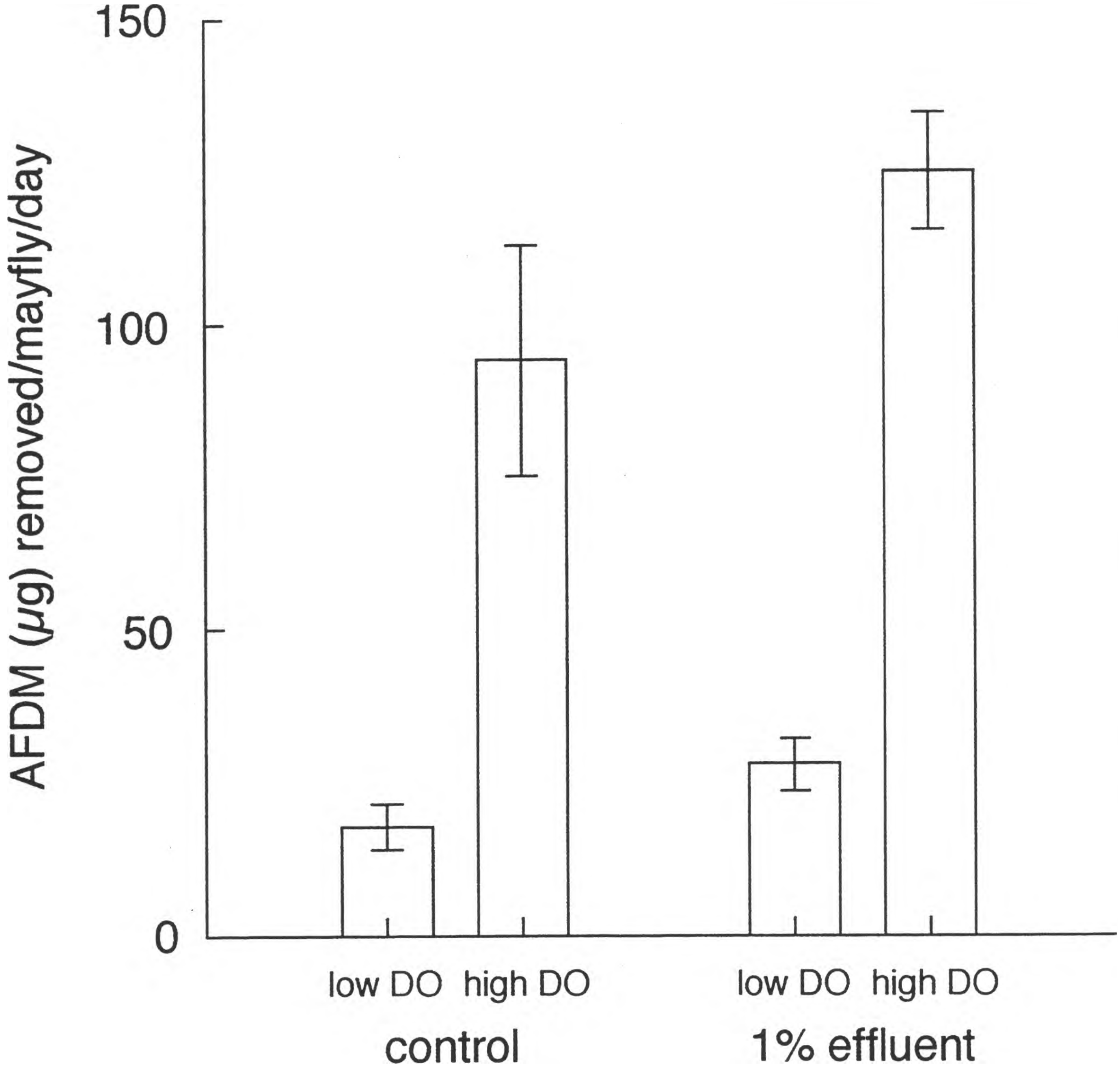

Table 2: Mayfly Food Removal Analysis of Variance for Two Concentrations (Control vs. 1% Effluent) and Two Dissolved Oxygen Levels (Low vs. High). Values used in analysis were calculated from amount of food removed each day averaged over 2 weeks.

Source of Variation	**SS**	**df**	**MS**	**F**	**P**
Concentration	2092.1	1	2092.1	3.498	0.080
Dissolved Oxygen	37565.5	1	37565.5	62.811	<0.001
Concentration x Dissolved Oxygen	537.3	1	537.3	0.898	0.357
Error	9569.2	16	598.1		

Figure 3: Survival of Mayflies over 14 Day Experimental Period (±1 SE) at Two Concentrations (Control, 1% Effluent) and Two Dissolved Oxygen (DO) Levels. Points staggered to separate error bars. N = 5 replicate streams per treatment.

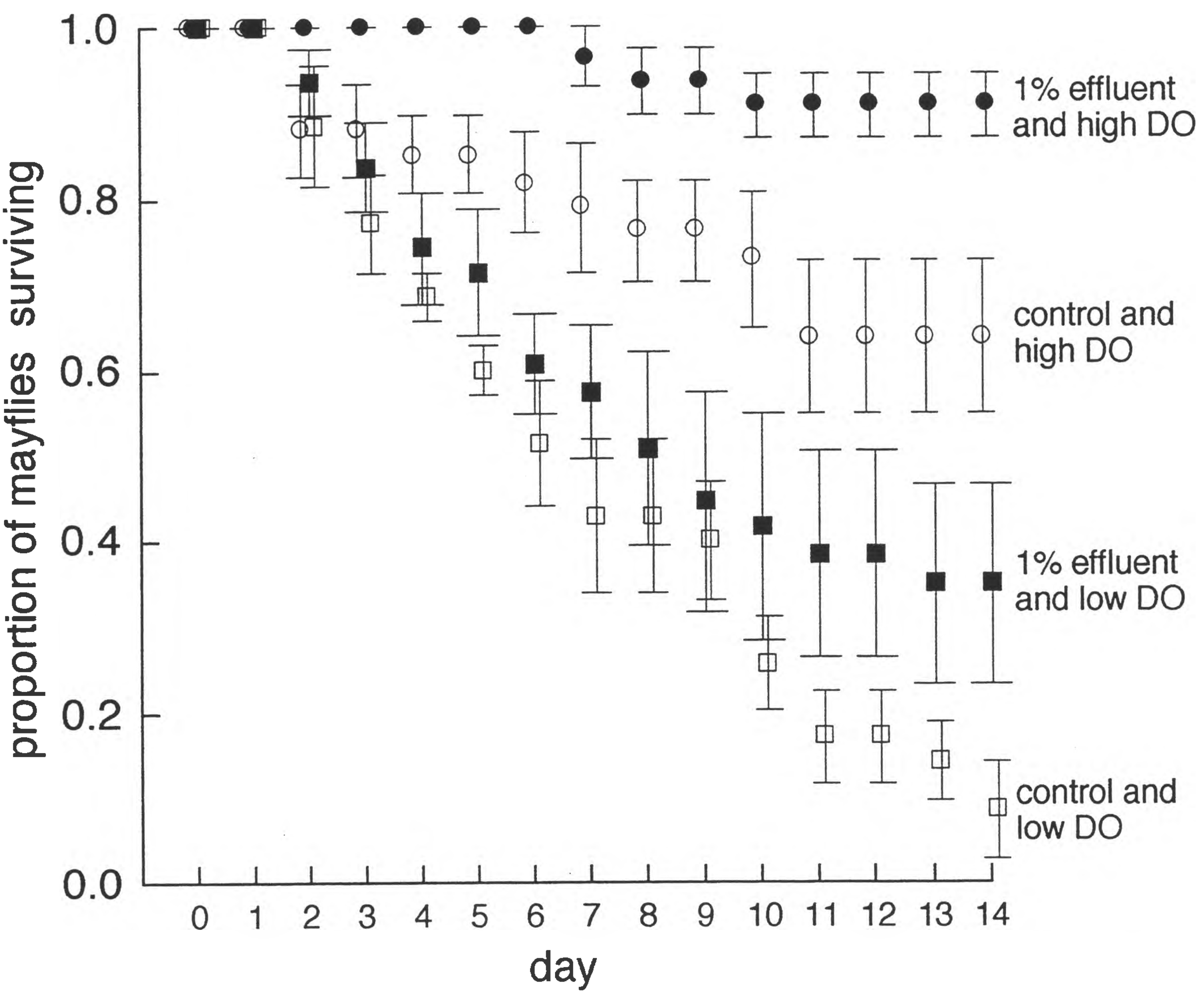

Table 3: Mayfly Survival Analysis of Variance at Two Concentrations (Control vs. 1% Effluent) and Two Dissolved Oxygen Levels (Low vs. High). Values used in analysis (proportion surviving in each stream on day 14) were arcsine-square root transformed.

Source of Variation	SS	df	MS	F	P
Concentration	0.8673	1	0.8673	14.182	0.002
Dissolved Oxygen	2.6271	1	2.6271	42.956	<0.001
Concentration x Dissolved Oxygen	0.0018	1	0.0018	0.029	0.868
Error	0.9785	16	0.0612		

4.0 DISCUSSION AND CONCLUSIONS

The low DO levels used in this experiment clearly stressed the mayflies in several ways leading to altered microhabitat positioning behavior, reduced feeding rate, and decreased survival. Based on previous field measurements, the lower DO level (5 mg/L) was most likely typical of the levels currently experienced by *B. tricaudatus* in the Athabasca River during winter under-ice conditions. Reports by Alberta Environmental Protection during the last seven years have typically cited minimum water column DO levels of 7-8 mg/L, with a low of 6 mg/L in 1989 (Noton and Shaw, 1989; Noton and Allan, 1994). Diurnal cycling of DO levels due to photosynthesis have usually lead to daily fluctuations of _0.5 mg/L, although fluctuations as great as 2 mg/L have been observed (Noton and Allan, 1994). Oxygen-depleted ground water or tributary flow into the Athabasca River may further locally depress water column DO levels to below 3 mg/L (Shelast and Brayford, 1995). Work with trout spawning substrata in other rivers has shown that DO levels in the substratum may be 3 mg/L lower than those measured in the overlying water column (Chapman, 1986). Because mayflies and other benthic invertebrates typically move at least part way down into the substratum, they may also experience these lowered DO levels. Thus, some individuals in the Athabasca River may be exposed to DO levels significantly lower than 5 mg/L.

Chronic stress due to insufficient oxygen delivery to the gills and reduced food intake probably explains the increased mortality caused by the low levels of DO during the experiment. Survivorship in the field may be even lower due to the indirect negative effects of the altered positioning behavior caused by low DO levels. The mayflies used in our experiment moved to more elevated positions in the artificial streams following exposure to low DO levels, probably to facilitate respiration by increasing water flow past the gills. Previous studies suggest that this response would lead to increased mortality due to predation. Benthic invertebrates such as *B. tricaudatus* typically seek refuge from visual predation by fish by moving under stones, particularly during daylight hours (Culp and Scrimgeour, 1993), the diel period when our data was collected (Fig. 1). Other studies have demonstrated increased predation upon benthic invertebrates when they are driven into more exposed locations by low DO levels (Rahel and Kolar, 1990; Pihl et al., 1992; see also Wiley and Kohler, 1980).

Consequently, in the field, some of the negative effects of low DO levels may be greater than observed in our experiments, due to the potential for increased predation in addition to the more direct negative effects of exposure to DO levels even lower than used in our experiments. On the other hand, field differences in other environmental conditions may lessen the negative effects that we measured. A longer acclimation period to low DO levels in the field may allow benthic invertebrates to compensate partially for a lowered oxygen supply via physiological changes. Furthermore, field temperatures even lower than we were able to incorporate into the experimental apparatus may reduce metabolism (and, thus, oxygen demand) sufficiently to further lessen the negative impacts of low DO levels. In addition, the potential negative effects of low DO levels on predators themselves should be considered when evaluating effects on predator-prey interactions.

In contrast to the negative low DO effects, the 1% effluent treatment increased survival, at least over the 2 wk course of the experiment. The effluent-associated increase in survival at high DO levels (relative to the control concentration) was partly a consequence of unusually low survival (33%) in one replicate stream in the control concentration-high DO treatment. But the effluent-enhanced increase in survival at low DO levels may have been due to the possible stimulation of an increase in feeding rate by the effluent; this may have partly offset the even greater reduction in feeding rate caused by the low DO levels. In an earlier study, we found that pulp mill effluent can stimulate increased short-term growth and development of *B. tricaudatus* (Lowell et al., 1995b). One of the mechanisms that we proposed for the growth stimulation was an effluent-induced increase in feeding rate. We also suggested other possible mechanisms for this effect, including a) increased nutritive value of the periphyton and b) hormonal or other growth-stimulating effects of the compounds in pulp mill effluent. Further work is required, however, to determine whether these growth-enhancing short-term effects of pulp mill effluent involve trade-offs against other longer-term fitness measures, such as fecundity.

5.0 RECOMMENDATIONS

Based on these results and data available in the literature, the negative impacts on *B. tricaudatus* (and possibly other benthic invertebrates) of pulp mill/sewage effluent, such as currently produced at Hinton, would more likely be due to reductions in DO levels than due to toxicant effects, at least over the short-term. DO levels in the Athabasca River during the winter may already be low enough to have deleterious effects on some benthic invertebrates and, where possible, further reductions should be avoided.

Assessments of potential present and future impacts would benefit from further study including both field measurements of DO levels and more extensive laboratory experiments. *In situ* measurements of DO levels in the substratum, especially during the winter, would be particularly useful. Follow-up experiments in the laboratory would help provide a more complete picture of effluent effects. For example, impacts may be modified by differences in developmental stage of the larvae and length of exposure, including acclimation time to low DO levels. Different species, as well as individuals collected from different riverine environments, are also likely to vary in their tolerance to toxicants and low DO levels. Higher contaminant concentrations experienced closer to the effluent outfall, and during periods when the effluent to river discharge ratio is particularly high, may also modify impacts. In addition, effluent properties can change temporally both within and among mills. Finally, studies of the combined effects of effluent and low DO levels on fish-invertebrate/predator-prey interactions would further facilitate predictions of future impacts of pulp mill and sewage effluents on northern rivers commonly covered by winter ice.

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NORTHERN RIVER BASINS STUDY

SCHEDULE A - TERMS OF REFERENCE

Project 2618-D1: Impacts of Low Winter Dissolved Oxygen on Sensitivity of Benthic Invertebrates to Pulp Mill Effluent

I. BACKGROUND & OBJECTIVES

Effluents from pulp mills are the major point sources of contaminant and nutrient loading on the Peace and Athabasca rivers. Pulp mill effluents contain a wide variety of compounds which can have differing effects on aquatic organisms and communities in receiving waters (McLeay 1987). For example, increased concentrations of nutrients and contaminants from effluent discharges may cause deterioration in water quality, subsequently affecting the community composition and abundance of benthic invertebrates, a major source of food for riverine fish. It remains difficult to predict the effect on ecosystem health from further loadings to the system because the effluents contain: (1) nutrients which may stimulate the growth of algal and microbial food supplies of invertebrates, and (2) contaminant stressors which may reduce invertebrate growth and production. Furthermore, one of the environmental variables most likely to modify the effects of pulp mill effluent is the level of dissolved oxygen in the river. In the Athabasca River, dissolved oxygen varies substantially through the year, with the lowest levels being observed under ice conditions in the winter. Previous studies suggest that contaminant toxicity increases with decreasing dissolved oxygen concentration, but further study is required to determine the overall effect of pulp mill effluent during the critical winter period when dissolved oxygen levels are at their lowest.

Benthic invertebrates are a major source of food for many species of fish inhabiting northern rivers in Alberta. Therefore, regulating pulp mill effluent to protect aquatic resources requires answers to the following questions: (1) What are the effects of pulp mill effluents on invertebrate populations? (2) Do the contaminant (negative) or the nutrient enhancement (positive) effects predominate? (3) How are these effects modified by the periodic extremes in dissolved oxygen levels experienced by both invertebrates and fish in these rivers, particularly during the low levels in winter?

The purpose of this project is to measure the response of a common northern river invertebrate (mayfly) to the combined impacts of pulp mill effluent and low winter dissolved oxygen levels. These experiments will be conducted during the winter using artificial streams supplied with effluent collected from the Weldwood Canada Ltd. pulp mill (Hinton) on the Athabasca River. This project will help determine (1) the concentrations of dissolved oxygen that are required seasonally to protect the various life stages of aquatic invertebrates in the Athabasca River. This research will link the artificial stream studies on the impacts of pulp mill effluent on benthic invertebrates (2615-D1) with the question of dissolved oxygen requirements for benthic organisms. Ultimately, the results of this study will contribute information needed to determine dissolved oxygen guidelines for the protection of fish and other organisms, one of the final products proposed by the Nutrients Component of the NRBS.

II. GENERAL REQUIREMENTS

The experiments will be conducted under laboratory conditions using artificial streams located at the National Hydrology Research Institute, Saskatoon. Artificial streams will be arrayed in a 2x2 factorial design to measure the interacting effects of pulp mill effluent and dissolved oxygen level on mayflies. The artificial streams will be of the same design as that used in previous experiments with mayflies (Lowell *et al.* 1994, 1995). The mayflies to be used will be from the genus *Baetis,* because (1) this group is very common and widely distributed in the northern rivers of Alberta, and (2) there is a good deal of background information from previous experimental studies on this genus.

The experimental treatments are to consist of two effluent concentrations; (1) control river water, and (2) a combination of river water/effluent characteristic of complete mix levels downstream of Hinton. Treatments will also consist of two dissolved oxygen levels; (1) a low concentration typical of the levels found in the Athabasca River during winter, and (2) a high concentration typical of saturation levels found during the rest of the year. There will be five replicates for each experimental treatment. The experiments are to be run for approximately two to six weeks, depending upon the length of time required for the mayflies to develop at a low experimental temperature (<5°C). Algal food will be supplied *ad libitum* to standardize the amount of food available to the mayflies in the different experimental treatments. The mayfly response variables to be measured include the following:

> **survival** - proportion of mayflies surviving at the end of the experiment; **growth** - increase in total dry weight; **development** - degree of development as measured by the relative length of the wing pads (i.e., they become proportionally longer as development progresses); **moulting** - the number of moults is another means of measuring growth, and; **behaviour**- the frequency of drifting (a behaviour that influences mayfly vulnerability to fish predators).

III. REPORTING REQUIREMENTS

1. A brief progress report is to be submitted to the Component Coordinator by **January 31, 1995**.

2. Ten copies of the Draft Report along with an electronic disk copy are to be submitted to

the Component Coordinator by **March 31, 1995**.

3. Three weeks after the receipt of review comments on the draft report, the Contractor is to provide the Component Coordinator with two unbound, camera ready copies and ten cerlox bound copies of the final report along with an electronic version.

4. The Contractor is to provide draft and final reports in the style and format outlined in the NRBS document, "A Guide for the Preparation of Reports," which will be supplied upon execution of the contract.

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

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

   a) Times Roman 12 point (Pro) or Times New Roman (WPWIN6.0) font.
   b) Margins; are 1" at top and bottom, 7/8" on left and right.
   c) Headings; in the report body are labelled with hierarchical decimal Arabic numbers.
   d) Text; is presented with full justification; that is, the text aligns on both left and right margins.
   e) Page numbers; are Arabic numerals for the body of the report, centred at the bottom of each page and bold.

   - If photographs are to be included in the report text they should be high contrast black and white.
   - All tables and figures in the report should be clearly reproducible by a black and white photocopier.
   - Along with copies of the final report, the Contractor is to supply an electronic version of the report in Word Perfect 5.1 or Word Perfect for Windows Version 6.0 format.
   - Electronic copies of tables, figures and data appendices in the report are also to be submitted to the Project Liaison Officer along with the final report. These should be submitted in a spreadsheet (Quattro Pro preferred, but also Excel or Lotus) or database (dBase IV) format. Where appropriate, data in tables, figures and appendices should be geo-referenced.

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

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

7. The presentation package of 35 mm slides is to comprise of one original and four duplicates of each slide.

IV. DELIVERABLES

1. A comprehensive project report that presents and discusses the results of the experiments, linking the laboratory responses to instream conditions and the impacts to benthic invertebrate communities and the fish species that prey on them.

2. Ten to twenty-five 35 mm slides that can be used at public meetings to summarize the project, methods and key findings.

V. CONTRACT ADMINISTRATION

This project has been proposed by the Nutrients Component of the NRBS (Nutrients Component Leader - Dr. Patricia Chambers, NHRI, Saskatoon).

The Scientific Authorities for this project are:

Dr. Richard Lowell, Dr. Joseph Culp and Dr. Patricia Chambers
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Questions of a technical nature should be directed to them.

The NRBS Component Coordinator for this project is:

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Questions of an administrative nature should be directed to him

VI. LITERATURE CITED

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