ECOLOGICAL EFFECTS OF A BLEACHED KRAFT PULP MILL EFFLUENT ON BENTHIC BIOTA OF THE ATHABASCA RIVER

A Thesis Submitted to the College of
Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the Department of Biology
University of Saskatchewan
Saskatoon

By

Cheryl Linda Podemski

Spring 1999

UNIVERSITY OF SASKATCHEWAN

College of Graduate Studies and Research

SUMMARY OF DISSERTATION

Submitted in partial fulfillment

of the requirements for the

DEGREE OF DOCTOR OF PHILOSOPHY

by

Cheryl Linda Podemski

Department of Biology
University of Saskatchewan

Spring 1999

Examining Committee:

Dr. D.H. De Boer	Dean/Associate Dean, Dean's Designate, Chair College of Graduate Studies and Research
Dr. L.C. Fowke	Chair of Advisory Committee, Department of Biology
Dr. J.M. Culp	Supervisor, National Water Research Institute, Environment Canada
Dr. F. Messier	Department of Biology
Dr. D.M. Lehmkuhl	Department of Biology
Dr. J. Romo	Department of Plant Sciences

External Examiner:

Dr. W.H. Clements
Associate Professor
Department of Fishery and Wildlife Biology
Colorado State University
Fort Collins, CO 80523

Ecological Effects of a Bleached Kraft Pulp Mill on Benthic Biota of the Athabasca River

The effects of nutrient enrichment and contaminant toxicity from a bleached kraft mill effluent (BKME) on the benthic biota of the Athabasca River were assessed using an integrated assessment approach. Components of the research included a stream mesocosm experiment, field observations, and chronic and behavioral laboratory bioassays using mayfly species from the receiving environment. Effluent and nutrient additions to stream mesocosms resulted in marked increases in periphyton biomass, greater abundance and size of some grazing insects, and altered diatom and insect community composition. In all cases, responses to 1% BKME and the nutrient addition were remarkably similar, indicating that the primary effect of this discharge was nutrient enrichment. These findings were corroborated by observations in field samples from reference and exposed river reaches.

Laboratory bioassays addressed the effect of exposure to BKME in water alone, or both water and food, on feeding behavior of the mayfly *Ameletus subnotatus*. BKME in water had no effect on feeding, but exposure through both food and water resulted in decreased consumption in BKME treatments; contrary to expectations, mayflies preferentially fed on diatoms exposed to BKME. Exposed diatom cultures contained more lipid and bacteria than control algae, and these observations, combined with results from feeding bioassays, indicated that exposure to effluent increased algal food quality.

Chronic toxicity of the effluent was assessed for *A. subnotatus* and *Baetis tricaudatus*. In both taxa, exposure to effluent in food and water increased growth,

supporting the conclusion that BKME exposure increased food quality of algae. First instar *B. tricaudatus* were sensitive to BKME, with mortality at concentrations above 1%, while mortality in older nymphs increased at 7%. Effluent-exposure had no effect on hatching success of *B. tricaudatus*. *A. subnotatus* exhibited increased mortality at 1%. Increased mortality with faster growth indicates that these insects may not expend resources on defense.

This research demonstrates a new approach for assessing the environmental effects of BKME release, and has resulted in new understanding of the impacts of this industry on the benthic community of this and perhaps other, northern rivers.

BIOGRAPHICAL

January 10, 1966	Born in Edmonton, Alberta
June, 1988	B.Sc. (Distinction), Specialization in Zoology, University of Alberta
May, 1992	M.Sc., Zoology, University of Western Ontario

PUBLICATIONS

1) Culp J.M., C.L. Podemski, and K.J. Cash 1996. Teasing apart nutrient-contaminant impacts in river ecosystems: strategies for artificial stream research; pp. 83-88 *in*: P.R.G. Kramer, D.A. Jonkers, and L. van Liere. Interactions of Nutrients and Toxicants in the Foodchain of Aquatic Ecosystems RIVM report no. 703715001, National Institute of Public Health and the Environment, P.O. Box 3720 BA Bilthoven, The Netherlands.

2) Culp J.M., C.L. Podemski, K.J. Cash, and R.B. Lowell. 1996. Utility of field-based artificial streams for assessing effluent effects on riverine ecosystems. Journal of Aquatic Ecosystem Health. 5:117-124.

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purpose may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use that may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in the thesis in whole or in part should be addressed to:

Head of the Department of Biology
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5E2

ABSTRACT

The effects of nutrient enrichment and contaminant toxicity from a bleached kraft mill effluent (BKME) on the benthic biota of the Athabasca River were assessed using an integrated assessment approach. Components of the research included a stream mesocosm experiment, field observations, and chronic and behavioral laboratory bioassays using mayfly species from the receiving environment. Effluent and nutrient additions to stream mesocosms resulted in a 7-fold increase in periphyton biomass (Chl*a*), a doubling of insect abundance, approximately 30-100% increase in individual dry weight of some grazing insect taxa, and altered diatom and insect community composition. In all cases, responses to 1% BKME and an equivalent nutrient addition were remarkably similar, indicating that the primary effect of this discharge was nutrient enrichment. These findings were corroborated by observations in field samples from reference and exposed river reaches.

Laboratory bioassays addressed the effect of exposure to BKME in water alone, or both water and food, on feeding behavior of the mayfly *Ameletus subnotatus*. BKME in water had no effect on feeding, but exposure through both food and water resulted in 26-33% reduced consumption in BKME treatments. Contrary to expectations, when given a choice, mayflies preferentially fed on diatoms exposed to 1-7% BKME. Exposed diatom cultures contained more bacteria than control algae, and total lipid content of the diatoms ranged from 11.7% in the controls to 16.9% in 7% BKME. These observations, combined with results from feeding bioassays, indicated that exposure to effluent increased algal food quality.

Chronic toxicity of the effluent was assessed for *A. subnotatus* and *Baetis tricaudatus*. In both taxa, exposure to effluent in food and water increased size (total length) by 10-30%, supporting the conclusion that BKME exposure increased food quality of algae. First instar *B. tricaudatus* were sensitive to BKME, with $\geq$50% mortality at concentrations above 1%, while mortality in older

nymphs increased by at 7% BKME. Effluent-exposure had no effect on hatching success of *B. tricaudatus*. *A. subnotatus* exhibited increased mortality at 1%. Increased mortality with faster growth may indicate that these insects do not expend metabolic resources on defense.

This research demonstrates a new approach for assessing the environmental effects of BKME release, and has resulted in new understanding of the impacts of this industry on the benthic community of this and perhaps other, northern rivers.

ACKOWLEDGEMENTS

I would like to thank my supervisor, Dr Joseph M. Culp for his advice and support, the members of my Advisory Committee, Drs Messier, Lehmkuhl, Romo and Fowke for their guidance, and Dr. W.H. Clements for serving as my External Examiner. I am grateful for financial support from the Natural Science and Engineering Research Council, the University of Saskatchewan, the Northern River Basins Study, and Environment Canada. I would like to thank Weldwood of Canada Ltd., and staff of the technical division for their cooperation. Andy Hartery and Todd Andrews were particularly helpful, and thanks to Ian and Betty for all those early morning effluent deliveries. Maurice and Janet Hotte of Hinton, Alberta made my work easier by graciously offering access to their property.

Debbie Sargeant, Suzanne Sobey, and Trinh Luong provided very able and much appreciated assistance in the laboratory and/or field. I also extend a special thanks to John Mollison for his help with stream design and every other project that he lent his considerable skills to on my behalf. The very talented group of people who helped to build the artificial stream system at Hinton include: John Mollison, Carol Casey, Jim Banner, Tom Maxin, Bob Christie, Eric Marles, Nancy Glozier, Kelly Field, and Chris Perrin - thanks guys! Other people who deserve thanks for helping in the field or lab include: Gertie Hutchinson, Patricia Burgess, Kirk Sobey, George Swerhone, Daryl Halliwell, Kevin Himbeault, Jim Neary, Alec Dale, and Tessa Fauquier. Dr. Kevin Cash was a source of much advice and helpful discussion over the years, and Drs Patricia Chambers, Gary Scrimgeour, Marlene Evans and Rick Lowell all provided much appreciated information or advice. Thanks to Kevin, Donna, Katherine, Jason, Jim, Vanessa, Mari, Alec, Kirk, and the rest of the grad students and contractors at the National Hydrology Research Centre for creating a stimulating and supportive environment to work in. Thanks also to the support staff at NHRC for all of their generous help. Finally, heartfelt thanks and appreciation to Mark Ouellette for help in the field, typing hundreds of references, formatting, making all the nice figures in this thesis, and just for being there.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AFDM	Ash free dry mass
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AOX	Adsorbable organic halogen
ASB	Aerated stabilization basin
AST	Activated sludge treatment
BOD	Biological oxygen demand
BKME	Bleached kraft mill effluent
Chl*a*	Chlorophyll *a*
COD	Chemical oxygen demand
DO	Dissolved Oxygen
DOC	Dissolved organic carbon
EC_{50}	Median effect concentration
ECF	Elemental chlorine free
EDTA	Ethylene diamine tetraacetic acid
Hp	Horse power
LC_{50}	Median lethal concentration
LOEC	Lowest-observed effect concentration
LSD	Least significant difference
$N\text{-}NH_4^+$	Nitrogen in the form of ammonium
$N\text{-}NO_2+NO_3$	Nitrogen in the form of nitrite and nitrate
NOEC	No-observed effect concentration
NP	Nutrient (N and P) treatment
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal components analysis
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
PRIN	Principal component
sp.	Species
SRP	Soluble reactive phosphorus
TDP	Total dissolved phosphorus
TCDD	Terachlorodibenzo-*p*-dioxin
TCDF	Tetrachlorodibenzofuran
TCF	Totally chlorine free
TKN	Total Kjeldahl nitrogen
TP	Total phosphorus
TSS	Total suspended solids

Chapter 1

Introduction to the Research Topic and Overview of the Thesis

1.1 Introduction

About 120 pulp mills operate across Canada (Fig. 1.1), with most of these mills releasing effluent to surface waters. This effluent is a complex and highly variable mixture of hundreds of compounds, many of which remain unidentified (Suntio et al. 1988). Broad classes of compounds found in pulping effluent include phenolics, resin and fatty acids, polycyclic aromatic

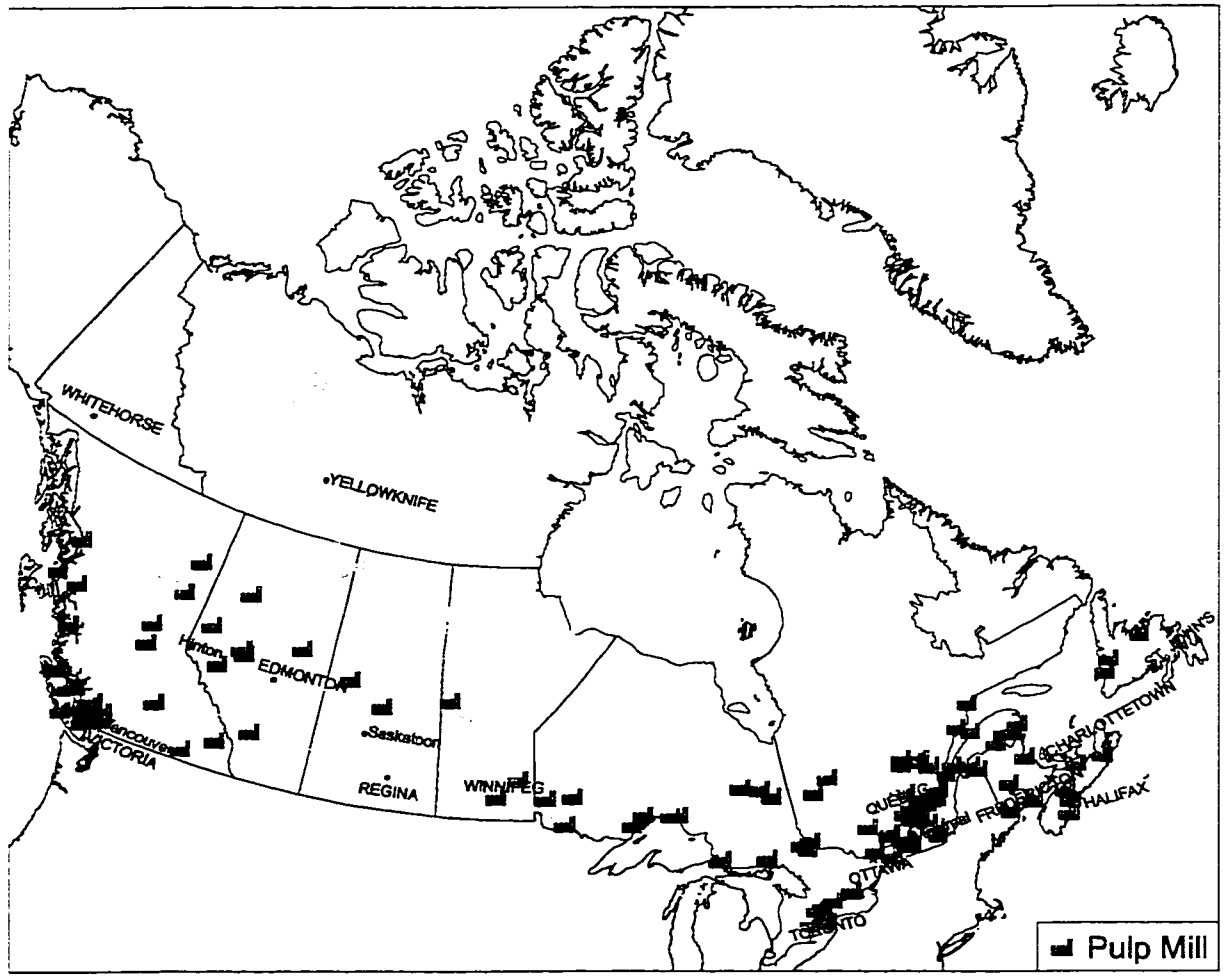


Figure 1.1. Location of pulp mills in the Dominion of Canada.

hydrocarbons (PAHs), and metals, as well as particulate and dissolved forms of carbon, phosphorus, and nitrogen (for a more detailed discussion of effluent components, see Appendices 1 and 2). Many of these compounds have demonstrated toxicity to aquatic life (e.g. Rogers 1973, Leach and Thakore 1973, 1975, 1976, Rogers et al. 1979, Hattulla et al. 1981, Neilson et al. 1984, Peterson and Peterson 1984, and Appendix 2), and pulping effluents have historically been associated with the loss of aquatic life (Whitney and Spindler 1959, Smith and Kramer 1963).

The pulp and paper industry has undergone substantial technology change in the last 20 years. As a result, effluents from mills with modern process and treatment systems are usually considered to be only weakly toxic (Walden 1976, Walden and Howard 1977, Solomon et al. 1993), meaning that exposure to undiluted effluent will not cause acute toxicity (McCubbin and Folke 1993). However, effluent discharge results in continuous long-term exposure to many compounds that are potentially harmful, and this chronic exposure may have deleterious effects on aquatic life.

Research into the environmental effects of pulping effluents has focused on issues such as biological and chemical oxygen demand (BOD, COD), colour, particulate matter, chlorinated organic compounds such as dioxins and furans, and identifying compounds responsible for acute toxicity of effluents. One aspect that has received little attention, and is perhaps due more study given the current reduction in effluent toxicity, is nutrient enrichment of receiving waters. Pulping effluents generally contain concentrations of nitrogen and phosphorus 1-2 magnitudes higher than their receiving waters; these compounds come from wood and from fertilizers added to enhance secondary treatment and, thus, reduce toxicity and oxygen demand of the effluent. As phosphorus and nitrogen are often limiting nutrients in aquatic systems, the addition of these compounds via effluent discharge can stimulate primary productivity. While severe eutrophication can lead to excessive algal growth, depletion of dissolved oxygen, and loss of species, at low to moderate levels of

enrichment increased primary productivity may lead to increased density, productivity, and diversity of consumers (e.g. Peterson et al. 1985, Hershey 1988, Johnson et al. 1990, Hart and Johnson 1990, Hinterleitner-Anderson et al. 1992, Hiltner and Hershey 1992, Harvey et al. 1998). However, because the regulation of effluents has always been toxicity-oriented, there has been a bias against recognizing stimulatory responses as an effect. Eutrophication of rivers by pulping effluent has only recently been recognized as an issue requiring attention (Bothwell et al. 1992).

Nutrient enrichment by pulp mill effluents not only creates confusion when value-judgements are incorporated into impact assessments, but also has the potential to mitigate or mask toxic effects of effluent. Feminella and Hawkins (1995) reviewed studies published between 1972-1993, of periphyton-herbivore interactions in streams and concluded that there was good evidence that abundance and growth of grazers are generally limited by periphyton abundance. Thus, while chronic toxic effects include decreased growth, increased mortality, and decreased reproduction, an increase in food availability may increase growth and reproduction, and reduce mortality of grazing insects. Nutrient additions and the resultant increase in primary production may also lead to "biomass dilution" of contaminants, reducing exposure to toxicants (Gunnarsson et al. 1995, Taylor et al. 1996).

The nutrient-contaminant interaction is depicted in Fig. 1.2. At low concentrations of effluent, nutrient concentrations will stimulate productivity and toxicity will be minimal, thus the net effect will be stimulation. As effluent concentrations increase, the effects of nutrient additions quickly plateau; periphyton growth rate saturation occurs at phosphorus (P) concentrations as low as 3-4µg/L in the Thompson River, British Columbia (Bothwell et al. 1985, 1989), and at concentrations of 2-5 µg/L P in the Athabasca River (Chambers 1996). However, as effluent concentrations increase, toxicity will strengthen, decreasing growth and/or reproduction and eventually increasing mortality. The interaction of nutrients and contaminants in pulp mill effluent is a subject about which we have limited understanding; it had not been investigated when this

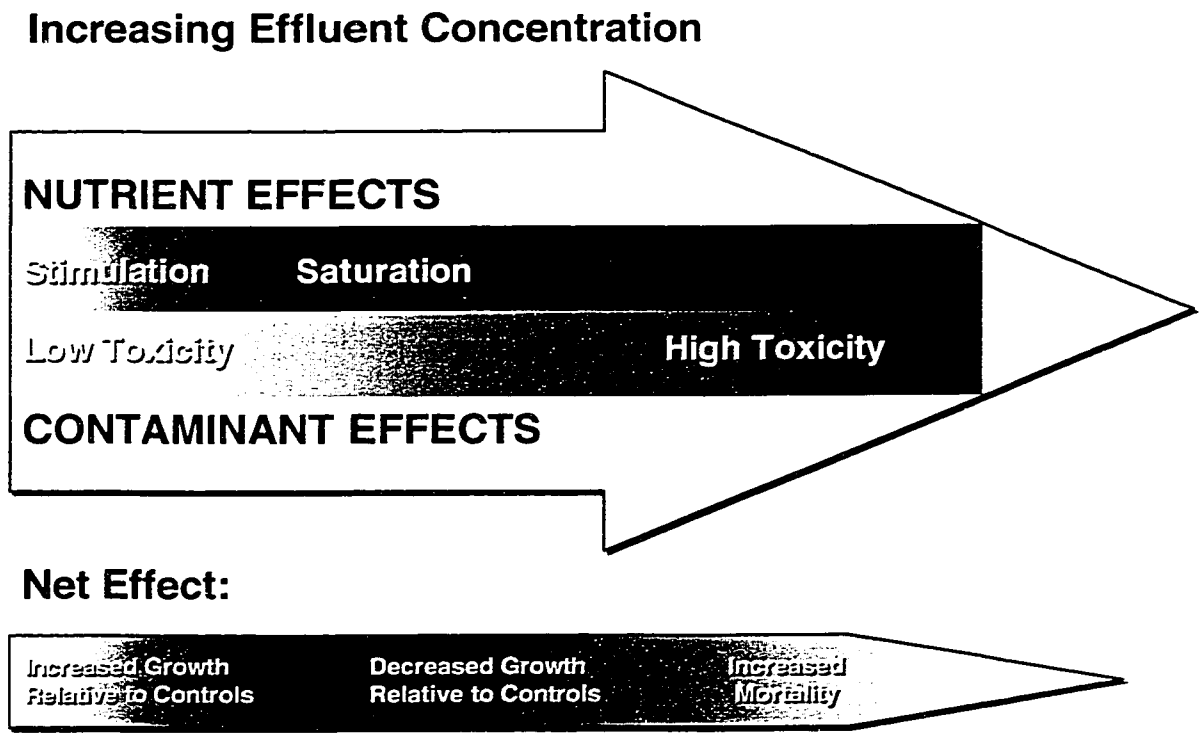


Figure 1.2. Schematic representation of the nutrient-contaminant interaction.

research was initiated (subsequently two studies have been published: Lowell et al. 1995,and Dubé and Culp 1996, 1997), and it is a focus of this thesis.

1.2 Outline of Research & Organization of the Thesis

Research into the effects of pulping effluent on non-vertebrate members of riverine communities has been almost exclusively in the form of biological surveys of upstream and downstream communities. Surveys are rarely able to infer causality, yet little experimental work has addressed the effects of effluent on riverine invertebrates. Experimental work that has been carried out has generally been done in poorly replicated, large experimental channels working at the community level (e.g. NCASI streams, Hall et al. 1991). As a result, although we may be able to say that density or community composition changes downstream of a discharge, we have difficulty assigning responsibility for changes to the discharge, and little understanding of the mechanisms responsible for observed changes. The primary goal of my research was to

examine effects of a modern pulp mill discharge in an integrated way to better understand the mechanisms through which nutrients and contaminants in the effluent affect the benthic community, and in particular, grazing insects. The term "integrated" is used to mean a research approach in which linked laboratory and field investigations are used to assess the effects of a discharge. Integrated assessment is a relatively new method that yields a more reliable measurement of environmental effects than either laboratory bioassays or field surveys are capable of producing alone (Chapman 1996, Hall 1996, Hall and Miner 1997).

Fig. 1.3 is a conceptual model of the mechanisms through which effluent may affect a simplified food chain; the mechanisms or effects that were addressed in this research are indicated. Nutrient enrichment of periphytic food resources will, in general, lead to faster growth, larger body size, shorter

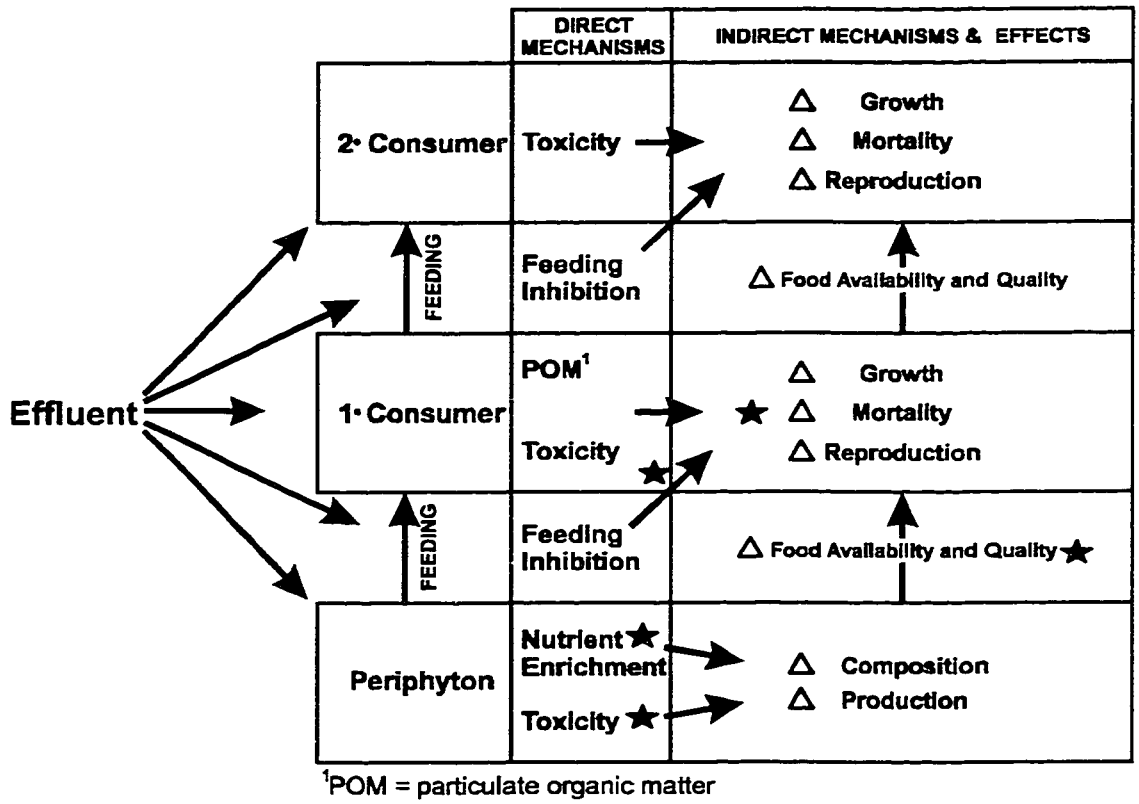


Figure 1.3. Conceptual model of the effects of pulp mill effluent on a simplified food chain. Mechanisms or effects that were addressed in this research are indicated by a ★.

generation time and/or increased fecundity of grazers. However, toxicants in pulp mill effluent may decrease growth rates (and therefore increase time to reproduction), decrease reproductive success, and increase mortality rates. The net effects of the interaction of these components will determine the effects on the population. At the community level, different sensitivities to toxicants may change community composition. Increased primary production may also change community composition, because changes in productivity of the system and the physical habitat provided by primary producers may confer a competitive advantage on a different suite of species.

Table 1.1 outlines the various objectives of my research, gives a brief description of my approach to meeting these objectives, and identifies where they are found in the thesis. Chapter 2 describes the study site for this research, the Athabasca River at the town of Hinton, Alberta; the operations and effluent composition of Weldwood of Canada, Hinton Division's bleached kraft pulp mill; and reviews what is known about the impact of this discharge on the Athabasca River. The results of field observations and an experiment designed to separate the effects of nutrients from those of contaminants on community composition and growth of algae and grazing insects are discussed in Chapter 3.

Laboratory bioassays were conducted to examine sublethal and chronic effects of effluent, and to look at mechanisms responsible for these effects. The species used for these bioassays were the mayflies *Baetis tricaudatus* and *Ameletus subnotatus*, both identified in the field mesocosm work as numerically important community members. Sublethal effects include behavioral changes such as alterations in foraging activity and predator avoidance; and changes in parameters such as generation time, body size, and fecundity.

The sublethal effect investigated was the effect of effluent on feeding behavior. Nutrient enrichment may partially or completely mitigate increased costs of toxicant exposure by increasing availability of food resources. However, toxicants may affect energy intake; a reduction in feeding rates is a

well-documented sublethal response in a variety of aquatic invertebrates (e.g., McNaught 1989, Naylor et al. 1989, Férnandez-Casalderry et al. 1994, Wicklum and Davies 1996). This toxicant-induced decrease in energy intake is largely responsible for changes in energy available for production (Baird 1990, Naylor et al. 1989, Maltby and Naylor 1990). Thus, compounds in pulp mill effluent may not only affect food resource availability, but also the utilization of those resources. A change in feeding behavior in response to exposure to pulp mill effluent is likely because effluent contains specific compounds, such as resin acids, that are known to affect insect feeding. Resin acids are secondary compounds produced by trees that are "washed out" in the pulping process (see Appendix 1, section 4). The natural function of these compounds is to protect the tree from herbivore damage through antifeedant and other insecticidal activities. Although they are "aimed" at terrestrial insects, it is possible that these compounds may have similar effects on aquatic insects. If so, increased primary production resulting from nutrient enrichment by effluent may not actually represent increased food resources for grazing insects.

To conduct feeding experiments, I first needed to determine if the food supply, in this case the pennate diatom *Navicula*, could be cultured in effluent, and if exposure to effluent would affect rates of growth. This information was required because it was necessary to present animals with an equal biomass of food to avoid possible confounding of a functional response with effluent effects on feeding rate. Algae were cultured in effluent rather than exposing it for a short period of time before the feeding trial because: (1) there are no published accounts of the uptake kinetics of effluent compounds, and (2) I felt that this technique would more properly simulate natural exposure downstream of a continuous discharge. An experiment testing the effects of effluent on growth rates of *Navicula*, and what was learned about how *Navicula* differs in quality as food after being cultured in effluent are presented in Chapter 4. The effects of effluent on feeding of *Ameletus subnotatus*, a grazing mayfly, are discussed in Chapter 5.

Effects of toxicants on life history parameters are often attributed to increased energetic costs associated with detoxification/repair and the resulting reallocation of resources (Sibley and Calow 1989, Calow and Sibley 1990, Calow 1991,1992). An animal may respond in many ways to a non-lethal exposure to a toxicant (Calow 1991, Sibley 1996, Fig. 1.4): (1) the animal may initiate evasive behavior to avoid exposure; (2) exposure to the toxicant may be tolerated through the use of a variety of physiological defensive mechanisms that prevent damage, for example, active transport, the induction of detoxification enzymes (Terriere 1984), and the production of mucus (Wicklum and Davies 1996) or sequestering proteins (e.g. metallothionens); and finally, (3) damage may occur and may be repaired. All of these mechanisms, behavioral, defensive, and repair, cost energy that is then no longer available for growth and/or reproduction (Calow 1991). The energy available for production, i.e., energy intake minus total metabolic costs, is known as "scope

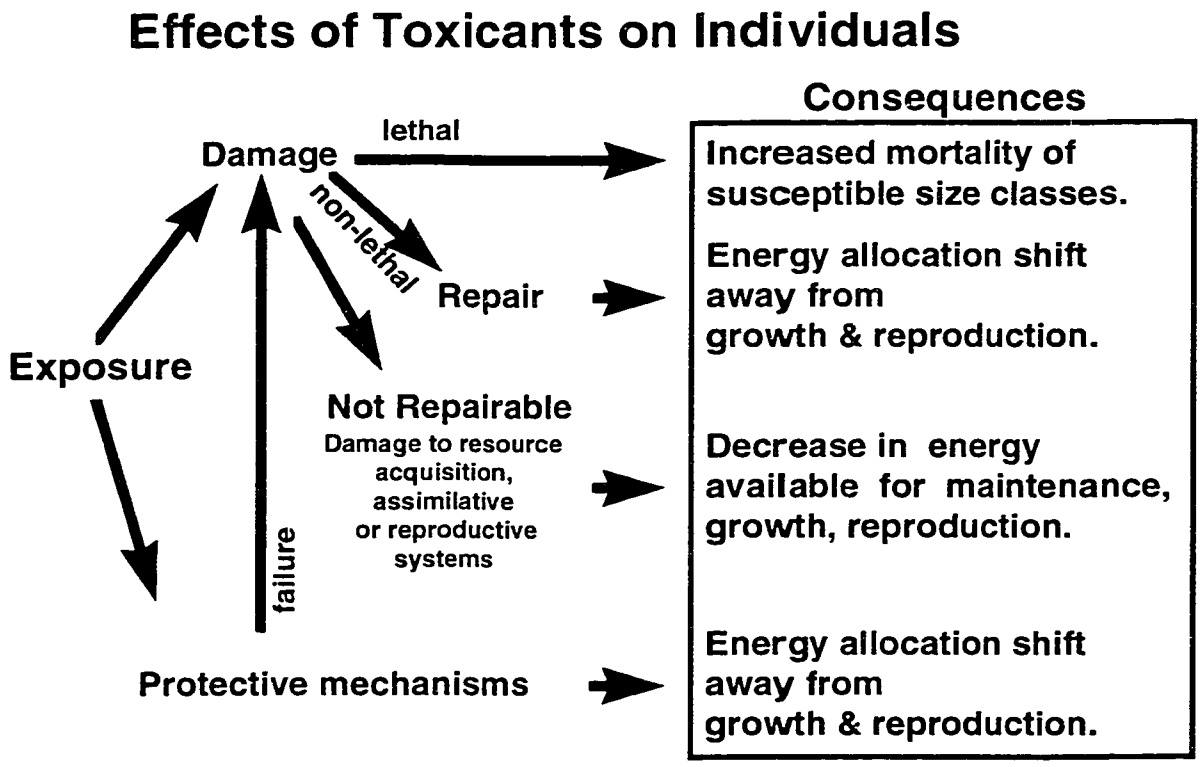


Figure 1.4. Toxicant effects on individuals and consequences to life table parameters.

for growth" (Walker et al. 1996), and the measurement of toxicant effects on scope for growth is an accepted approach in physiological ecotoxicology (e.g., Bayne et al. 1979, Naylor et al. 1989, Maltby and Naylor 1990, Wicklum and Davies 1996). Changes in scope for growth may be measured indirectly by examining toxicant-induced alterations in growth rate, reproduction and mortality. Measurement of these life table parameters is believed to be one of the most sensitive indicators of anthropogenic stress (Schindler 1987). Chapter 6 discusses bioassays designed to look at the effects of chronic exposure to pulp mill effluent on growth, mortality, and/or reproduction of *Ameletus subnotatus* and *Baetis tricaudatus* respectively. All results are summarized and directions for future research are presented in Chapter 7.

Finally, many appendices containing supporting information are included. Readers unfamiliar with the operations of a pulp mill, impact assessment as applied to this industry, and effluent composition are directed to Appendix 1. Appendix 2 contains a list of compounds commonly reported in kraft pulp mill effluent and, where available, the concentration of those compounds in effluent and their ecotoxicity. Appendix 2 illustrates the point that, although there are numerous compounds in effluent that have demonstrated toxicity to aquatic life, in general, these compounds are found in concentrations orders of magnitudes smaller than required to cause acute toxicity.

Table 1.1. Organization of the thesis: objectives, description of research approach, hypotheses tested, and location in the thesis.

Objective	Description	Hypothesis Tested	Chapter
Provide background information on sampling location and mill.	Description of Athabasca River at Hinton, review of environmental impact assessments of the mill's receiving environment, description of mill's process, effluent treatment, and effluent composition.		2
Compare effects of whole BKME with effects of nutrient addition on the insect and periphyton community of the Athabasca River.	Artificial stream study comparing effects of BKME and NP additions on growth rates and community composition of aquatic insects and periphyton. Concurrent field sampling done to "validate" study.	**Artificial Stream Study** H_0: Control = BKME = NP H_{a1}: Control ≠ BKME = NP (no contaminant effects) H_{a2}: Control ≠ BKME ≠ NP (contaminant effects present) **Field Sampling** H_0: upstream = downstream H_a: upstream ≠ downstream	3
Measure effects of BKME on cultured diatoms to determine if culturing times will have to be adjusted to produce equal biomass in controls and treatments for feeding experiments.	Laboratory experiment; culturing Navicula (pennate benthic diatom) in 0, 1, 3, 5, and 7% BKME. Rate of biomass increase compared.	**Biomass accrual regression** H_0: β1 of control = β1 of BKME treatments H_a: β1 of control ≠ β1 of BKME treatments	4
Compare composition of diatoms cultured in control media and effluent.	Bacterial content, carbon/nitrogen content, lipid content, and metal content of diatoms from biomass experiment (objective 3) were compared.	**Lipid, C/N, metal content** H_0: Control = BKME treatments H_a: Control ≠ BKME treatments	4

Table 1.1 continued.

Objective	Description	Hypothesis Tested		Chapter
Measure effects of BKME in water on feeding rate of Ameletus subnotatus.	Laboratory bioassay; Ameletus subnotatus nymphs fed Navicula while in 0, 1, 3, or 7% BKME. Amount eaten in fixed time period compared.	H_0: Ha:	Control = 1% = 3% = 7% Control ≠ 1% ≠ 3% ≠ 7%	5
Measure effects of BKME in algae on feeding rate of Ameletus subnotatus.	Laboratory bioassay; Ameletus subnotatus nymphs fed Navicula cultured in 0, 1, or 7% BKME. Amount eaten in fixed time period compared.	H_0: H_a:	Control = 1% = 7% Control ≠ 1% ≠ 7%	5
Measure effects of BKME in algae on food choice of *Ameletus subnotatus.*	Laboratory bioassay; *Ameletus subnotatus* nymphs offered choice of *Navicula* that had been cultured in 0, 1, 5, or 7% BKME. Amount eaten in fixed time period compared.	H_0: H_a:	Control = 1% = 7% Control ≠ 1% ≠ 7%	5
Measure effects of BKME on growth rate of *Ameletus subnotatus.*	Laboratory chronic exposure, animals fed *Navicula* cultured in effluent and effluent added to stream water. Growth and mortality compared with control.	H_0: H_a:	Control = 1% = 7% Control ≠ 1% ≠ 7%	6
Measure effects of BKME on growth rate, time to emergence, and fecundity of *Baetis tricaudatus*	Laboratory chronic exposure. Animals fed *Navicula* cultured in effluent and effluent added to stream water. Growth, mortality and fecundity compared with controls.	H_0: H_a:	Control = 1% = 7% Control ≠ 1% ≠ 7%	6
Measure BKME effects on hatching success / survival of 1st instar *B. tricaudatus.*	Laboratory exposure of *in vitro* fertilized eggs until hatching. Hatch success and mortality of 1st instars compared.	H_0: Ha:	Control =Treatments (1, 5, 10, 15, 20, 50%) Control ≠ Treatments	6
Summary of research.				7

Table 1.1 continued.

Objective	Description	Hypothesis Tested	Chapter
Literature review	Literature review of environmental effects of pulp mill effluent.		A1
	Review of pulp mill operations, process origins of effluent components, and effluent composition.		
Kraft mill effluent composition	Table of compounds reported in kraft pulp mill effluent: concentration in effluent, the log octanol water partition coefficient, and ecotoxicity of each compound.		A2
Weldwood effluent composition	Effluent composition for Weldwood of Canada, Hinton Division for the years 1993-1995		A3
Artificial Stream System	Description of stream system as used in research presented in Chapter 3.		A4
Algal taxonomy methods	Methods for enumeration and identification of algal species as described by laboratory		A5

1.3 Literature Cited

Baird, D. J., I. Barber, and P. Calow. 1990. Clonal variation in general responses of *Daphnia magna* Straus to toxic stress. I. Chronic life-history effects. Functional Ecology 4: 399-407.

Bayne, B. L., M. N. Moore, J. Widdows, D. R. Livingstone, and P. Salkeld. 1979. Measurement of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. Philosophical Transactions of the Royal Society of London Series B 286: 563-581.

Bothwell, M. L. 1985. Phosphorus limitation of lotic periphyton growth rates: an intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). Limnology and Oceanography 30: 527-542.

Bothwell, M. L., G. Derkson, R. N. Nordin, and J. M. Culp. 1992. Nutrient and grazer control of algal biomass in the Thompson River, British Columbia: a case history of water quality management. Pages 253-266 in R. D. Robarts and M. L. Bothwell, eds. *Aquatic Ecosystems in Semi-arid Regions: Implications for Resource Management.* Environment Canada, Saskatoon.

Bothwell, M. L., S. Jasper, and R. J. Daley. 1989. Phosphorus control of algal production and biomass in the Thompson River, British Columbia. National Hydrology Research Institute.

Calow, P. 1991. Physiological costs of combating chemical toxicants: ecological implications. Comparative Biochemistry and Physiology 100**C**: 3-6.

Calow, P. 1992. The three Rs of ecotoxicology. Functional Ecology 6: 616-619.

Calow, P., and R. M. Sibley. 1990. A physiological basis of population processes: ecotoxicological implications. Functional Ecology 4: 283-288.

Chambers, P. A. 1996. Nutrient enrichment in the Peace, Athabasca and Slave rivers: assessment of present conditions and future trends. Synthesis Report 4, Northern River Basins Study. Edmonton, Alberta.

Chapman, P. M. 1996. Recent perspectives in integrated monitoring. Pages 651-657 in M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Dubé, M. G., and J. M. Culp. 1996. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Environmental Toxicology and Chemistry 15: 2019-2027.

Dubé, M. G., and J. M. Culp. 1997. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Water Science and Technology 35: 339-345.

Feminella, J. W., and C. P. Hawkins. 1995 Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. Journal of the North American Benthological Society 14: 465-509.

Fernández-Casalderrey, A., M. D. Ferrando, and E. Andreu-Moliner. 1994. Effects of sublethal concentrations of pesticides on the feeding behavior of *Daphnia magna*. Ecotoxicology and Environmental Safety 27: 82-89.

Gunnarsson, J., D. Broman, P. Jonsson, M. Olsson, and R. Rosenberg. 1995. Interactions between eutrophication and contaminants: towards a new research concept for the European aquatic community. Ambio 24:383-385.

Hall, T. J. 1996. A review of recent studies in North America using integrated monitoring approaches to assessing bleached kraft mill effluent effects on receiving waters. Pages 599-611 in M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Hall, T. J., and R. A. Miner. 1997. Integrated long-term receiving water study methodology development. 35: 315-320.

Hall, T. J., R. K. Haley, and L. E. LaFleur. 1991. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental stream channels. Environmental Toxicology and Chemistry 10: 1051-1060.

Hart, D. D., and C. T. Robinson. 1990. Resource limitation in a stream community: phosphorus enrichment effects on periphyton and grazers. Ecology 71: 1494-1502.

Harvey, C. J., B. J. Peterson, W. B. Bowden, A. E. Hershey, M. C. Miller, L. A. Deegan, and J. C. Finlay. 1998. Biological responses to fertilization of Oksrukuyik Creek, a tundra stream. Journal of the North American Benthological Society 17: 190-209.

Hattula, M. L., V. -M. Wasenius, H. Reunanen, and A. U. Arstila. 1981. Acute toxicity of some chlorinated phenols, catechols and cresols to trout. Bulletin of Environmental Contamination and Toxicology 26: 295-298.

Hershey, A. E., A. L. Hiltner, M. A. J. Hullar, M. C. Miller, J. R. Vestal, M. A. Lock, S. Rundle, and B. Peterson. 1988. Nutrient influence on a stream grazer: *Orthocladius* microcommunities respond to nutrient input. Ecology 69: 1383-1392.

Hiltner, A. L., and A. E. Hershey. 1992. Blackfly (Diptera: Simuliidae) response to phosphorus enrichment of an arctic tundra stream. Hydrobiologia 240: 259-265.

Hinterleitner-Anderson, D., A. Hershey, and J. A. Schuldt. 1992. The effects of river fertilization on mayfly (*Baetis* sp.) drift patterns and population density in an arctic river. Hydrobiologia 240: 247-258.

Johnston, N. T., C. J. Perrin, P. A. Slaney, and B. R. Ward. 1990. Increased juvenile salmonid growth by whole-river fertilization. Canadian Journal of Fisheries and Aquatic Sciences 47: 862-872.

Leach, J. M., and A. N. Thakore. 1973. Identification of the constituents of kraft pulping effluent that are toxic to juvenile coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 30: 479-484.

Leach, J. M., and A. N. Thakore. 1975. Acute toxicity to juvenile rainbow trout (*Salmo gairdneri*) of naturally occurring insect juvenile hormone analogues. Canadian Journal of Fisheries and Aquatic Sciences 32: 2556-2559.

Leach, J. M., and A. N. Thakore. 1976. Toxic constituents in mechanical pulping effluents. Tappi Journal 59: 129-132.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environmental Toxicology and Chemistry 14: 1529-1541.

Maltby, L., and C. Naylor. 1990. Preliminary observations on the ecological significance of the *Gammarus* 'scope for growth' assay: effects of zinc on reproduction. Functional Ecology 4: 393-397.

Maltby, L., C. G. Naylor, and P. Calow. 1990. Effect of stress on a freshwater benthic detritivore: scope for growth in *Gammarus pulex*. Ecotoxicology and Environmental Safety 19: 285-291.

McCubbin, N., and J. Folke. 1993. A review of literature on pulp and paper mill effluent characteristics in the Peace and Athabasca River basins. Project Report 15, Northern River Basins Study, Edmonton, Alberta.

McNaught, D. C. 1989. Functional bioassays utilizing zooplankton: A comparison. Hydrobiologia 188/189: 117-121.

Naylor, C., L. Maltby, and P. Calow. 1989. Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. Hydrobiologia 188/189: 517-523.

Neilson, A. H., A. S. Allard, S. Reiland, M. Remberger, A. Tärnholm, T. Viktor, and L. Lander. 1984. Tri- and tetra-chloroveratrol, metabolites produced by bacterial *o*-methylation of tri- and tetra-chloroguaiacol: an assessment of their bioconcentration potential and their effects on fish reproduction. Canadian Journal of Fisheries and Aquatic Sciences 41: 1502-1512.

Petersen, L. B. -M., and R. C. Petersen, Jr. 1984. Effect of kraft pulp mill effluent and 4,5,6 trichloroguaiacol on the net spinning behavior of *Hydropsche angustipennis* (Trichoptera). Ecological Bulletins 36: 68-74.

Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Locke, T.E. Ford, J. R. Vestal, V. L. McKinely, M. A. J. Hullar, M. C. Miller, R. M. Ventullo, and G. S. Volk. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229: 1383-1385.

Rogers, I., H. Mahood, J. Servici, and R. Gordon. 1979. Identifying extractives toxic to aquatic life. Pulp & Paper Canada 80: T286-T290.

Rogers, I. H. 1973. Isolation and chemical identification of toxic components of kraft mills wastes. Pulp & Paper Canada 74: 111-116.

Schindler, D. W. 1987. Detecting ecosystem responses to anthropogenic stress. Canadian Journal of Fisheries and Aquatic Sciences 44 (Suppl. 1): 6-25.

Sibley, R. M. 1996. Effects of pollutants on individual life histories and population growth rates. Pages 197-223 in M. C. Newman and C. H. Jagoe, eds. *Ecotoxicology: a heirarchical treatment.* Lewis Publishers, Boca Raton, FL.

Sibley, R. M., and P. Calow. 1989. A life-cycle theory of responses to stress. Biological Journal of the Linnean Society 37: 101-116.

Smith, L. L., and R. H. Kramer. 1963. Survival of walleye eggs in relation to wood fibers and *Sphaerotilus natans* in the Rainy River, Minnesota. Transactions of the American Fisheries Society 92: 220-234.

Solomon, K. R., H. L. Bergman, R. J. Huggett, D. Mackay, and B. McKague. 1993. A review and assessment of the ecological risks associated with the use of chlorine dioxide for the bleaching of pulp. Alliance for Environmental Technology.

Suntio, L. R., W. Y. Shiu, and D. Mackay. 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere 17: 1249-1290.

Taylor, W. D., E. Bentzen, B. Delucca, B. E. Hickie, D. R. S. Lean, D. Mackay, and G. Patterson. 1996. Empirical evidence for the importance of lake trophic status and food web in bioaccumulation in southern Ontario lakes. Pages 101-110 in P.R.G. Kramer, D. A. Jonkers, and L. van Liere, eds. *Interactions of nutrients and toxicants in the foodchain of aquatic ecosystems.* Rijksinstituut voor Volksgezondheid en Milieu, Zeist, Netherlands.

Terriere, L. C. 1984. Induction of detoxification enzymes. Annual Review of Entomology 29: 71-88.

Walden, C. C. 1976. The toxicity of pulp and paper mill effluents and corresponding measurement procedures. Water Research 10: 639-663.

Walden, C. C., and T. E. Howard. 1977. Toxicity of pulp and paper mill effluents. Tappi Journal 60: 122-125.

Walker, C. H., S. P. Hopkin, R. M. Sibly, and D. B. Peakall. 1996. *Principles of Ecotoxicology.* Taylor & Francis, London. 321p.

Whitney, A. N., and J. C. Spindler. 1959. Effects of kraft paper wastes on a Montana stream. Transactions of the American Fisheries Society 88: 153.

Wicklum, D., and R. W. Davies. 1996. The effects of chronic cadmium stress on energy acquisition and allocation in a freshwater benthic invertebrate predator. Aquatic Toxicology 35: 237-252.

Chapter 2
Study Site and the Pulp Mill

2.1. Athabasca River

The Athabasca River originates in the meltwaters of the Columbia ice fields, located in Jasper National Park, Alberta (Fig. 2.1). It travels approximately 1,231 km, draining a 159,000 km^2 watershed before emptying into Lake Athabasca. The river is unregulated and is fed largely by glacial meltwaters and mountain runoff, resulting in seasonally variable discharge with

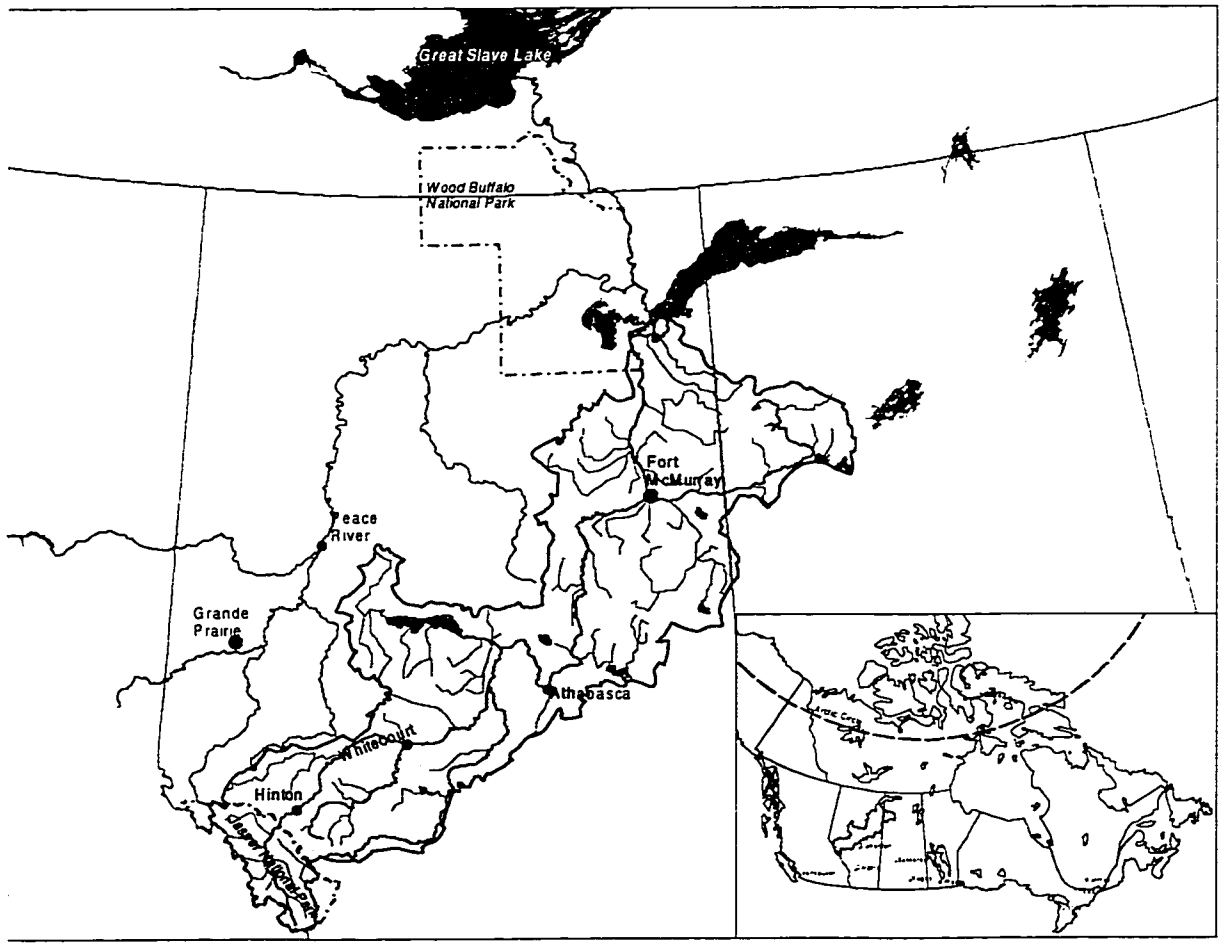


Figure 2.1. Athabasca River drainage.

peak flows in the spring and low flow between January and March. Near the headwaters, Athabasca River water quality reflects the glacial origin and sedimentary rock geology of its catchment. The water is alkaline, turbid, and hard, containing relatively high concentrations of calcium, magnesium, sulfates, and suspended solids, and low concentrations of dissolved organic carbon, and phosphorus (Table 2.1). As the river flows eastward, water hardness drops as tributaries dilute the river, and concentrations of nitrogen, sodium, chloride, silica, barium, and dissolved organic carbon gradually increase (Noton and Saffran 1995). The Athabasca River and its tributaries receive continuous effluent discharges from 5 pulp mills and 9 municipalities. An additional 40 municipalities and 2 oil sands extraction plants discharge effluent to the river or other water bodies within the catchment in spring and/or fall (Chambers 1996).

Table 2.1 Concentration of some major ions in the Athabasca River at Hinton (upstream of discharge, 1988-1993) compared to mean values for North American rivers.

	Mean concentration (mg/L)	
Parameter	Athabasca River[1]	North American Rivers[2]
Ca^{2+}	46.2	21.2
Mg^{2+}	14.4	4.9
SO_4^{2-}	65.5	18.0
HCO^{3-}	135.9	72.3
DOC	0.8	5.0
TDP	0.003	0.025

[1] Noton and Saffran (Appendix D1,1995);
[2]Allan (1995)

At Hinton, Alberta, the Athabasca is a shallow rocky bottomed river, with most areas less than 1m deep (R.L. & L. 1994). River banks are primarily composed of small and large boulders with irregular outcroppings forming occasional backwaters. The canopy is open (Plate 2.1) and there is little

riparian vegetation except in areas with erosional/depositional silt banks, which make up 11% of bank types (R.L & L. 1994). The dominant substratum type in the river channel is cobble, with some gravel and silt in slower moving backwaters. A hydrograph for the Athabasca at Hinton shows low flow periods extend from September to mid-May, with lowest discharge (mean = 30.6 m^3/sec, 1961-1990) occurring in March; and peak flows (mean = 501 m^3/sec, 1961-1990) occurring in June (Fig. 2.2). Water during high flow is a milky colour due to entrained rock flour. During low flow periods, the water is quite clear; light attenuation measurements indicate that the average depth of the euphotic zone is 2.8 m and, thus, periphyton could conceivably colonize the entire width of the channel (Chambers 1996). Periphyton growth is limited by turbidity and flow during high flow and by low temperatures and nutrient concentrations during low flow periods (Chamber 1996). The river's oligotrophic nature and large euphotic zone predispose it to respond to nutrient additions.

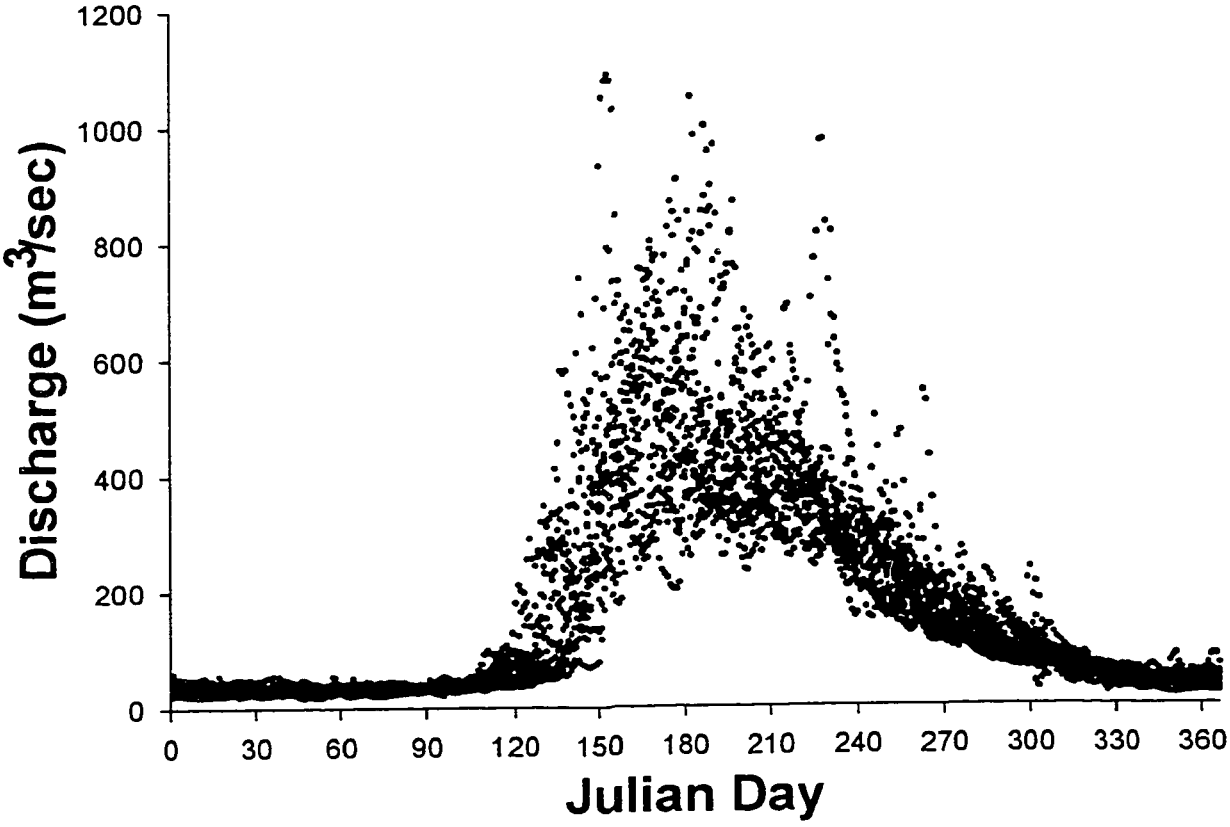


Figure 2.2. Athabasca River discharge at Hinton, Alberta, 1980-1994

Fish species in the Athabasca at Hinton include brook trout (*Salvelinus fontinalis*), bull trout (*S. confluentus*), Arctic grayling (*Thymallus arcticus*), rainbow trout (*Oncorhynchus mykiss*), mountain whitefish (*Prosopium williamsoni*), longnose sucker (*Catostomus catostomus*), burbot (*Lota lota*), white sucker (*C. commersoni*), longnose dace (*Rhinichthys cataractae*), lake chub (*Couesius plumbeus*), and spoonhead and slimy sculpins (*Cottus ricei* and *C. cognatus*) (R.L. & L. 1994, 1995). The macroinvertebrate community is composed primarily of Ephemeroptera (Baetidae, Ephemerellidae, Heptageniidae), Plecoptera (Perlidae, Capniidae), Diptera (Chironomidae, Athericidae), and Trichoptera (Hydropsychidae, Brachycentridae) (Anderson 1989, R.L. & L. 1993).

Plate 2.1. Athabasca River at Hinton, Alberta. Upstream view.

2.2 Weldwood of Canada, Hinton Division

Weldwood of Canada Ltd., Hinton Division, operates a large bleached kraft mill in Hinton, Alberta. This mill is the oldest one in the area, having started operations in 1957 as North Western Pulp and Power Ltd. The mill produces an average of 1150 air-dried 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% balsam fir. Weldwood harvests approximately 50% of its wood supply within its forest management area, with the remaining supply from 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 hydroxide and sodium sulfide, and are 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 this bleach sequence ($OD_{100}E_{OP}(DE_SD)$) to produce elemental chlorine free pulp (Golder 1994). Major changes affecting effluent discharge that have occurred within the mill since its start-up in 1957 are listed in Table 2.2.

The mill provides the town of Hinton with drinking water and also treats municipal sewage. Municipal effluent represents approximately 7.5% of the volume of the mill's effluent. Average water usage by the mill is 113,000 m^3/day, with approximately 110 m^3 of effluent produced for every tonne of air dried pulp produced. Effluent first passes into a 13,753 m^3 (61 m diameter) primary mechanical clarifier to remove solids. Primary treated effluent is then combined with municipal sewage, and the combined effluent flows into a 6.5 day aerated stabilization basin that has 41 mechanical aerators (2700 Hp total, Weldwood of Canada, Ltd. 1996). Granular 12-51-0 fertilizer is added to the basins to enhance secondary treatment, which generally achieves a 91.8% BOD reduction. Prompted by concerns about nutrient loading to the river, Weldwood has been reducing nutrient additions to the stabilization basins since 1992.

Since 1995, the basins have been fertilized only during winter months, with municipal sewage providing sufficient nutrients during the rest of the year (T. Andrews *pers comm.*). After passing through a quiescent zone to reduce suspended solids, treated effluent is then mixed with non-contact cooling water from the mill and discharged to the Athabasca River. At low winter flows (30-35 m^3), effluent comprises up to 3% of the river's discharge. Weldwood effluent is the second point source discharge to the river, sewage discharge from the town of Jasper, Alberta, being the first. Discharge from the town of Jasper represents 5% of the volume of the combined discharge at Hinton (Noton and Saffran 1995).

Table 2.2. Major changes in Weldwood process or equipment that may affect effluent quality or quantity (summarized from Golder 1994).

Date	Description of process or equipment change
1957	Pulp mill start up, production 91,000 tonnes/year, 780,000 hectare FMA
1966	Effluent diffuser installed in river bottom
1967	Mechanical clarifier installed, original settling basin converted to 5-day ASB
1975	Secondary treatment system expanded, ASB enlarged to 6.3 d retention, total aeration increased to 1425 Hp
1978-1979	Installation of low rate stream stripper, new line of black liquor evaporator and low odour recovery boiler
1957-1987	Pulp production increased in incremental stages to 193,000 tonnes/yr
1988	Installation of new brownstock screening system
1989-1990	Spill recovery systems installed
1989	Chlorine dioxide plant start-up, bleach sequence upgraded to C_DE0DED
1990 (June)	First run with 100% ClO_2 substitution
1990-1993	100% ClO_2 pulping conducted as 3-7 day runs making up 15-30% of total mill production
1990	Installation of oxygen delignification system
1991 (February)	ASB aeration increased to 3000 Hp
1993 (February)	Quiescent zone of ASB doubled to reduce total suspended solid (TSS) discharge
1993 (February)	Replacement of old (1967 vintage) aerators with new more efficient units (3000 Hp maintained)
1993 (June 30)	Complete elimination of elemental chlorine bleaching, bleach sequence $D_{100}E_{OP}(DE_SD)$
1993 (September)	Nutrient additions to ASB greatly reduced
1994	50% of evaporator contaminated condensate recycled to brownstock
1994	100% of evaporator contaminated condensate recycled to brownstock
1996 (July)	Discontinued use of liquid $S0_2$ in bleach plant
1996	Installation of 2 new 75 Hp aerators in ASB
1996	Installation of floating boom in quiescent zone of ASB to further reduce TSS
1997	Completion of spill containment system for weak black liquor and green liquor clarifiers

2.3 Effects of Weldwood Effluent on the Athabasca River

Weldwood effluent enters the river via a multiport diffuser located in the middle of the river channel. The effluent plume hugs the south bank (Plate 2.2), and is not completely mixed until 11 km downstream (TAEM 1996). The 20-30°C effluent raises river water temperature by approximately 1°C as far as 0.8 km downstream, and creates a winter ice-free zone extending approximately 1 km downstream of the diffuser (Plate 2.3). River water samples taken in March 1994 (low flow) as part of the mill's Environmental Effects Monitoring program, indicated that the effluent discharge increases concentrations of total Kjeldahl nitrogen, total phosphorus, orthophosphate, total dissolved phosphorus, dissolved organic carbon, sodium, and true colour, downstream of the outfall (TAEM 1996). A general description of effluent composition is in Table 2.3, and more detailed information is provided in Appendix 3 and Chapter 4.

Before the addition of a mechanical clarifier and aerated stabilization basin in 1967, effluent treatment consisted of a 3-4 day settling basin. Benthic surveys indicated that the invertebrate community immediately downstream of the outfall was dominated by oligochaetes and chironomids, indicating organic pollution. After modification of the effluent treatment system in 1975, the downstream community showed signs of recovery (SENTAR 1993). River sampling since 1980 has detected increased invertebrate density downstream of the outfall, and a community dominated by mayflies and chironomids at both upstream and downstream sites (SENTAR 1993, TAEM 1996, Golder 1996). Increased density of macroinvertebrates is likely due to the increase in periphytic algae that has been reported to occur for about 22 km downstream of the effluent outfall (TAEM 1989, 1991a,b, 1992, 1993). The mill's effluent contains phosphorus and nitrogen, the most common limiting nutrients in freshwaters. Upstream of the mill, algal growth is phosphorus limited (Perrin et al. 1995; Dale and Chambers 1995a,b; Scrimgeour et al. 1995). This nutrient limitation is relaxed at the Weldwood outfall and does not reappear until just upstream of Whitecourt (Scrimgeour and Chambers 1996).

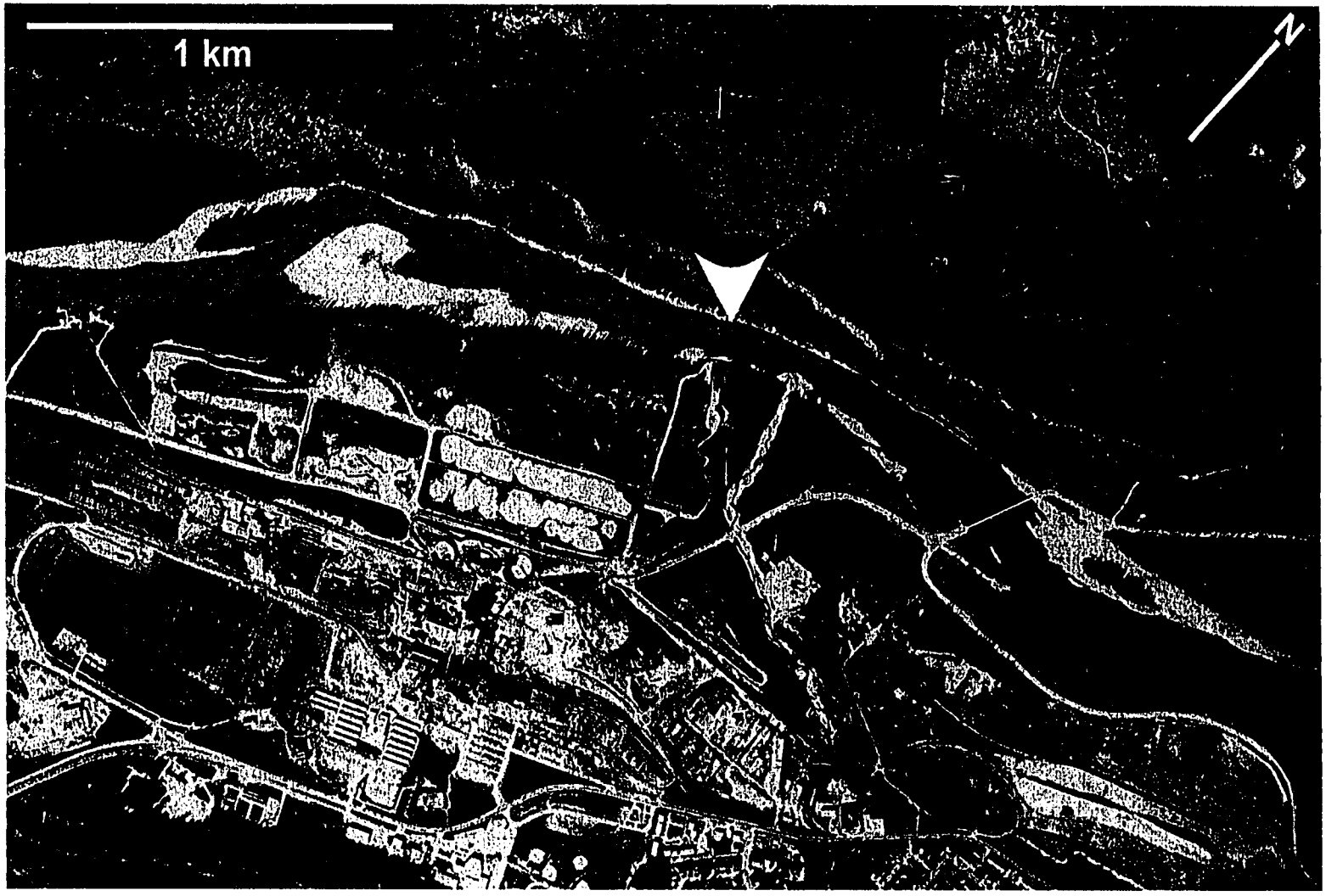


Plate 2.1. Effluent plume in the Athabasca River at Hinton (November, 1988). Photo courtesy of Airphoto Services, Alberta Environmental protection.

Plate 2.2. Ice-free lead on the Athabasca River, downstream of the Weldwood discharge (February 1989); photo courtesy of Airphoto Services, Alberta Environmental Protection.

Table 2.3. Range and mean values of Weldwood effluent BOD_5, pH, temperature, conductivity, TSS, TKN, NO_2, NO_3, NH_3, TP, DP, AOX, phenolics, and resin and fatty acid concentrations for the period of January 1993 - December 1995. Data are summarized from monthly effluent reports submitted to Alberta Environmental Protection in compliance with the mill's water license. Raw data and a more compete chemical characterization are available in Appendix 3.

Parameter	Range	Mean
BOD_5 (mg/L)	10.5 - 27.0	16.79
pH	7.4 - 8.4	7.9
Temperature (°C)	21 - 34	27
Conductivity (µmhos)	896 - 2491	1614
TSS (mg/L)	17 - 60	28
TKN (mg/L)	2.68 - 6.15	4.0
NO_2 (mg/L)	0.05 - 0.46	0.2
NO_3 (mg/L)	0.05 - 0.58	0.2
NH_3 (mg/L)	0.48 - 2.63	1.41
TP (mg/L)	0.33 - 1.46	0.7
DP (mg/L)	0.07 - 0.76	0.3
AOX (mg/L)	1.7 - 12.1	3.3
Total phenolics (µg/L)	ND - 80.9	0.055
Total Resin and Fatty Acids (mg/L)	ND - 0.79	0.057

Acute toxicity tests of Weldwood's effluent are conducted regularly as a part of the mill's license agreement, and indicate that full strength effluent is consistently non-toxic to rainbow trout fry and *Daphnia magna*. Chronic toxicity tests using fathead minnows (*Pimephales promelas*), *Ceriodaphnia dubia*, and *Selenastrum capricornutum* are a relatively recent addition to Weldwood's monitoring regime. The effluent displayed little or no toxicity to fathead minnows in 7-day exposures; the mean IC_{25} (concentration which reduces growth by 25%) was >100% (n=8) (Golder 1996). Effluent inhibited reproduction of *Ceriodaphnia* at concentrations ranging from 41-79%, with a mean IC_{25} of 58.3% (n=8). The green alga *Selenastrum* was most sensitive to

the effluent, showing growth inhibition (IC_{25}) at concentrations ranging from 14-82%. As the concentrations at which toxicity was observed are higher than those observed in the receiving environment (except in the mixing zone directly below the diffuser), it is believed that effluent inputs from the Weldwood mill do not result in toxicity problems in the Athabasca River (Golder 1996).

Weldwood effluent, like all pulp mill effluent, contains nutrients and contaminants, and both may affect organisms in the receiving environment. The consistent low toxicity of this effluent, and the considerable dilution that occurs, suggest that toxic effects of the discharge will be minimal. However, the toxicity of the effluent to indigenous species has not been determined, consequently, the possibility that chronic growth or reproductive effects may occur cannot be discounted. The most prominent effect of effluent additions to the Athabasca River is likely to be fertilization by phosphorus in the effluent; the river is oligotrophic, primary production is know to be phosphorus-limited, and the river has a large euphotic zone where primary production may take place (Chambers 1996). Stimulation of primary production, as well as the addition of particulate organic matter via effluent, may increase production at higher trophic levels, and mask any contaminant effects on these organisms. The separation of nutrient and contaminant effects is a difficult problem, as the removal of nutrients from effluent without affecting composition is problematic. An experiment comparing the effects of effluent with the effects of a comparable nutrient (N and P) addition on the benthic community and, thus, identifying the dominant influence of this discharge, will be presented in Chapter 3.

2.4 Literature Cited

Allan, J. D. 1995. Stream ecology: structure and function of running waters. Chapman & Hall, London.

Anderson, A. M. 1989. An assessment of the effects of the combined pulp mill and municipal effluents at Hinton on the water quality and zoobenthos of the Athabasca River. Environmental Quality Monitoring Branch, Environmental Assessment Division, Alberta Environment, Edmonton, Alberta.

Chambers, P. A. 1996. Nutrient enrichment in the Peace, Athabasca and Slave rivers: assessment of present conditions and future trends. Synthesis Report No. 4, Northern River Basins Study, Edmonton, Alberta.

Dale, A. R., and P. A. Chambers. 1995a. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, April and May, 1994. Project Report No. 67, Northern River Basins Study, Edmonton, Alberta.

Dale, A. R., and P. A. Chambers. 1995b. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, seasonal variation. Project Report No. 68, Northern River Basins Study, Edmonton, Alberta.

Golder Associates. 1996. Environmental effects monitoring Cycle I final report for Weldwood of Canada Limited, Calgary, Alberta.

Noton, L. R., and K. A. Saffran. 1995. Water quality in the Athabasca River System 1990-1993. Alberta Environmental Protection, Edmonton, Alberta.

Perrin, C. J., P.A. Chambers, and M. L. Bothwell. 1995. Growth rate and biomass responses of periphytic algae to nutrient enrichment of stable and unstable substrata, Athabasca River. Project Report No. 46, Northern River Basin Study, Edmonton, Alberta.

R. L. & L. Environmental Services Ltd. 1993. Aquatic macroinvertebrate identifications, upper Athabasca River, spring, 1992. Project Report No. 5, Northern River Basin Study, Edmonton, Alberta.

R. L. & L. Environmental Services Ltd. 1994. A general fish and riverine habitat inventory, Athabasca River, April to May, 1992. Project Report No. 32, Northern River Basin Study, Edmonton, Alberta.

R. L. & L. Environmental Services Ltd. 1995. A general fish and riverine habitat inventory, Athabasca River, May, 1994. Northern River Basin Study, Edmonton, Alberta.

Scrimgeour, G., and P. A. Chambers. 1996. Identification of spatial and temporal patterns in nutrient limitation with herbivory effects, Wapiti, Smoky and Athabasca rivers, 1994. Project Report No. 53, Northern River Basins Study, Edmonton, Alberta.

Scrimgeour, G. J., P. A. Chambers, J. M. Culp, and C. L. Podemski. 1995. Identification of spatial and temporal patterns in nutrient limitation, Athabasca River, October to December, 1993. Project Report No. 49, Northern River Basins Study, Edmonton, Alberta.

SENTAR Consultants Ltd. 1993. Overview of the long-term changes in benthic macroinvertebrates in the Athabasca River near Hinton. Prepared for Weldwood of Canada Limited, Hinton Division, Saskatoon, Saskatchewan.

TAEM Ltd. 1989. Biological and water quality survey of the Athabasca River, 1989. Prepared for Weldwood of Canada Limited, Saskatoon, Saskatchewan.

TAEM Ltd. 1991a. Biological and water quality survey of the Athabasca River, 1990. Prepared for Weldwood of Canada Limited, Saskatoon, Saskatchewan.

TAEM Ltd. 1991b. Biological and water quality survey of the Athabasca River, April 1991. Prepared for Weldwood of Canada Limited, Saskatoon, Saskatchewan.

TAEM Ltd. 1992. Biological and water quality survey of the Athabasca River, April 1992. Prepared for Weldwood of Canada Limited, Saskatoon, Saskatchewan.

TAEM Ltd. 1993. Biological and water quality survey of the Athabasca River, October 1992. Prepared for Weldwood of Canada Limited, Saskatoon, Saskatchewan.

TAEM Ltd. 1996. Biological and water quality survey of the Athabasca River, 1994. Prepared for Weldwood of Canada Limited, Saskatoon, Saskatchewan.

Weldwood of Canada Limited, Hinton Division. 1996. Emergency spill response plan: annual report, Hinton, Alberta.

Chapter 3

Effects of BKME on the Athabasca River- An Artificial Stream Study

3.1 Introduction

Pulp and paper mill effluents contain a complex array of compounds and 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 (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, high levels of nitrogen and phosphorus contained in pulp mill effluents have produced significant changes in primary productivity in some rivers in western Canada and the United States (Bothwell 1992). Given our poor understanding of the nutrient and contaminant effects of pulp mill effluents on riverine biota, it is difficult to predict impacts on aquatic systems receiving these effluents, and to set rigorous regulatory guidelines (Owens 1991).

A variety of inhibitory compounds in bleached kraft mill effluent (BKME) are derived from the wood (e.g., resin acids, phenolics and PAHs). Resin acids often provide terrestrial plants with resistance to insect herbivores, and can inhibit feeding and growth (Chapman 1974, Elliger et al. 1976, Rosenthal and Janzen 1979, Wagner et al. 1983, Harborne 1990, Xie et al. 1993). Catechol, a phenolic lignin residual, can reduce food assimilation (Reese and Beck 1976) and cause developmental abnormalities and increased mortality in mosquito larvae (Desmarchelier and Fukuto 1974). Chlorinated lignin residuals may affect feeding in hydropsychid caddisflies by causing the production of deformed feeding-net structures (Petersen and Petersen 1984). Similarly, pulp mill effluent inhibits feeding in a variety of aquatic organisms (McLeese 1973,

Cooley 1977, Crane and Maltby 1991, Bitton et al. 1995). Thus, inhibitory compounds in BKME may lead to a suite of ecological effects, including decreased feeding, growth, and fecundity of riverine grazers.

Alternatively, nutrients (N and P) can counter or modify the effect of contaminants in BKME. Addition of limiting nutrients can increase food resources for invertebrates, which are an important nutritional base for riverine fish. This elevated production of plant material may increase invertebrate growth and fecundity (Hershey et al. 1988). Nutrient stimulation of primary production can also alter the amount and route of exposure of aquatic biota to contaminants through biomass dilution of contaminants (Taylor et al. 1996) and increased sedimentation rates (Kramer et al. 1996). Addition of nutrients can reduce toxicity of contaminants to primary producers (Lozano and Pratt 1994), and nutrient-related changes in algal community structure (i.e., increased predominance of inedible blue-green algae) may affect bioaccumulation in higher trophic levels (Kramer et al. 1996). Finally, contaminants can reduce grazing rates (toxic anorexia) thereby increasing the biomass of primary producers (toxic eutrophication) (Scholten et al. 1996).

The Athabasca River is oligotrophic in its upper regions, with soluble reactive phosphorus concentrations (SRP) usually below 2 µg/L (Chambers 1996). Dale and Chambers (1995a,b) reported that additions of phosphorus as low as 1 µg/L to this system increased periphyton biomass. A combined pulp mill and municipal effluent outfall at Hinton, Alberta is the first continuous discharge to the river. Nutrient loading from the Weldwood outfall (combined with Hinton municipal effluent) causes a 33% and 58% increase in TP and TN, respectively (Chambers 1996), thus nutrient addition via bleached kraft mill effluent is likely to significantly affect this nutrient-poor system. Since 1990, effluent from this mill has consistently shown low toxicity in bioassays (SENTAR 1997). Given the low toxicity of this effluent and the oligotrophic nature of the river, the impact of nutrients in the effluent may outweigh contaminant effects. If nutrient enrichment from pulp mill effluent has a more significant impact on this

system than contaminants, the current practice of not regulating effluent nutrient content (Chambers 1996) may need to be re-evaluated.

3.2 Objectives and Experimental Design

My main research objectives were to measure the effects of a bleached kraft pulp mill effluent on benthic biota of the Athabasca River and, through a comparison with an equivalent nutrient addition, determine if the primary effect of effluent was nutrient enrichment or whether significant evidence of toxicity also existed. Specifically, the effects of an addition of BKME to a 1% dilution and that of an addition of nutrient (nitrogen and phosphorus) contained in the effluent were compared. A 1% dilution was chosen because, based on historical flow data, this is the approximate dilution in the Athabasca during early autumn when this experiment was conducted (Fig. 3.1). A comparison of the control and nutrient treatments provided a measurement of nutrient effects,

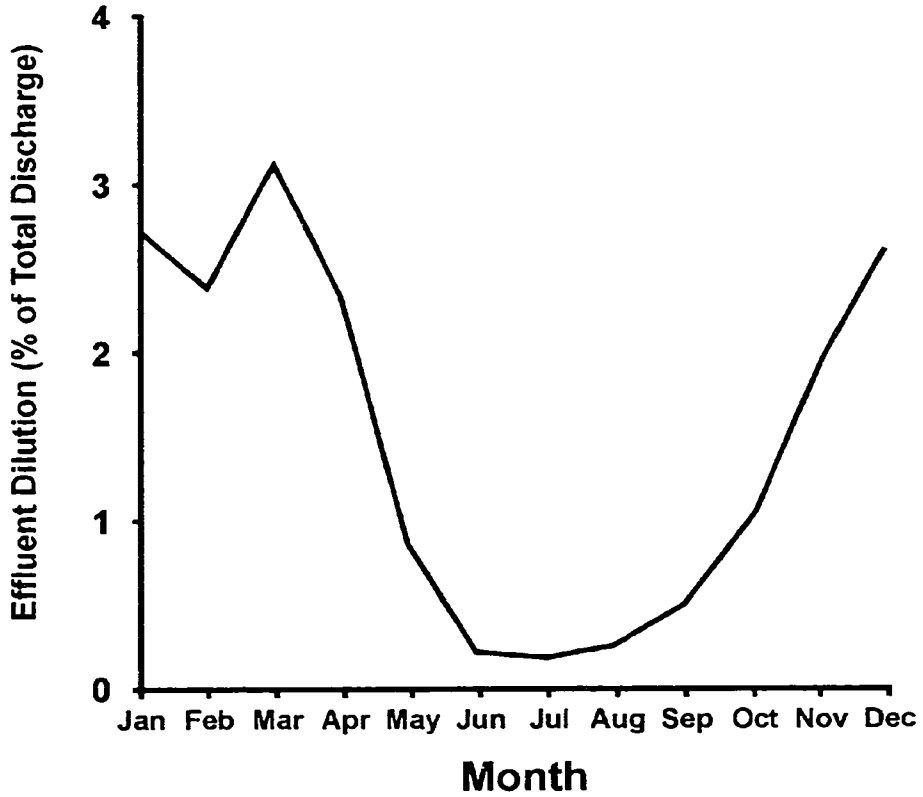


Figure 3.1. Seasonal variation in effluent (Weldwood outfall) dilution in the Athabasca River at Hinton, Alberta.

and a comparison of the nutrient and effluent treatments provided a measure of contaminant effects. Although this approach does not address the question of an interaction in a statistical sense, there was no feasible means to provide contaminants separately, and thus complete a factorial experiment. Response variables included community composition and biomass (measured as ash-free dry mass (AFDM) and chlorophyll *a* (Chl*a*)) of periphyton, abundance and community composition of benthic insects, and biomass of abundant invertebrates including specific mayfly and stonefly taxa. Autumn was chosen as the season for study because it is a period of low flow when effluent concentration is relatively high, water temperatures are adequate for algal growth, and turbidity is low. Thus, autumn is predicted to be a time of maximum effluent influence.

The experiment was carried out in an artificial stream system designed specifically for this work. Inferential hypothesis testing through field assessments is often impossible due to the challenge of obtaining true replicates in a lotic environment, and because of the high degree of spatial heterogeneity typical of benthic communities (Stewart-Oaten et al. 1992; Cooper and Barmuta 1993, Buikema and Voshell 1993, Forbes and Forbes 1994). Artificial streams allow control of environmental conditions, eliminating confounding factors and reducing "noise" due to spatial heterogeneity (McIntire 1993). Most importantly, treatments (e.g., addition of effluent) are applied to true replicates, allowing uncompromised hypothesis testing. Concurrent field sampling for the same response variables provides weight-of-evidence for concluding that field patterns are caused by effluent discharge, and provides some reassurance that the experimental system adequately simulated the natural environment.

3.3 Methods

3.3.1 The Stream System

This section provides a brief description of the experimental stream facility (Plate 3.1); a detailed description of the system is available in Appendix

4. The stream system was constructed beside the Athabasca River to allow the experimental streams to experience ambient temperature, water quality, and light regimes. The stream system consisted of 16 circular, 0.9 m^2 surface area tanks made of polyester fiberglass. Theses tanks were placed in pairs on tables that were 74 cm high. Water from the river was pumped into a 378 L polyethylene head tank placed on a 1.2 m high platform, and gravity-fed through a system of pipes to the stream tanks. Gate valves controlled water flow to individual streams, and allowed flow rate calibration for each stream. Water flow to each stream was set at 2 L/min and residence time for each tank was about 2 h. Water depth in the tanks was maintained at 27 cm by an overflow drain that was screened to limit emigration of insects; each stream contained 227 L of water. Current in each stream was created by a belt-driven propeller. The head tank and all water delivery lines were wrapped with heat tape and insulated to allow the system to be operated in the late autumn when freezing temperatures often occurred. Waste-water from the streams was returned directly to the river.

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. Effluent and nutrient solutions were heated (25-30°C) and stored in insulated containers to prevent the < 2 mm supply lines from freezing. Tubes carrying treatment solutions were threaded through foam pipe insulation to the streams, then fed into the water delivery spout.

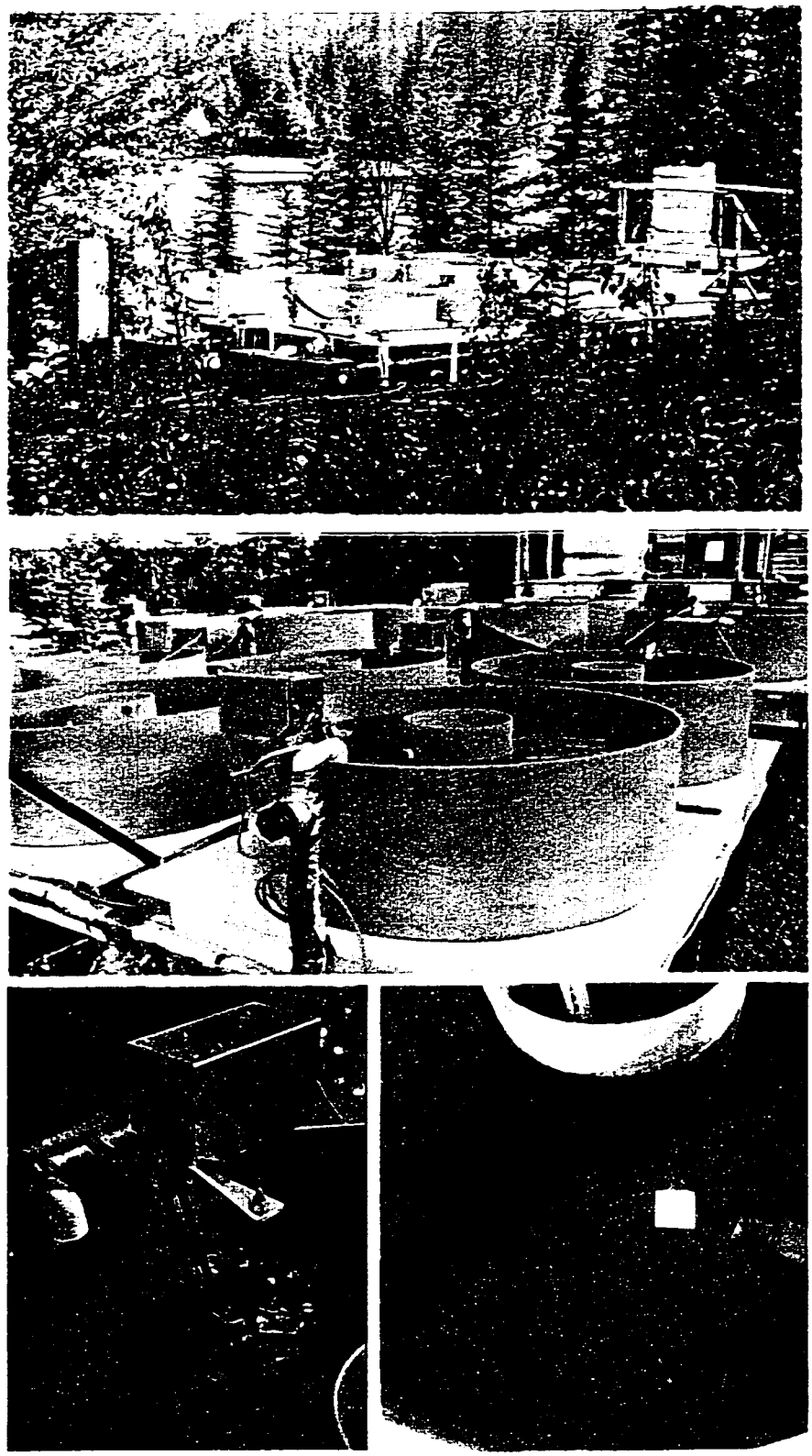


Plate 3.1 Artificial stream system: (a) stream system; (b) close-up of individual streams; (c) motor and propeller used to create water flow; and (d) stream substratum and tiles used for periphyton sampling.

3.3.2 Methods of Artificial Stream Operation

A standardized benthic environment was created in each stream to simulate riffle substrate found along the upper Athabasca River, upstream of the Weldwood mill outfall. The bottom of each stream was covered with an 8 cm layer of washed gravel (1-2 cm diameter stones) upon which 10 larger stones (‰ surface area = 535 cm^2) from the river were placed. These stones stocked the streams with a natural community of periphyton. Unglazed porcelain tiles (23.5 cm^2) were used to provide a standardized substratum for periphyton development and accumulation. During collection, the stones were enclosed with a 0.1 m^2 U-net (Scrimgeour et al. 1993), carefully lifted from the streambed and placed into a container (two stones per container) with 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. The stones and their biota were immediately transported and randomly assigned to the artificial streams. An additional 30 benthic samples (biota only) were collected from a second upstream riffle and 2 samples were randomly assigned to each stream. This random allocation of 12 samples per stream ensured that invertebrate composition was initially similar among streams. Sampling sites and location of the stream system with respect to the mill's outfall are shown in Plate 3.2. One additional stream was not seeded with invertebrates so that immigration of invertebrates via inflow water could be estimated. Ten days after initial colonization of the streams, the peristaltic pumps were started and the experiment began.

The distribution of water velocities in the streams was characterized in the laboratory with a substratum similar to the Athabasca experiment, using a Nixon Instruments ® velocity meter. Mean velocities (± SE) recorded at 21 locations around the stream were similar for all 3 water 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.

Mean velocity in the field experiments (above stones) was similar to laboratory tests (‰ = 0.26 ± 0.01 m/sec, n= 150), and was also similar to water velocity measured above stones at a similar water depth in the Athabasca River at Hinton (water velocity ‰ = 0.26 ± 0.01 m/sec, water depth ‰ = 24.8 ± 0.72 cm, n = 30) while the experiment was being conducted. Visual observations of dye traces indicated that full mixing occurred within the first quarter of the stream length (Appendix 4).

Water temperature was monitored with Ryan® thermographs placed in a stream and the head tank. Temperatures in the head tank reflected the temperature of incoming river water. A comparison of data from the 2 thermographs indicated that 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, the streams were cooler at night and warmer during the day as compared to the incoming river water (Fig. 3.2). The maximum instantaneous difference between water temperature in the river and the streams was < 5 °C.

Streams were inspected daily and drain screens were brushed clean to prevent clogging and overflow of the streams. Motors were lubricated with non-toxic, Permatex Superlube ® (food grade USDA H1) every 3-6 d to prevent seizing of the propeller shafts. The 10 cm water delivery lines were flushed regularly to remove silt and sand deposits. Water and treatment solution flows to each stream were calibrated regularly. Effluent and nutrient delivery tubing had to be inspected for blockages and wear from the pump heads, and changed as required.

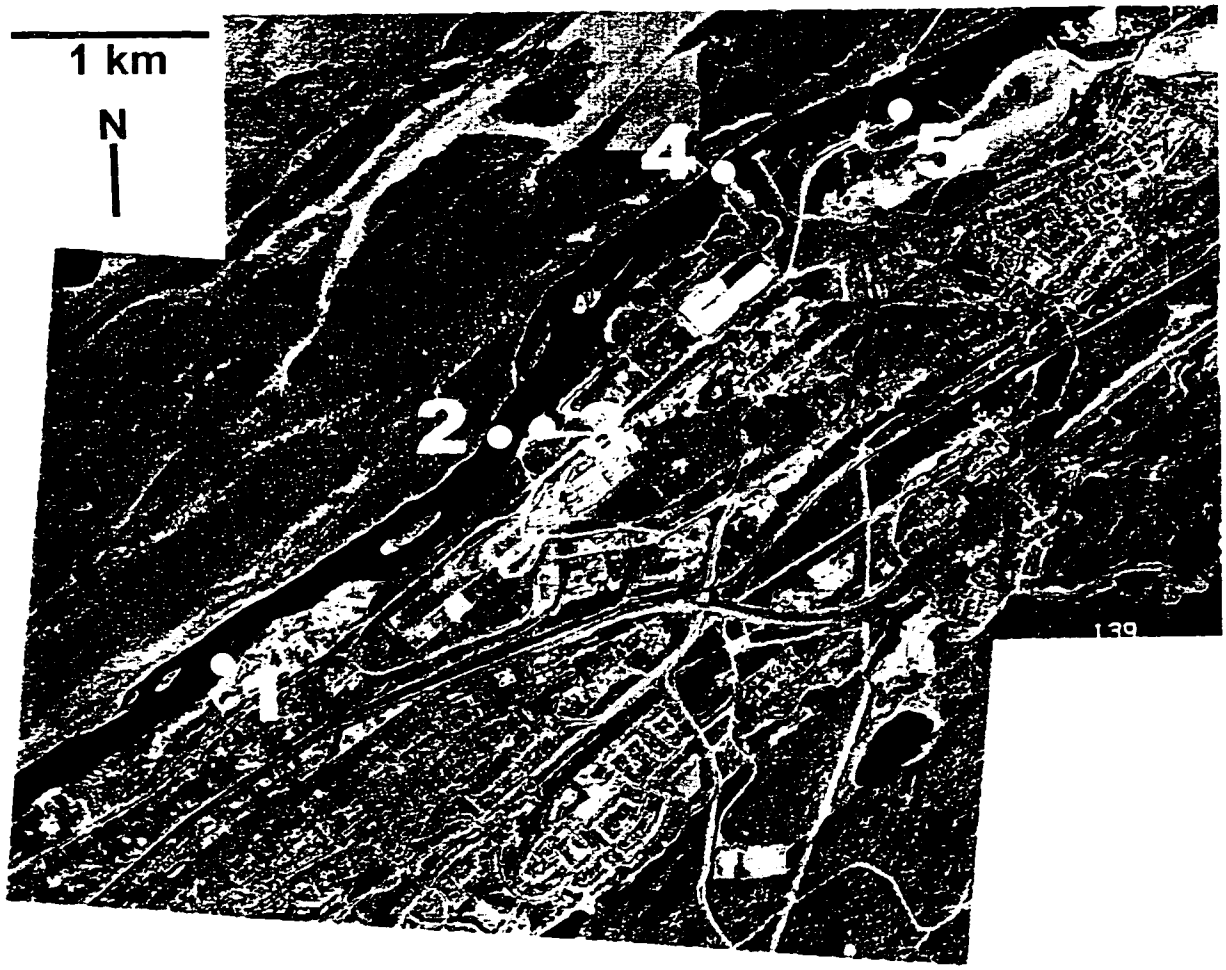


Plate 3.2 Aerial Photograph of Hinton, Alberta (August 1996), with the following locations indicated: (1) and (2) upstream riffles where rocks and invertebrates were collected to seed the streams; (3) location of the artificial stream system; (4) mill effluent. Photo courtesy of Airphoto Services, Alberta Environmental Protection.

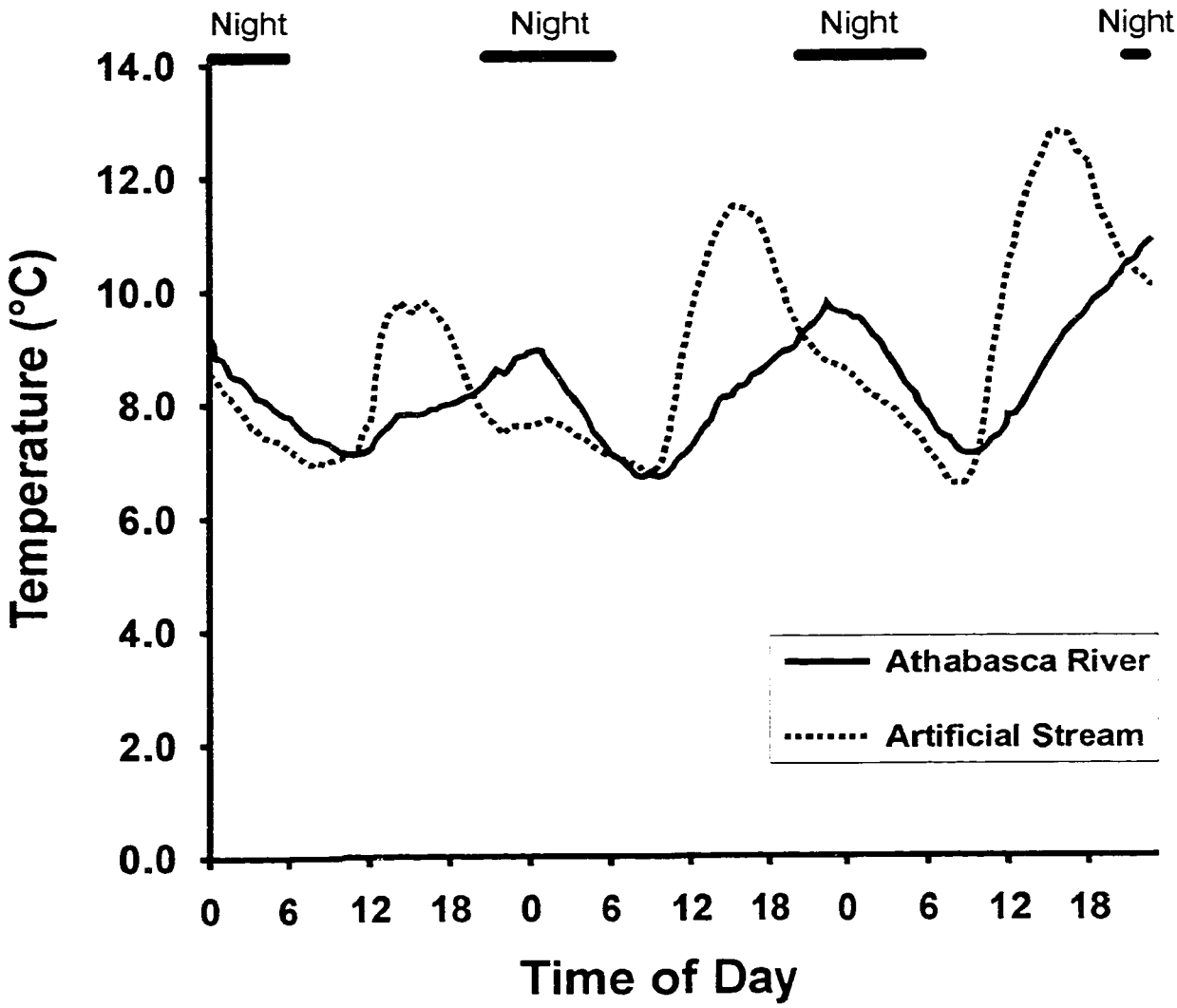


Figure 3.2. Temperature of incoming river water and in the artificial streams.

3.4 Treatments

The experiment 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 N and P (NP treatment) found in the effluent (i.e., same N and P concentrations as in the 1% effluent). Replicate streams (n = 5) were randomly assigned to each treatment, and the experiment ran for 28 d. 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 immediately shipped, on ice, to the University of Alberta, Limnology Laboratory,

to be analyzed for soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen [N as ammonium ($N\text{-}NH_4^+$) and N as nitrate + nitrite ($N\text{-}NO_2+N\text{-}NO_3$)]. Throughout the thesis the dissolved inorganic nitrogen components, ammonium and nitrate + nitrate, are abbreviated as $N\text{-}NH_4^+$ and $N\text{-}NO_2+NO_3$, respectively. A solution with concentrations equivalent to the median values of the effluent samples was added to the NP streams for 8 d, followed by a 1 d nutrient spike application containing concentrations equivalent to the highest effluent value recorded during the previous 8 d. A nutrient spike was used because effluent concentrations were highly variable (Fig. 3.3) and Bothwell (1992) showed that periphyton communities were able to utilize nutrient spikes to achieve higher long-term growth rates. 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. Median and spike effluent concentrations are listed in Table 3.1.

Table 3.1. Median and spike concentrations (µg/L) of SRP, $N\text{-}NO_2+NO_3$, and $N\text{-}NH_4^+$ during the 28d experiment. 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	Treatment	Nutrient Concentration		
		SRP	NO_2+NO_3	NH_4^+
		µg/L		
1-8	median	218	548	1502
9	spike	240	761	1578
10-17	median	222	683	1311
18	spike	219	251	3390
19-26	median	161	245	942
27	spike	307	217	2762
28	median	257	151	1965

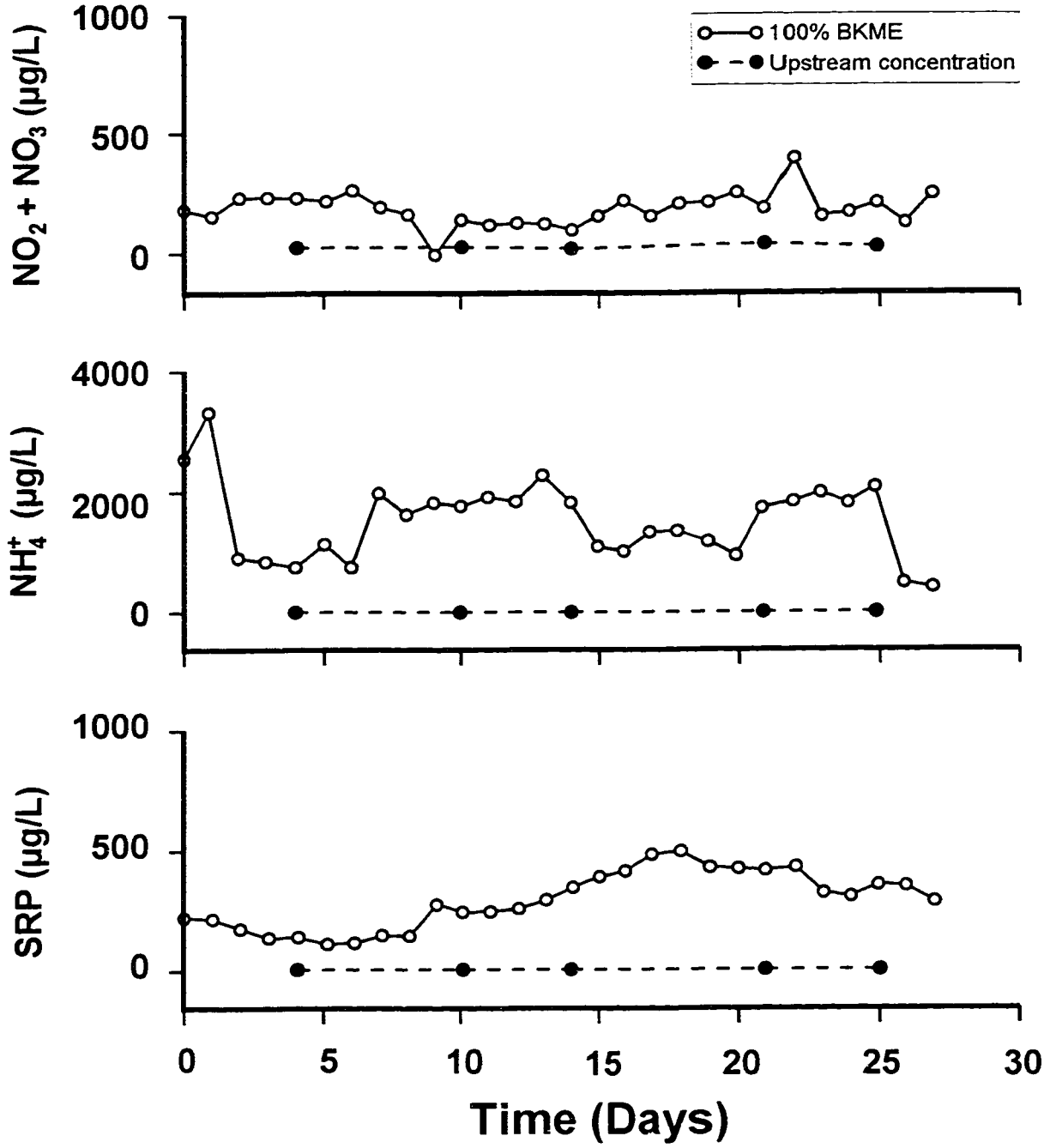


Figure 3.3. Concentrations of NO_2+NO_3, NH_4^+, and SRP in effluent and in the upstream river water during the experiment.

3.5 Data Collection

Water samples were collected from each experimental stream once every 5 d. 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 N analysis were placed in polystyrene bottles. Samples for TDP and SRP were filtered through pre-washed 0.45 mm Millipore filters; TP and TDP samples were digested and analyzed by Menzel and Corwin's (1965) potassium persulfate method. Samples for $N\text{-}NO_2+NO_3$ were filtered through pre-washed 0.45 mm Millipore membrane filters. A Technicon autoanalyzer (Stainton et al. 1977) was used to analyze for $N\text{-}NH_4^+$ and $N\text{-}NO_2+NO_3$. Alkalinity and bicarbonate were determined following standard methods (APHA 1985).

Periphyton samples were collected every 5 d from 1 randomly selected stone and one tile in each replicate stream. 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 a tile was sampled. Periphyton samples were placed in vials and held on ice until frozen later the same day. Samples were collected from rocks in a similar manner at sites in the river, 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 experiment, periphyton samples from a rock in 4 streams per treatment and from 4 rocks in the river above and below the effluent outfall were collected in the same manner as samples for biomass determination, and were preserved in Lugol's solution for later taxonomic

identification. At the downstream site, samples were collected from both north and south banks as the effluent is incompletely mixed at this point (see Chapter 2, Plate 2.1). Identification and enumeration of the algal community was done by a research laboratory specializing in this field. Detailed methods for enumeration and identification of these samples are in Appendix 5.

For analysis of contaminants, samples of periphyton were collected from the sides of 2 streams in the nutrient and effluent treatments. The NP treatment served as a control for exposure to pulp mill contaminants as there was insufficient material for analysis of the control treatments. Samples were collected with a clean, acetone and hexane-rinsed metal spatula, placed in clean, acetone and hexane-rinsed glass bottles, and kept on dry ice while being transported to the laboratory. These samples were shipped to Zenon Environmental Laboratories, Vancouver, British Columbia, for analysis of chlorophenolics and PAHs, and to Enviro-Test Laboratories, Edmonton, Alberta, for analysis of resin acids, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans.

All invertebrates in the streams 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). Benthic samples were also collected in the river, upstream and downstream of the effluent discharge. Invertebrates were preserved immediately in 10% formalin. The majority of invertebrates were insects, thus, analysis focused on this taxonomic group. Samples were sorted under 12x magnification, identified and enumerated. All insects were identified to genus whenever possible (taxonomic references included Merritt and Cummins (1984), and Stewart and Stark (1993)), however, due to the large numbers of immature animals, many individuals were identified only to family. Size of numerically dominant taxa (excluding Chironomidae) was estimated by measuring thorax length 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, allowing them to cool in a dessicator and then weighing them on a CAHN C-31 Series microbalance.

3.6 Statistical Analysis

The Chl*a* content and AFDM of periphyton on rocks and tiles at the end of each experiment was compared with a one-way ANOVA after the data were checked for heteroscedasticity and normality. Transformations were applied if necessary. Means were compared with Fisher's protected Least Significant Difference (LSD) with (α=0.05). Principal component analysis (PCA) was used to identify treatment-related patterns in periphyton community composition. Community composition data was first converted to percent abundance as the large range in the raw data would have resulted in the first component representing changes in abundance rather than community composition.

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. The data set was reduced in this manner after it was observed that relatively unimportant genera (i.e., found in low abundance and in a few streams) were influential in the genera-level PCAs. Thorax lengths and weights of the insects were compared by one-way ANOVA after checking for heteroscedasticity and normality, and applying transformations if necessary. Mean comparisons were done using Fisher's protected LSD with α=0.05. Kruskal-Wallis analysis was used if transformations were not successful in reducing heteroscedasticity.

3.7 Results

3.7.1 Effluent and Water Chemistry

Soluble reactive phosphorus (SRP), N-NO_2+NO_3, and N-NH_4^+ were measured in the effluent each day throughout the 28 d experiment (Fig. 3.3). With the exception of one observation for N-NO_2+NO_3, effluent concentrations

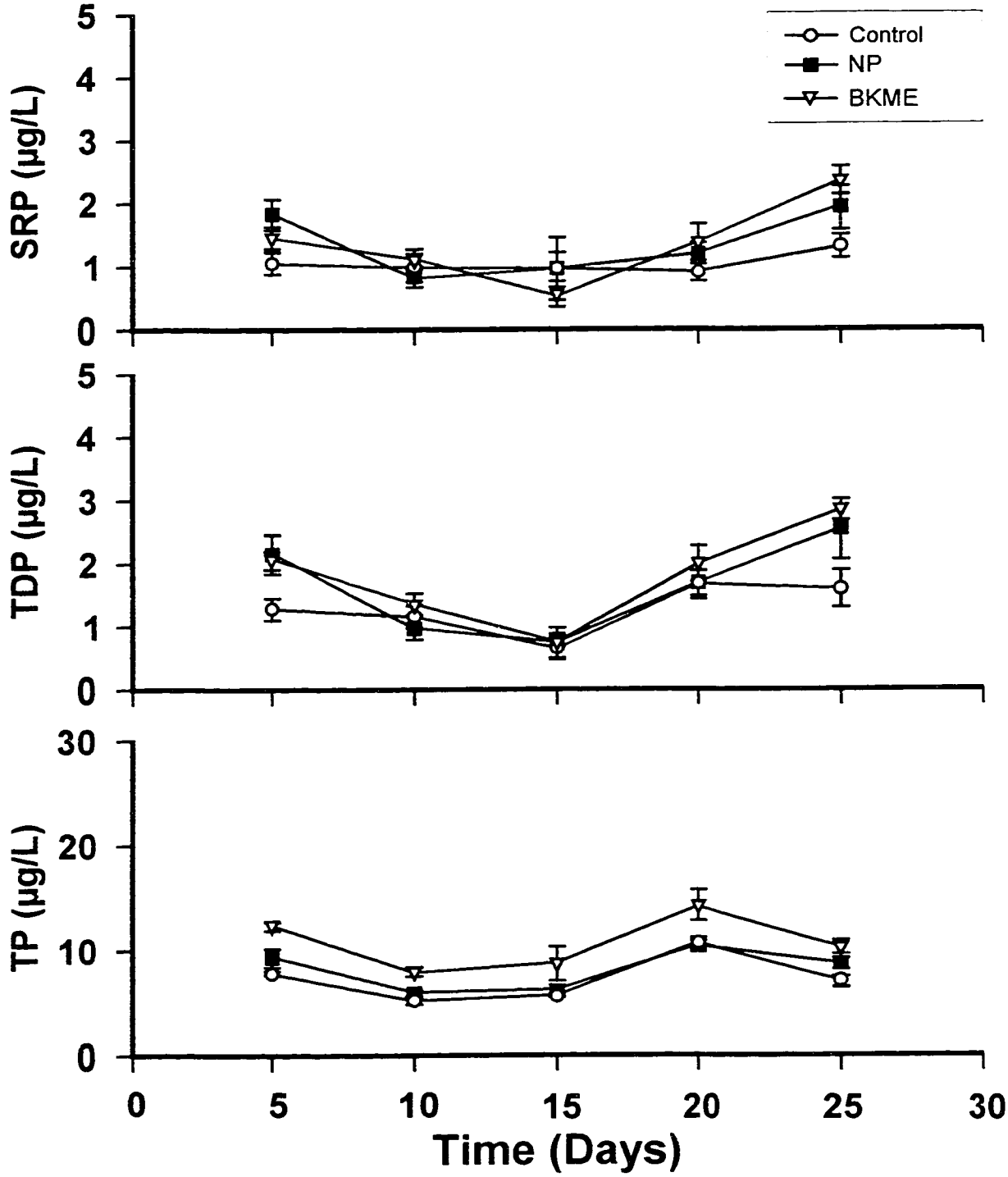


Figure 3.4. Mean (± SE) water column SRP, TDP, and TP concentrations (µg/L) in the control, nutrients (NP) and BKME treatments during the 28 d experiment

were always greater than those in the Athabasca River upstream of the effluent discharge. All three variables fluctuated widely throughout the experiment; the value of a particular variable sometimes changed up to 1 order of magnitude over a 24 h period. The greatest 24 h change was 153 to 282 µg/L for SRP (7-8 October), 187 to 18 µg/L for $N\text{-}NO_2+NO_3$ (7-8 October), and 2211 to 588 µg/L for $N\text{-}NH4^+$ (24-25 October). Over the course of the experiment, effluent concentrations of SRP, $N\text{-}NO_2+NO_3$, and $N\text{-}NH_4^+$ ranged between 122-515 µg/L, 18-430 µg/L, and 315-2365 µg/L, respectively.

Most of the total dissolved phosphorus (TDP) in all three treatments was in the form of SRP (Fig. 3.4). Total dissolved phosphorus concentrations never exceeded 23% of the total phosphorus (TP) in any treatment, thus ,> 75% of the TP was in 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. Total phosphorus in effluent streams was generally higher than in NP and control streams. Additionally, $N\text{-}NO_2+NO_3$ and $N\text{-}NH_4^+$ concentrations were similar across treatments despite the fact that the NP and BKME treatments were enriched with nitrogen (Fig. 3.5). As expected, nutrient concentrations in the streams had a narrower range relative to the effluent samples (Figs 3.4 and 3.5), 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 $N\text{-}NO_2+NO_3$, and 7 to 25 µg/L for $N\text{-}NH_4^+$. For all treatments, $N\text{-}NO_2+NO_3$, and $N\text{-}NH_4^+$ were at higher concentrations at the end of the experiment compared with initial values (Fig. 3.5). Conductivity, alkalinity, bicarbonate and pH (Table 3.2) were similar among treatments except for the higher conductivity in effluent streams.

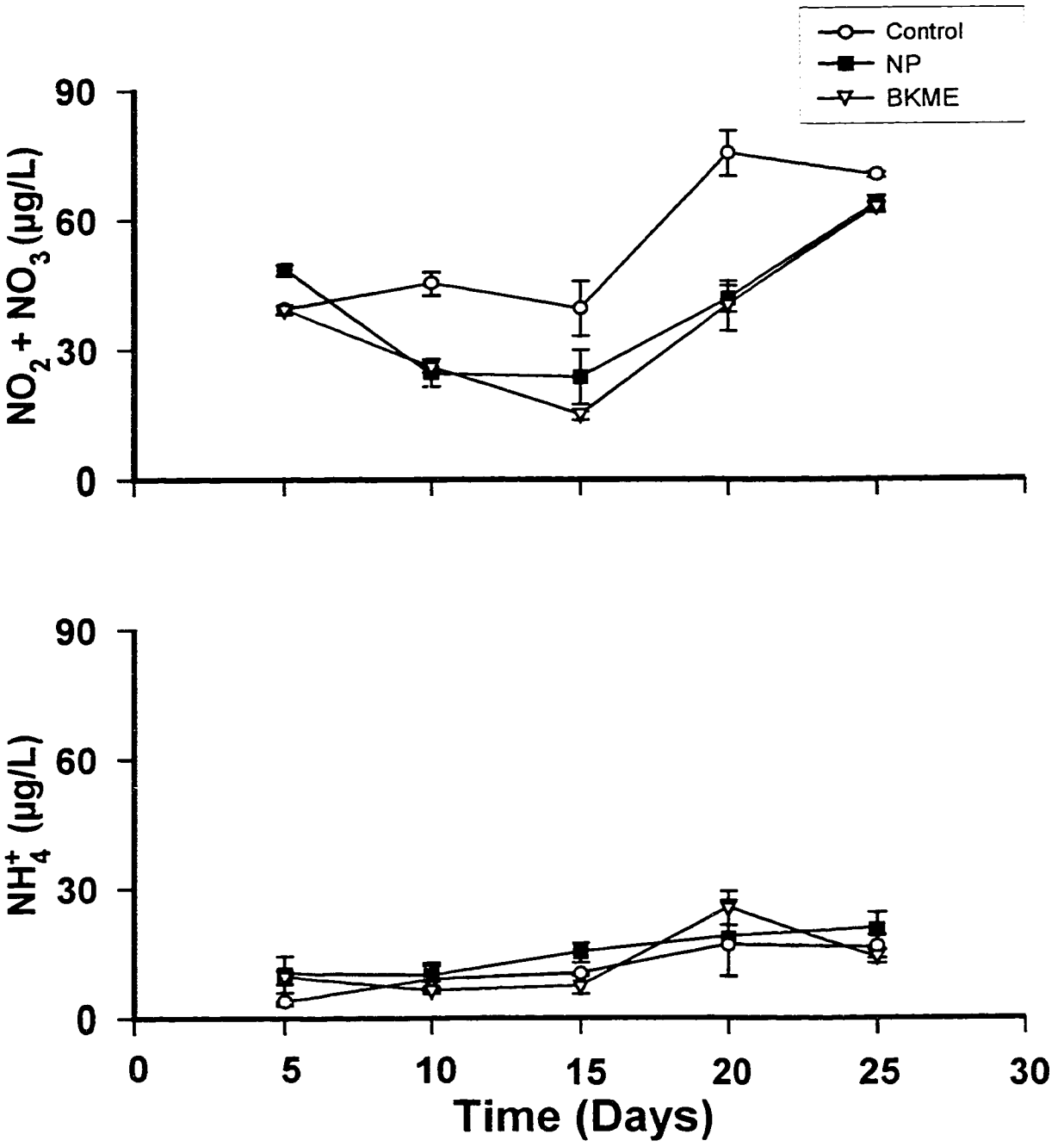


Figure 3.5. Mean (±SE) water column N-NO_2+NO_3 and N-NH_4^+ concentrations in the control, NP and BKME treatments during the 28 d experiment.

Table 3.2. Mean (±SE) conductivity (µmhos/cm), alkalinity (mg/L as $CaCO_3$), bicarbonate (mg/L), and pH in the control, nitrogen-phosphorus (NP), and 1% BKME treatments.

VARIABLE	TREATMENT		
	CONTROL	NP	1% BKME
Conductivity (µmhos/cm)	315 ± 12	314 ± 13	328 ± 13
Alkalinity (mg/L as $CaCO_3$)	130 ± 2	129 ± 1	131 ± 2
Bicarbonate (mg/L)	159 ± 3	152 ± 3	155 ± 4
pH	8.1 ± 0.1	8.2 ± 0.1	8.2 ± 0.1

3.7.2 Periphyton in Artificial Streams and the Athabasca River

3.7.2.1 Biomass

Periphyton Chl*a* on rocks and tiles increased rapidly in streams receiving either 1% BKME or NP additions compared to control streams (Fig. 3.6). After 25 d, periphyton Chl*a* on tiles in effluent- and nutrient-treated streams was approximately 30 times greater than in control streams (Fig. 3.6). There was a significant treatment effect ($F_{2,12}$=242.77, p<0.001) with NP and BKME treatments significantly different from the controls (p<0.050). There was no difference in the Chl*a* content of effluent and nutrient streams. Significant differences among treatments were also found in the Chl*a* content of periphyton on rocks ($F_{2,12}$=7.14, p< 0.009; Fig. 3.6). Again, Chl*a* in control streams was significantly lower than that of the effluent treatment (p<0.050), and the Chl*a* content of periphyton in the BKME and NP streams did not differ.

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<0.050) (Fig. 3.7.). Biomass levels were significantly higher in streams receiving BKME or nutrient additions compared with control streams (p<0.05), and AFDM in the

effluent and NP streams were similar. Measurements of AFDM on tiles were not made 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, final periphyton AFDM on rocks was similar among treatments ($F_{2,12}$=0.87, p>0.445).

Mean (±SE) periphyton biomass (expressed as Chl*a*) on Athabasca River rocks was 58.5 (±12) µg/cm^2, 0.8 km downstream of the effluent discharge, almost 9 times higher than the mean biomass on rocks at the upstream reference site (‰= 6.60 µg/cm^2 ± 1.8; $t_{4,\ 0.05/2}$ = -4.21, p= 0.014). There was no significant difference between the AFDM on rocks at upstream (‰= 5.66 ±1.1 µg/cm^2) and downstream sites (‰= 7.32 ±1.4 µg/cm^2; $t_{4,\ 0.05/2}$=0.9, p>0.372; Fig. 3.8).

3.7.2.2 Diatom Community Composition

Periphyton communities in the artificial streams and the river were numerically dominated by diatom species. In the stream system, diatom species richness declined from an average of 41.5 species in control streams, to 31.8 and 26.5 in the NP and BKME treatments, respectively. A PCA of the community composition data showed treatment separation along the first axis (Fig. 3.8a), which accounted for 89% of the variation in the data set. An examination of eigenvectors indicated that only 2 taxa, *Diatom tenuis* and *Achnanthes minutissima*, contributed to this axis. *Diatom tenuis*, a numerically dominant member of the algal community, increased from 12.36% in the controls to 36.3% and 51.0% of total abundance in the NP and BKME treatments, respectively (Fig. 3.8b). The relative abundance of *Achnanthes minutissima* decreased from 22.8% in the controls to 13.1% and 7.7% of total abundance in the NP and BKME treatments, respectively.

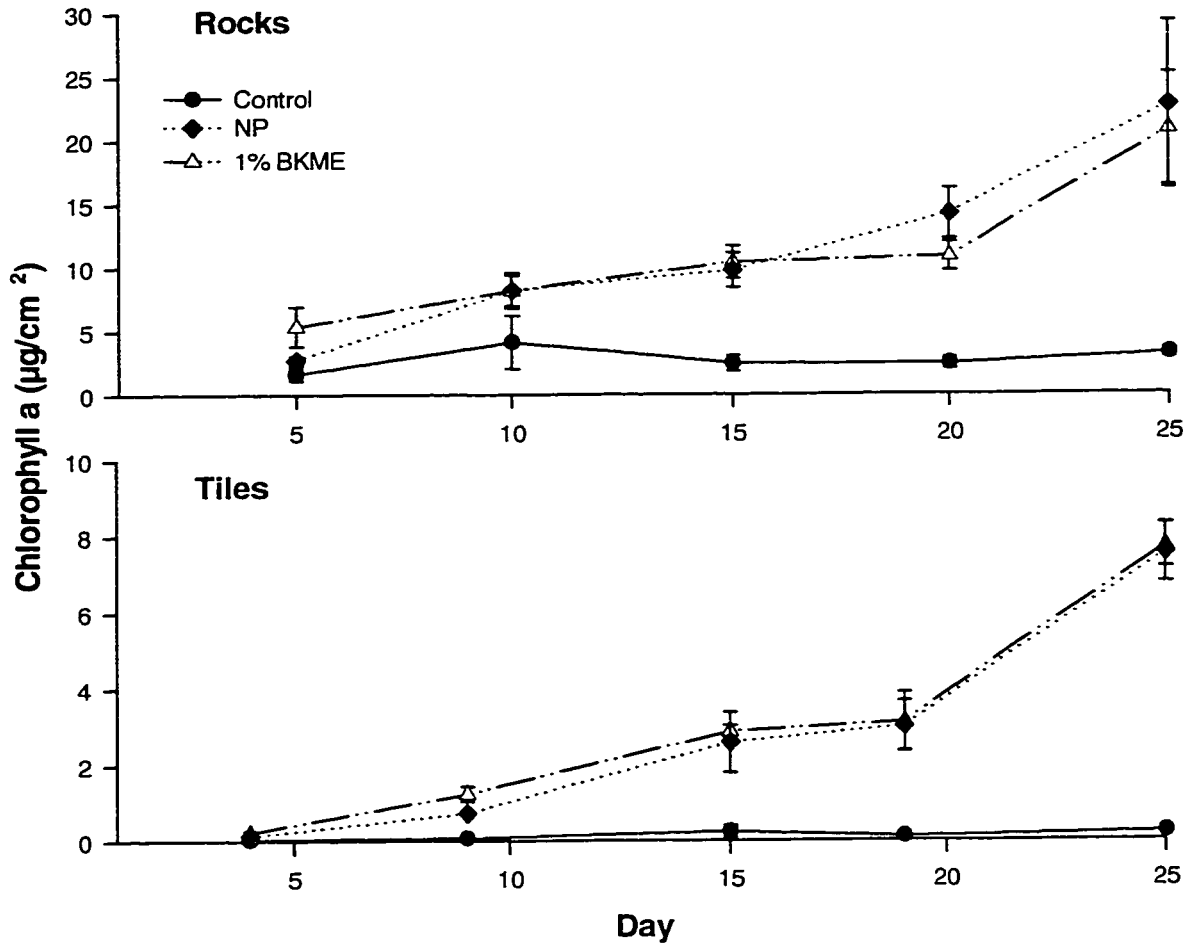


Figure 3.6. Increase in periphyton Chl*a* (µg/cm^2 ± SE) on tiles in the control, NP, and BKME treatment, as measured every 5 d during the 28 d experiment.

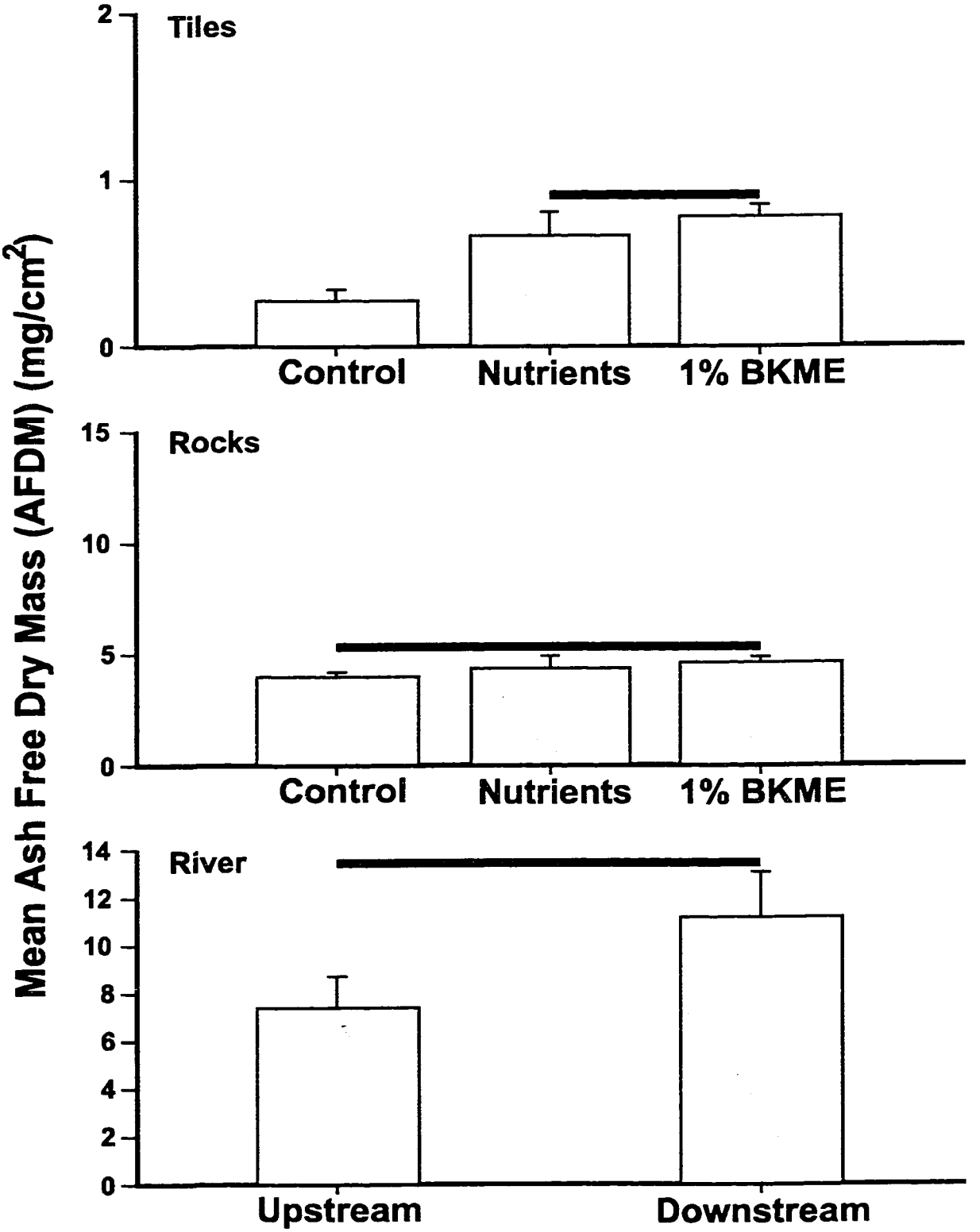


Figure 3.7. Final (day 25) periphyton AFDM ($mg/cm^2 \pm$ SE) on tiles and rocks in the control, NP, and BKME treatments in the artificial stream experiment, and on rocks in the Athabasca River, upstream and downstream of the effluent outfall. Bars connect means that are not significantly different at α=0.05.

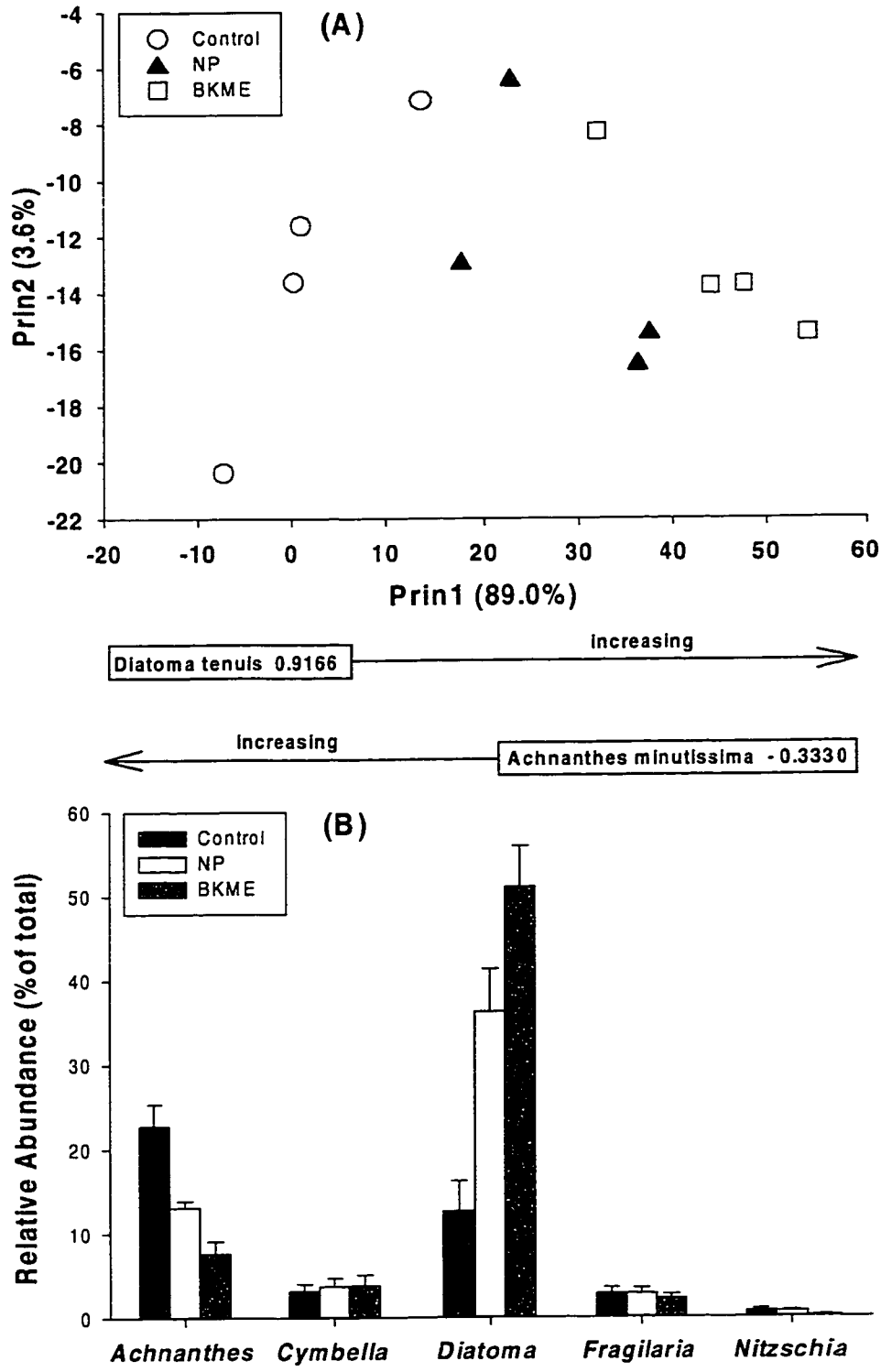


Figure 3.8. Analysis of diatom community composition on rocks: (A) location of samples on the first and second principal component axes, variance accounted for by each axis and taxa with eigenvectors greater than 0.25 on the first axis are indicated; and (B) relative abundance of five most common diatom taxa in the control, NP, and BKME treatments.

Diatom species richness on rocks in the Athabasca River declined similarly with exposure to effluent, averaging 38.2 species at the upstream site and 33.8 and 29.0 species at the downstream site on the north and south banks, respectively. A PCA on the community composition data accounted for 69.8% of the variation in data in the first two axes. Upstream and downstream sites separated along the first axis, which accounted for 48.7% of variation in the data (Fig. 3.9). *Achnanthes minutissima* at the upstream site, was relatively more abundant, similar to the control streams, while periphyton at the downstream site had a greater relative abundance of the diatoms *Fragilaria vaucheriae* and *Cymbella silesiaca* (Fig. 3.10). Community composition at the downstream north bank was intermediate between the upstream and downstream south-bank sites, and was similar to the BKME and NP treatment streams. North-bank periphyton had a lower relative abundance of *Achnanthes minutissima* and higher relative abundance of *Diatoma tenuis* than the upstream site and had a greater relative abundance of *Achnanthes minutissima*, and less *Fragilaria vaucheriae* and *Cymbella silesiaca* than south-bank periphyton.

3.7.2.3 Periphyton Contaminant Analysis

Contaminant analysis was done on only 4 streams (2 exposed 2 non-exposed), thus statistical analysis was not attempted. Results suggest that there was little difference in the dioxin and furan content of exposed and non-exposed periphyton (Table 3.3). Exposed periphyton had a higher concentration of dehydroabietic acid than unexposed periphyton, and there was a slightly higher concentration of phenanthrene, the only PAH detected, in unexposed periphyton.

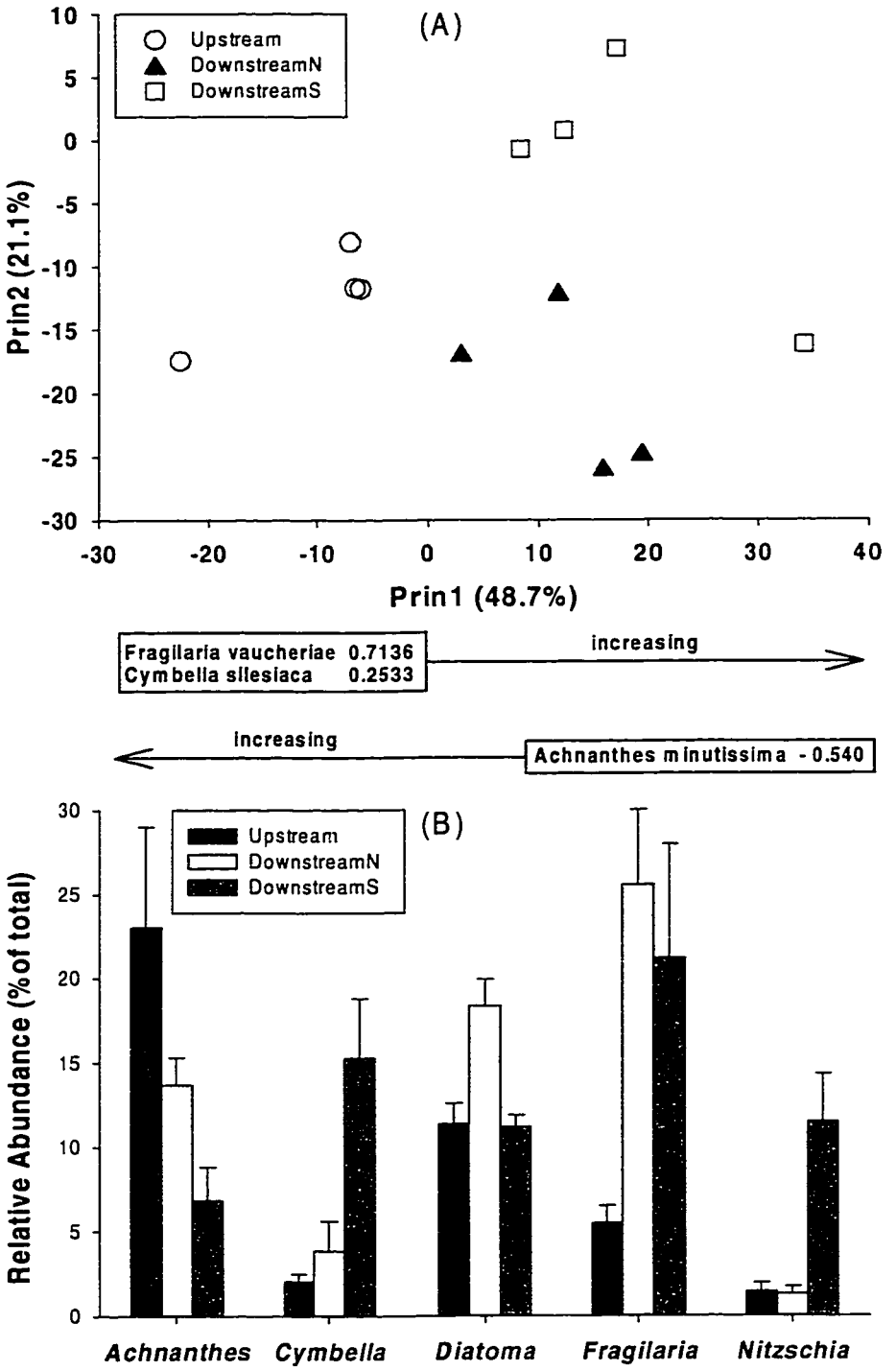


Figure 3.9. Diatom community composition on rocks in the Athabasca River, upstream and downstream (north and south banks) of the outfall: (A) location of samples on the first and second principal component axes, with variance accounted for by each axis and taxa with eigenvector loadings greater than 0.25 on the first axis indicated; and (B) relative abundance of five most common diatom taxa at the upstream and downstream (north and south banks) sites. Note that the downstream north bank (N) site receives less exposure to effluent than the south bank (S) (see Chapter 2).

Table 3.3. Concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (pg/g), resin acids (µg/L), and PAH's (µg/g) in periphyton samples collected from 2 nutrient (unexposed) and 2 BKME (exposed) streams at the end of the experiment; (ND = nondetectable, *=did not meet ratio criteria).

	Unexposed		Exposed	
	sample 1	sample 2	sample 1	sample 2
Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans (pg/g)				
DiCDD	ND	ND	ND	ND
TriCDD	ND	ND	ND	ND
TDCC	ND	ND	ND	ND
PeCDD	ND	ND	ND	ND
HxCDD	ND	ND	ND	ND
HpCDD	0.2	0.2	0.4	0.3
OCDD	3.3	2.6	2.9	4.7
DiCDF	ND	ND	ND	ND
TriCDF	ND	ND	0.2	ND
TDCF	ND	ND	ND	ND
PeCDF	ND	ND	ND	ND
HxCDF	ND	ND	ND	ND
HpCDF	ND	ND	ND	ND
OCDF	0.5	0.3	ND	ND
Resin Acids (µg/g)				
Abietic acid	ND	ND	ND	0.36*
Dehydroabietic acid	0.20	ND	0.63	0.44
12,14 dichlorodehydroabietic	ND	ND	ND	0.019*
12-chlorodehydroabietic acid	ND	ND	ND	0.022*
14-chlorodehydroabietic acid	ND	ND	ND	ND
PAH's (µg/g)				
Benz(a)anthracene	ND	ND	ND	ND
Dibenz(a,h)anthracene	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND
Benzo(b+k)fluoranthene	ND	ND	ND	ND
Benzo(g,h,I)perylene	ND	ND	ND	ND
Pyrene	ND	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND
Ideno(1,2,3-c,d)pyrene	ND	ND	ND	ND
Acenaphthene	ND	ND	ND	ND
Acenaphthylene	ND	ND	ND	ND
Anthracene	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	ND
Fluorene	ND	ND	ND	ND
Naphthalene	ND	ND	ND	ND
Phenanthrene	0.011	0.013	0.006	0.006

3.7.3 Insect Abundance and Biomass

Total insect abundance was significantly increased by the 1% BKME and NP treatments ($F_{2,12}$=5.15, p<0.024) (Fig. 3.10). Five taxa, *Ameletus* and baetid mayflies, capniid and nemourid stoneflies, 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) were more abundant in the 1% BKME and NP treatments relative to the controls (Fig. 3.10). Abundance of the remaining three taxa was similar among all treatments.

The first axis of a principal components analysis ordination (PCA) explained 25.9% of the variation in community structure among replicate samples of the treatments (Fig. 3.11). The nutrient and effluent treatments changed insect community composition as replicates of these treatments overlapped with one another more than with control replicates along the first axis. This axis indicates that replicates from the NP and 1% BKME treatments tended to have more baetid and ephemerellid mayflies, capniid and chloroperlid stoneflies, and chironomids. In contrast, brachycentrid caddisflies were more abundant in control streams. 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 (i.e. no. of families) was similar among treatments, ranging between 12-13.

Immigration to the streams, likely though the water supply, was measured in a stream that had been treated as a control stream, except that river rocks placed in this stream were first scrubbed clean to ensure that no animals or eggs were transferred. At the end of the experiment, this stream contained 275 insects: 235 baetid mayflies, 32 chironomids, 5 capniid stoneflies, 2 unidentified stoneflies, and 1 *Taenionema* (Plecoptera, Taenioptyerigidae). Emigration from the streams, measured by running drain lines through sieves overnight (approximately 12 hours) was minimal, averaging 1.3 animals (always a baetid or a capniid) per stream.

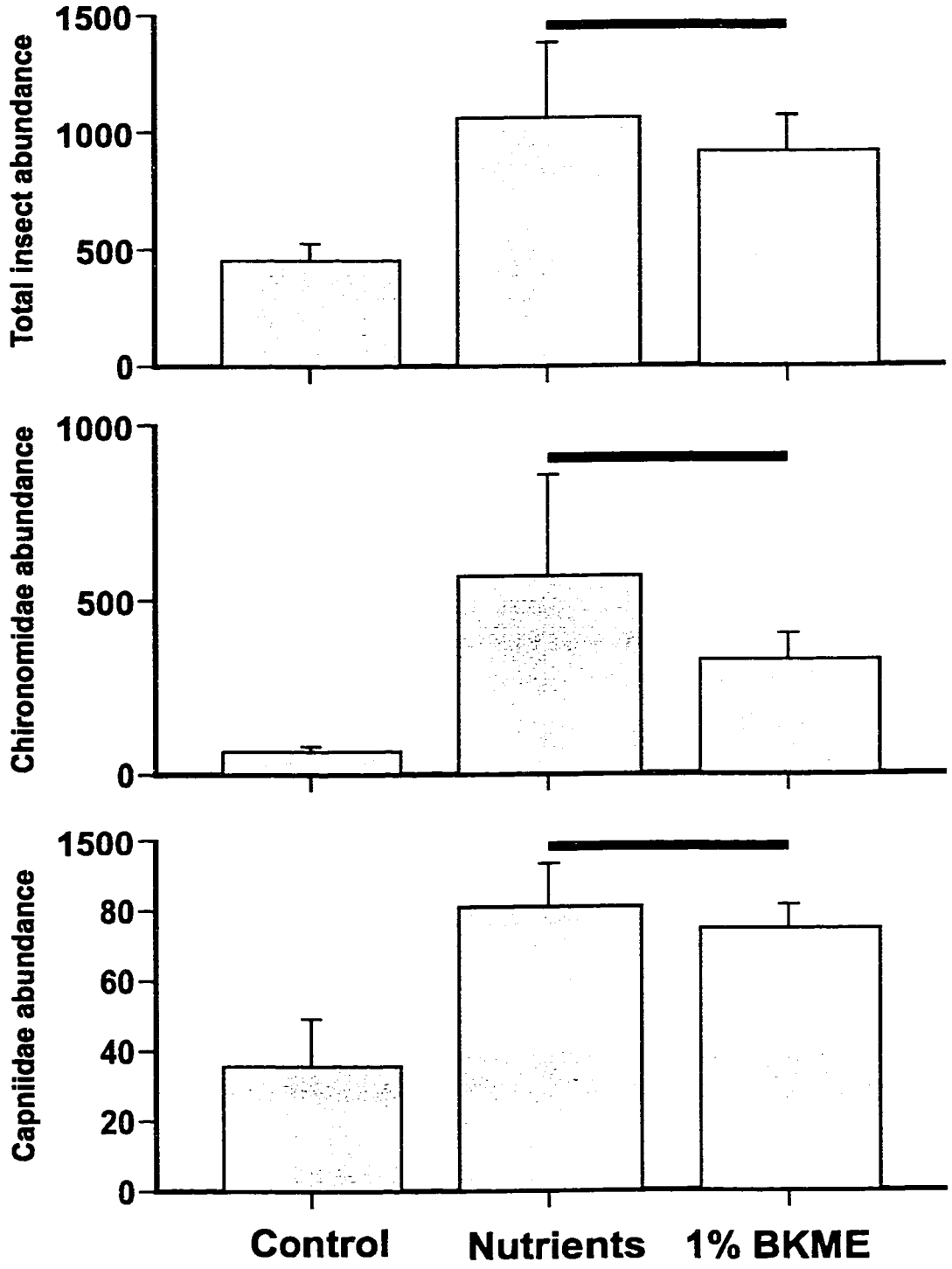


Figure 3.10. Total numbers (per stream) of insects, chironomids and capniid stoneflies in the control, NP, and BKME treatments. Bars connect means that are not significantly different from each other using Fisher's protected LSD (α=0.05).

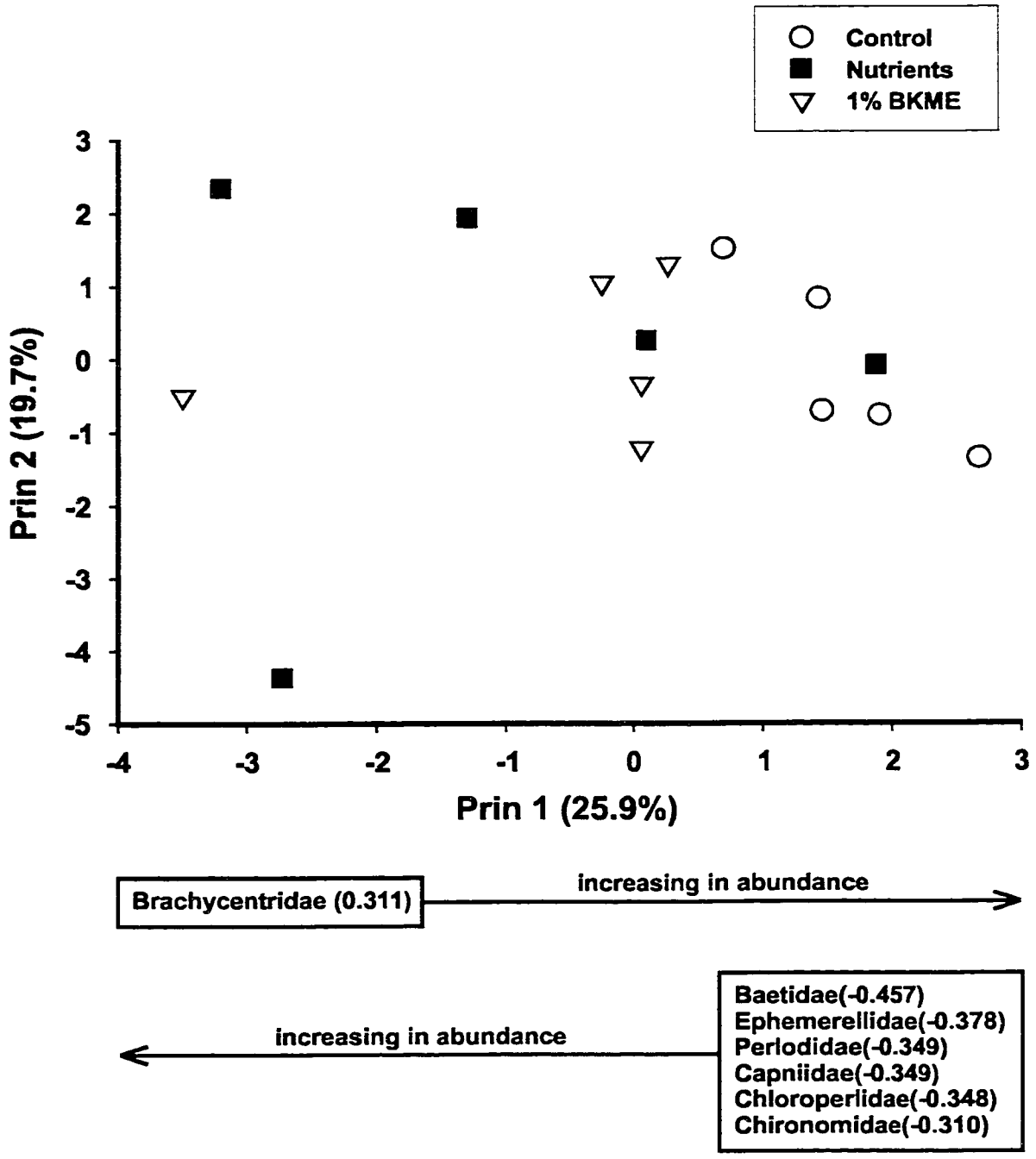


Figure 3.11. Ordination (PCA) of insect community composition in the control, NP, and BKME treatments. The proportion of total variation accounted for by each axis and taxa with high loading (eigenvectors) on the first axis are indicated.

Insect weight was significantly affected by experimental treatments in these three taxonomic groups: *Ameletus* ($F_{2,11}$= 5.57, p=0.021), Capniidae ($F_{2,12}$=12.55, p= 0.001) and Nemouridae ($F_{2,12}$= 16.76, p<0.001). Mean thorax length was also affected by treatment: Ameletus ($F_{2,11}$= 5.57, p= 0.021), Capniidae ($F_{2,12}$=7.53, p=0.008) and Nemouridae ($F_{2,12}$= 8.41, p<0.005). For these taxa, individuals in streams receiving effluent or nutrient additions were longer and heavier when compared to nymphs from control streams (p<0.05). Furthermore, insect sizes in effluent and nutrient addition streams were similar (Fig. 3.12).

In the Athabasca River, *Ameletus* were heavier at the downstream site (t-test, $t_{\alpha=0.05/2,\ 26}$=2.46, p=0.021), as were Ephemerellid mayflies (Kruskal-Wallis, H=13.93, DF=1, p<0.001), and capniid stoneflies (Kruskal-Wallis, H=35.39, DF=1, p<0.001). There was no difference in the weights of baetid mayflies (t-test, $t_{\alpha=0.05/2,\ 191}$=0.30, p=0.77) from upstream and downstream sites.

Benthic insect community composition data collected upstream and downstream of the effluent discharge during autumn 1992 (TAEM 1993) were compared to artificial stream data. Total insect density was higher 0.8 km below the effluent discharge relative to the reference site ($t_{8,\ 0.05/2}$=-4.41, p<0.05) (Fig. 3.14), as were the densities of the Chironomidae ($t_{8,\ 0.05/2}$=-3.44, p<0.05), Capniidae ($t_{8,\ 0.05/2}$=-2.89, p<0.05) and the Baetidae ($t_{8,\ 0.05/2}$=-2.60, p<0.05) (Fig. 3.14). 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. 3.13). Discharge of BKME 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

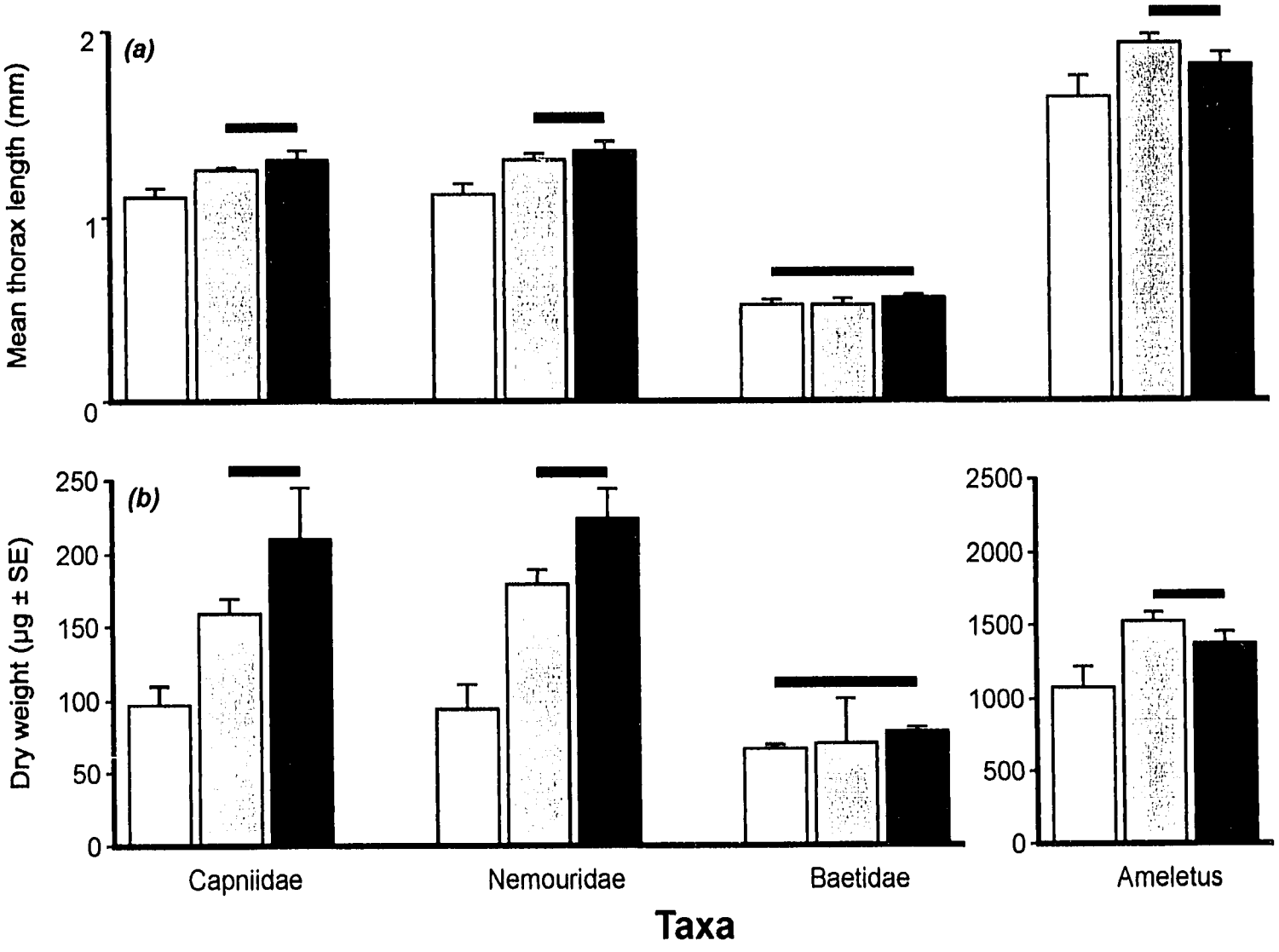


Figure 3.13. Mean *(a)* thorax length (mm), and *(b)* individual dry weight (µg) of the stoneflies, Capniidae and Nemouridae, and the mayflies Baetidae and *Ameletus*, in the control (□), nitrogen-phosphorus (NP) (□) and 1% BKME (■) treatments; bars connect means that are not significantly different at p=0.05.

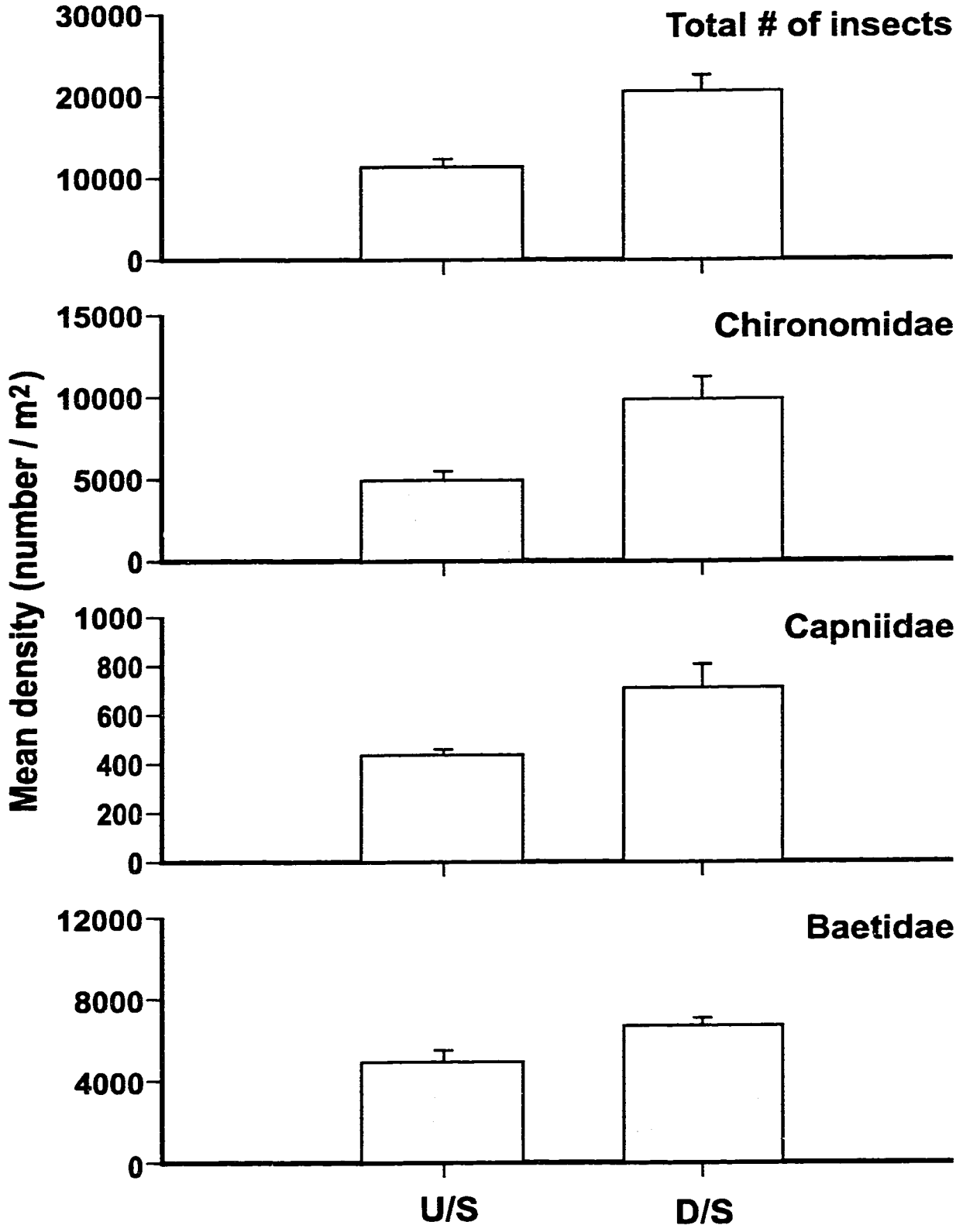


Figure 3.12. Mean density (no./m^2 ± SE) of insects, chironomid midges and capniid stoneflies at sites 2.1 km upstream (U/S) and 0.8 km downstream (D/S) of the BKME discharge at Hinton, Alberta (data from TAEM 1993).

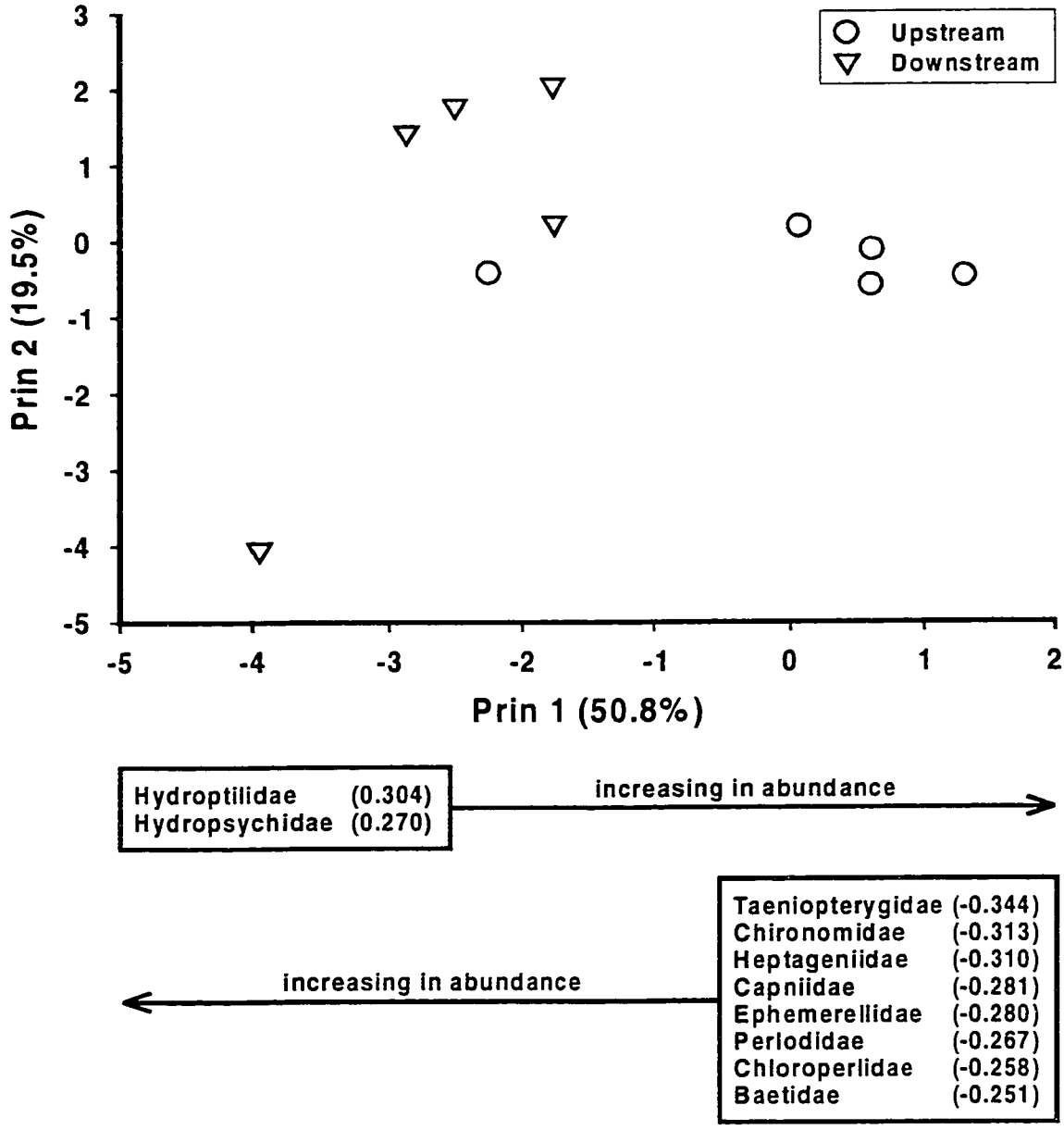


Figure 3.13. Ordination (PCA) of autumn 1992 insect communities at sites 2.1 km above and 0.8 km below the BKME discharge at Hinton, Alberta (data from TAEM 1993). Proportion of total variation accounted for by each axis and taxa with eigenvectors $\geq$ 0.25 on axis 1 are indicated.

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. 3.13). These in-river changes in benthic insect communities are similar to community shifts attributed to the effects of the NP and 1% BKME treatments in the autumn 1993 experiment (Fig. 3.11). 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).

3.8 Discussion

Pulp mill effluents contain a variety of compounds that can have inhibitory effects on aquatic organisms in receiving waters (McLeay 1987). Alberta pulp mills are modern by international standards, and discharge weakly toxic effluents that normally do not cause acute lethality (McCubbin and Folke 1993, SENTAR 1997). Thus, 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 chronic variety, such as reductions in growth rates or fecundity. 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).

Experimental results, along with in-river observations, suggests that the Weldwood combined discharge had a significant effect on benthic biota of the upper Athabasca River. Furthermore, this research provides strong evidence that the dominant effect of this discharge was that of nutrient enrichment and stimulation of food web productivity. Both the NP and BKME treatments stimulated primary production of the largely diatom algal community. These experimental findings corresponded with in-river trends, where periphyton

biomass was approximately 9x higher downstream of the outfall, relative to the upstream reference site. 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 (Figs 3.6, 3.7). The rapidity of the response to nutrient or effluent additions suggests that algal community biomass downstream of the effluent discharge can recover rapidly after scouring disturbance events (e.g., annual summer peak flows). On average, background SRP concentration in the river upstream of the effluent outfall was 1 µg/L. Phosphorus enrichment from effluent during the experiment usually exceeded 2-3 µg/L SRP with excursions to 5 µg/L on several days. Thus, the periphyton community response is consistent with Dale and Chambers' (1996) observation that the addition of low P concentrations (2-5 µg/L) can cause substantial increases in periphytic algae at this location along the Athabasca River.

Algal community composition is sensitive to water quality (Round 1991, Dixit et al. 1992), and has been used to monitor or infer changes in trophic status, pH, salinity, organic pollution, and sedimentation (Watanabe et al. 1986, Dixit et al. 1992, Zeeb et al. 1994, Kelly 1998). In the stream experiment and the Athabasca River, exposure to BKME or nutrients resulted in reduced species richness and an altered community composition. The relative abundance of the diatom *Achnanthes minutissima* was reduced by the addition of effluent or nutrients to the experimental streams, and was similarly reduced downstream of the effluent outfall in the Athabasca River. A reduction in *A. minutissima* in response to a BKME addition occurred in a similar experiment, performed on the Fraser River at the Northwood pulp and paper mill at Prince George, British Columbia (Cash and Culp 1996). *Achnanthes minutissima* is a relatively small species that is typical of oligotrophic to mesotrophic, well oxygenated waters (Lowe 1974). The diatom *Diatoma tenuis* responded to effluent and nutrient additions in the stream experiment, becoming the numerically dominant taxa in both treatments. However, in the Athabasca

River, this species increased at one of the downstream sites, but not the other. In contrast, the relative abundance of two diatom species, *Fragilaria vaucheriae* and *Cymbella silesiaca*, showed clear increases at both downstream sites, but showed little change in the stream experiment. The relative abundance of *F. vaucheriae* also increased in response to an effluent addition in the Fraser River work (Cash and Culp 1996). *Fragilaria vaucheriae* is reported to be typical of eutrophic or moderately polluted waters (Lowe 1974). Although community composition differed somewhat between the stream system and the river, in a second, longer (42d) experiment (Podemski and Culp, unpublished data) *A. minutissima* and *F. vaucheriae* responded similarly in the experimental system and the river, suggesting that *F. vaucheriae* requires a longer duration experiment for effluent responses to be evident.

Besides increasing the quantity of periphytic food, effluent and nutrient additions may increase periphyton quality through shifts in algal community composition. *Achnanthes minutissima*, the numerically dominant species in the control treatments and at the upstream site, is a small adnate (i.e., tightly adherent) species, while *F. vaucheriae* and *D. tenuis* are larger filament-forming species (Patrick and Reimer 1966). *Achnanthes minutissima* and other adnate diatoms are less accessible to grazers (Sumner and McIntire 1982, Peterson 1987) and mats dominated by these species are, thus, a lower quality resource. Effluent discharges appear to shift the algal mat away from dominance by adnate forms, thereby enhancing the quality of food available to grazers.

The artificial stream study provides 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). Abundance of insects, and specifically of 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. 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. Anderson's (1989, 1991) field studies also noted an increase in mayflies and midges downstream of the effluent outfall. 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. The Athabasca River upstream of the BKME discharge is phosphorus-limited and oligotrophic, and the current effluent-loads provide levels of nutrient enrichment that increases benthic riverine productivity without the biotic changes associated with severe eutrophication.

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 is transferred to consumer trophic levels (McLeay 1987, Hall et al. 1991, Dubé 1995, Lowell et al. 1995). In the Athabasca River experiment, sizes of herbivores and detritivores were increased, suggesting the nutrient enrichment response is not restricted to algae and their grazers. Effluent biosolids may represent a supplemental food supply for animals that normally ingest particulate organic matter (NCASI 1978, Dubé and Culp 1996,1997). Although experiments conducted in the Thompson River, British Columbia, 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 the artificial stream experiment where insect size in the NP and BKME treatments was similar. Results from the experimental streams agreed with those of in-river sampling, which detemined that capniid stoneflies and the mayflies, *Ameletus* and *Ephemerella*, were heavier downstream of the effluent discharge. Lack of a growth response in baetid mayflies, in the experimental streams and the river, may be explained by examining data from

the experimental stream that was not seeded with invertebrates. By the end of the experiment, this stream contained a total of 275 animals, 235 of which were small baetid mayflies. The other experimental streams contained an average of 160, 272, and 376 baetid mayflies in the control, NP and BKME treatments, respectively. These data suggest that the majority of baetid mayflies in all streams were the result of immigration, likely via the water supply but possibly the result of eggs laid in the streams during the experiment. The small size of the insects (mean thorax length was approximately 0.5 mm), suggests that these animals may be only recent recruits and may not have been exposed to effluent long enough for a growth response to occur.

If the BKME treatments had deleterious effects on the benthic biota, these would be expected to be manifested through reductions in insect growth, fecundity, and diversity, and through an increasing dominance of pollution tolerant taxa. 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 size in the control streams was always lower than the NP or BKME treatments, and size was never lower in the effluent streams than in NP streams. In addition, the fact that size was similarly increased in the NP and BKME treatments indicates that nutrient enrichment effects were not masked by deleterious contaminant effects. Effluent exposure also produced no measurable effect of contaminants at the community level. Shifts in community composition in the NP and BKME treatments were similar and, thus, largely reflect the abundance of taxa responding to increased periphytic resources. McCubbin and Folke (1993) indicate that effluents discharged to the Athabasca River exhibit low toxicity, but may have the potential to produce chronic effects. This study provides evidence that effluent loadings to the upper Athabasca River in autumn 1993, produced no measurable chronic toxicity effects at the population or community level. I caution that the experiment did not examine

reproductive effects or the bioaccumulation of lipophilic contaminants that may not affect insects, but could be concentrated by higher consumers including fish and birds.

Despite the common perception that pulp mill effluents may induce chronic toxicity in the benthic biota, this study does not provide evidence that this is occurring in the Athabasca River. The experimental exposure of mesocosms to BKME concentrations equivalent to present loadings to the upper Athabasca River produced no measurable chronic toxicity as measured by insect size or community structure. The results instead suggested that BKME-associated increases in SRP were responsible for increased periphytic algal biomass and, thus, increased food availability that had direct effects on the abundance and growth of aquatic insects. Effects of BKME and NP treatments were similar, suggesting that 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. Pulp mill effluents 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). In the upper Athabasca River, phosphorus limits primary production (Scrimgeour and Chambers 1996), and biomonitoring programs have noted increased biomass of periphyton and abundance of many benthic invertebrates, including some pollution sensitive taxa, for several kilometres below Hinton, Alberta. Because insects are an important food source for many fish, the nutrient-induced stimulation of secondary producers in the benthos is probably transferred upward through the food web. Gibbons et al. (1995) reported that an insectivorous fish (spoonhead sculpin) was larger and in better condition downstream of the effluent discharge. Under the present nutrient regime in the upper Athabasca River, effluent loading provides levels of nutrient enrichment that increase benthic riverine productivity, but do not cause biotic changes normally associated with severe eutrophication. Pulp mill effluent is currently not regulated for nutrient content (Chambers 1996), however, nutrient

enrichment can have profound impacts on aquatic systems. With improvements in pulping and treatment technologies, toxicity of effluent is no longer as great a concern, thus, enrichment effects may become a new focus for reducing this industry's impact on aquatic resources.

3.9 Literature Cited

(APHA) American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1985. Standard methods for the examination of water and wastewater. 14th Edition. Washington, D.C. 1193p.

Anderson, A. M. 1989. An assessment of the effects of the combined pulp mill and municipal effluents at Hinton on the water quality and zoobenthos of the Athabasca River. Report prepared by the Environmental Quality Monitoring Branch, Environmental Assessment Division, Alberta Environment, Edmonton, Alberta.

Anderson, A. M. 1991. An overview of long-term zoobenthic monitoring in Alberta Rivers (1983-1987). Environmental Assessment Division, Alberta Environment.

Bitton, G., K. Rhodes, B. Koopmen, and M. Cornejo. 1995. Short-term toxicity assay based on daphnid feeding behavior. Water Environmental Research 67: 290-293.

Bothwell, M. L. 1992. Eutrophication of rivers by nutrients in treated kraft pulp mill effluent. Water Pollution Research Journal of Canada 27: 447-472.

Buikema, A. and J. R. Voshell. 1993. Toxicity studies using freshwater benthic macroinvertebrates. Pages 340-395 *in* D.M. Rosenberg and V. H. Resh (Eds.). Biomonitoring and benthic invertebrates. Chapman and Hall, New York.

Cash, K. J., and J. M. Culp. 1996. The effects of bleached kraft pulp-mill effluent on periphyton tissue. National Hydrology Research Institute, Saskatoon, Saskatchewan.

Chambers, P. A. 1996. Nutrient enrichment in the Peace, Athabasca and Slave rivers: assessment of present conditions and future trends. Synthesis Report No. 4. Northern River Basins Study, Edmonton, Alberta.

Chapman, R. F. 1974. The chemical inhibition of feeding by phytophagous insects: a review. Bulletin of Entomological Research 64: 339-363.

Cooley, J. M. 1977. Filtering performance of *Daphnia retrocurva* in pulp mill effluent. Journal of the Fisheries Research Board of Canada 34: 863-868.

Cooper, S. D. and L. Barmuta. 1993. Field experiments in biomonitoring. Pages 399-441 *in* D.M. Rosenberg and V. H. Resh (editors). Biomonitoring and benthic invertebrates. Chapman and Hall, New York.

Crane, M., and L. Maltby. 1991. The lethal and sublethal responses of *Gammarus pulex* to stress: sensitivity and sources of variation in an in situ bioassay. Environmental Toxicology and Chemistry 10: 1331-1339.

Dale, A. R. and P. A. Chambers. 1996. Growth Rate and Biomass Responses of Periphytic Algae to Phosphorus Enrichment in Experimental Flumes, Athabasca River, Seasonal Variation, 1993 and 1994. Project Report No. 68. Northern River Basins Study, Edmonton, Alberta.

Dale, A. R., and P. A. Chambers. 1995a. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, April and May, 1994. Northern River Basins Study, Edmonton, Alberta.

Dale, A. R., and P. A. Chambers. 1995b. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, seasonal variation. Northern River Basins Study, Edmonton, Alberta.

Desmarchelier, J. M., and T. R. Fukuto. 1974. Toxicological effects produced by some 1,3-benzodioxoles, catechols, and quinones in *Culex* mosquito larvae. Journal of Economic Entomology 67: 153-158.

Dixit, S. S., J. P. Smol, J. C. Kingston, and D. F. Charles. 1992. Diatoms: powerful indicators of environmental change. Environmental Science and Technology 26: 23-33.

Dubé, M. G. 1995. Effects of bleached kraft pulp mill effluent on periphyton and chironomid abundance in the Thompson River, British Columbia. M.Sc. Thesis. University of Saskatchewan.

Dubé, M. G., and J. M. Culp. 1996. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Environmental Toxicology and Chemistry 15: 2019-2027.

Dubé, M. G., and J. M. Culp. 1997. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Water Science and Technology 35: 339-345.

Elliger, C. A., D. F. Zinkel, B. G. Chan, and A. C. Waiss. 1976. Diterpene acids as larval growth inhibitors. Experientia 32: 1364-1366.

Feder, H. M. and T. H. Pearson. 1988. The benthic ecology of Loch Linnhe and Loch Eil, a sea-loch system on the west coast of Scotland: V. Biology of the dominant soft-bottom epifauna and their interaction with the infauna. Journal of Experimental Marine Biology and Ecology 116: 1051-1060.

Forbes, V. E. and T. L. Forbes. 1994. Ecotoxicology in theory and practice. Chapman and Hall, New York.

Gibbons, W., K. Munkittrick and W. Taylor. 1995. Suitability of small fish species for monitoring the effects of pulp mill effluent on fish populations of the Athabasca River. Project Report No. 100. Northern River Basins Study, Edmonton, Alberta.

Hall, T. J., R. K. Haley and L. E. LaFleur. 1991. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental stream channels. Environmental Toxicology and Chemistry 10: 1511-1540.

Hansson, S. 1987. Effects of pulp and paper mill effluents on coastal fish communities in the Gulf of Bothnia, Baltic Sea. Ambio 16: 344-348.

Harborne, J. B. 1990. Role of secondary metabolite in chemical defense mechanisms in plants. p. 126-139 *in* D. J. Chadwick and J. Marsh (editors). Bioactive compounds from plants. Ciba Foundation Symposium 154. 242 p.

Hershey, A. E., A. L. Hiltner, M. A. J. Hullar, M. C. Miller, J. R. Vestal, M. A. Lock, S. Rundle, and B. Peterson. 1988. Nutrient influence on a stream grazer: *Orthocladius* microcommunities respond to nutrient input. Ecology 69: 1383-1392.

Kelly, M. G. 1998. Use of the trophic diatom index to monitor eutrophication in rivers. Water Research 32: 236-242.

Kramer, P. R. G., D. A. Jonkers, and L. van Liere. 1996. Interactions of nutrients and toxicants in the foodchain of aquatic ecosystems. Rijksinstituut voor Volksgezondheid en Milieu, Zeist, Netherlands.

Lowe, R. L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. U.S. Environmental Protection Agency, Cincinnati, OH.

Lowell, R. B, J. M. Culp and F. J. Wrona. 1995. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environmental Toxicology and Chemistry 14: 1529-1541.

Lozano, R. B. and J. R. Pratt. 1994. Interaction of toxicants and communities: the role of nutrients. Environmental Toxicology and Chemistry 13: 361-368.

McCubbin, N. and J. Folke. 1993. A review of literature on pulp and paper mill effluent characteristics in the Peace and Athabasca river basins. Project Report 15. Northern River Basin Study, Edmonton, Alberta.

McIntire, C. D. 1993. Historical and other perspectives of laboratory stream research. Pages 318-323 *in* G. A. Lamberti and A. D. Steinman (editors). Research in artificial streams: applications, uses, and abuses. Journal of the North American Benthological Society 12:313-384.

McLeay, D. J. 1987. Aquatic toxicity of pulp and paper mill effluent: A review. Environmental Protection Series Report 4/PF/1. Environment Canada, Ottawa, Ontario.

McLeese, D. W. 1973. Response of lobsters *Homarus americanus* to odor solution in the presence of bleached kraft mill effluent. Journal of the Fisheries Research Board of Canada 30: 279-282.

Menzel, D. W. and N. Corwin. 1965. The measurement of total phosphorus in seawater based on liberation of organically bound fractions by persulfate oxidation. Limnology and Oceanography 10: 280-282.

Merritt, R. W. and K. W. Cummins 1984. An introduction to the aquatic insects. Second Edition. Kendall and Hunt Publishing. Dubuque, Iowa. 722p.

NCASI. 1978. A study of the fate of suspended solids from a full scale treatment plant in receiving waters. National Council of the Paper Industry for Air and Stream Improvement, Inc., Research Triangle Park, NC.

Owens, J. W. 1991. The hazard assessment of pulp and paper effluents in the aquatic environment: a review. Environmental Toxicology and Chemistry 10: 1511-1540.

Patrick, R., and C. W. Reimer. 1966. *The Diatoms of the United States.* Monographs of the Academy of Natural Science, Philadelphia.

Petersen, B. M. and R. C. Petersen. 1984. Effect of kraft pulp mill effluent and 4, 5, 6 trichloroguaiacol on the net-spinning behavior of *Hydropsyche angustipennis* (Trichoptera). Ecological Bulletin 36: 68-74.

Peterson, C. G. 1987. Gut passage and insect grazer selectivity of lotic diatoms. Freshwater Biology 18: 455-460.

Reese, J. C., and S. D. Beck. 1976. Effects of allelochemicals on the black cutworm, *Agrotis ipsilon*: effects of catechol, l-dopa, dopamine, and chlorogenic acid on larval growth, development, and utilization of food. Annals of the Entomological Society of America 69: 68-72.

Rosenberg, D. M. and V. H. Resh. 1993. Biomonitoring and benthic invertebrates. Chapman and Hall, New York. 488p.

Rosenthal, G. A. and D. H. Janzen. 1979. Herbivores: Their interaction with secondary plant metabolites. Academic Press Inc., New York. 718p.

Round, F. E. 1991. Diatoms in river-monitoring studies. Journal of Applied Phycology 3: 129-145.

Scholten, M. C.T., E. M. Foekema, and R. G. Jak. 1996. Toxic anorexia of daphnids: a critical factor in the development of eutrophication problems. Rijksinstituut voor Volksgezondheid en Milieu, Zeist, Netherlands.

Scrimgeour, G. J. and P. A. Chambers. 1996. Identification of spatial and temporal patterns in nutrient limitation with herbivory effects, Wapiti, Smoky and Athabasca Rivers, 1994. Project Report No. 96. Northern River Basins Study, Edmonton, Alberta.

Scrimgeour, G. J., J. M. Culp and N. E. Glozier. 1993. An improved technique for sampling lotic invertebrates. Hydrobiologia 254: 65-71.

SENTAR Consultants Ltd. 1997. A synthesis of information on ecotoxicity of pulp mill effluents in the Peace, Athabasca and Slave River Basins. Northern River Basins Study, Edmonton, Alberta.

Stainton, M. P., M. J. Capel and F. A. J. Armstrong. 1977. The chemical analysis of freshwater. 2nd edition. Miscellaneous Special Publication of the Fisheries Environment, Canada 26, 180p.

Stewart, K. W. and B. P. Stark. 1993. Nymphs of North American stonefly genera (Plecoptera). University of North Texas Press, Denton, Texas. 460.p.

Stewart-Oaten, A., J. R. Bence, and C. W. Osenberg. 1992. Assessing effects of unreplicated perturbations: no simple solutions. Ecology 73:1396-1404.

Sumner, W. T., and C. D. McIntire. 1982. Grazer-periphyton interactions in laboratory streams. Archive für Hydrobiologie 93: 135-157.

TAEM. 1993. Biological and water quality survey of the Athabasca River, October 1992. Prepared for Weldwood of Canada Ltd., Hinton Division.

Taylor, W. D., E. Bentzen, B. Delucca, B. E. Hickie, D. R. S. Lean, D. Mackay, and G. Patterson. 1996. Empirical evidence for the importance of lake trophic status and food web in bioaccumulation in southern Ontario lakes. Pages 101-110 in P. R. G. Kramer, D. A. Jonkers, and L. van Liere, eds. *Interactions of nutrients and toxicants in the foodchain of aquatic ecosystems*. Rijksinstituut voor Volksgezondheid en Milieu, Zeist, Netherlands.

Wagner, M. R., D. M. Benjamin, K. M. Clancy, and B. A. Schun. 1983. Influence of diterpene resin acids on feeding and growth of larch sawfly, *Pristiphora erichsonii* (Hartig). Journal of Chemical Ecology 9: 119-127.

Walden, C. C. and T. E. Howard. 1977. Toxicity of pulp and paper mill effluents: a review of regulation and research. TAPPI 60: 122-125.

Watanabe, T., K. Asai, and A. Houki. 1986. Numerical water quality monitoring of organic pollution using diatom assemblages. 9th Diatom-Symposium: 123-141.

Xie, Y., M. B. Isman, Y. Feng, and A. Wong. 1993. Diterpene resin acids: major active principles in tall oil against variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae). Journal of Chemical Ecology 19: 1075-1084.

Zeeb, B. A., C. E. Christie, J. P. Smol, D. L. Findlay, H. J. Kling, and H. J. B. Birks. 1994. Responses of diatom and chrysophyte assemblages in lake 227 sediments to experimental eutrophication. Canadian Journal of Fisheries and Aquatic Sciences 51: 2300-2311.

Chapter 4.

Toxicant Interactions with Food Algae: A Missing Link Between Laboratory and Field Effects?

4.1 Introduction

Single-species laboratory bioassays are frequently criticized for being unrealistic, overly simplified, and producing data with limited relevance or capacity to extrapolate to field conditions (Cairns 1983, Kimball and Levin 1985, Malins 1989, Maltby and Calow 1989, Forbes and Forbes 1994). To reduce variability and facilitate toxicant comparisons, these bioassays are conducted under conditions that are rigorously controlled, and, as a consequence, artificial compared to field conditions. For example, during chronic toxicity testing, the diet of invertebrate species (e.g. *Daphnia*, *Ceriodaphnia*, *Baetis*) often includes cultured algae (e.g. Cowgill 1987, Cowgill et al. 1988, Enserink et al. 1990, Environment Canada 1992, Lowell and Culp 1996, Himbeault 1995). Typically, algae are cultured without exposure to test substances and uneaten material is removed regularly. This approach is problematic because it assumes not only that bioconcentration is the only important uptake route, but that an interaction between toxicant and algae does not occur or is not relevant to the effect of the toxicant on test animals. In the natural environment, however, multiple trophic levels may be exposed and affected by stressors, and organisms may take up contaminants via bioconcentration or ingestion. The predictive ability of chronic bioassays might therefore be improved by exposing both trophic levels (e.g. 1° producer→consumer) to toxicants. This procedure would allow toxicants to partition to algae, resulting in uptake via ingestion or bioconcentration by test animals, thereby allowing the direct and indirect effects of toxicants to be included.

Algae react to toxicants with changes in growth rate, photosynthetic activity, and pigment content (Torres and O'Flaherty 1976, Soto et al. 1977, Blanck 1985, Blanck and Wängberg 1988, Visviki and Rachlin 1994, Parrish 1995, Lewis 1995, El Jay 1996, Genter 1996, Hoaglund et al. 1996). Algae also react to nutrient or toxicant stress with a change in cellular macromolecule content. Cellular content of lipid, carbohydrate, and protein can respond to a variety of factors including cation deficiency, heavy metal exposure, and organic contaminants (Table 4.1). The importance to chronic bioassay results of these changes in cellular composition is that it can represent a change in nutritional quality that can affect the consumer's growth and reproduction (e.g., Kiørboe 1989, Groeger et al. 1991, Sterner 1993, Sterner and Hessen 1994). Furthermore, nutritional content and/or food availability can affect consumer response to toxicants (Mehrle et al. 1974, Sosnowski et al. 1979, Phillips and Buhler 1979, Hilton and Hodson 1983, Dixon and Hilton 1985, Holdway and Dixon 1985, Cowgill 1987, Hickie and Dixon 1987, Dahlgren 1988, Hickie et al. 1989, LaRocca et al. 1994, Farkas et al. 1996). As responses measured in chronic toxicity testing usually include mortality, growth, or reproduction, it is clear that the exposure of food algae to the test substance may produce an important interaction that is currently being ignored.

The research presented in this chapter focuses on the effects of a bleached kraft mill effluent (BKME) on algae used as food for test animals, and the possible consequences of this exposure to bioassay results. Specifically, the influence of BKME exposure on important growth and food quality variables of a pennate diatom, *Navicula* sp., including biomass, carbon:nitrogen ratio, total lipid content, and elemental composition of the algae were examined. This alga has been used as a food source for mayflies in bioassays of municipal and pulp mill effluent (Himbeault 1995, Lowell and Culp 1996). In my research on the environmental effects of pulp mill effluent, I proposed to increase the realism and, thus, the accuracy of bioassays conducted on mayflies by culturing

Table 4.1. Biochemical response of algae species to nutrient and/or toxicant stress.

Reference	Algal Species	Stress	Response
MacCarthy and Patterson 1974	*Chlorella sorokiniana*	K deficiency Mg deficiency Ca deficiency	↑ lipid, nitrogen ↑ nitrogen no effect
Soto et al. 1977	*Chlamydomonas angulosa*	Naphthalene crude oil extract	↓protein, ↑carbohydrate, ↑lipid ↓protein, ↑carbohydrate, ↑lipid
Piorreck et al. 1984	*Chlorella vulgaris* *Scenedesmus obliquus*	N deficiency	↑ lipid and ↓ protein
Ben-Amotz et al. 1985	*Ankistrodesmus sp.* *Botryococcus braunii* *Botryococcus braunii* *Dunliella salina* *Dunliella salina* *Isochrysis sp.* *Isochrysis sp.* *Nannochloris sp.*	N deficiency N deficiency NaCl N deficiency NaCl N deficiency NaCl N deficiency	↓protein, ↑lipid ↑carbohydrate ↑lipid ↓protein ↓protein, ↓lipid ↑carbohydrate ↑protein, ↓lipid ↓carbohydrate ↓protein, ↑lipid ↑carbohydrate ↓protein, ↑lipid ↑carbohydrate ↑lipid ↑carbohydrate
Sicko-Goad et al. 1989b	*Cyclotella meneghiana*	1,2,3-Trichloro-benzene	↑ lipid
Sicko-Goad et al. 1989a	*Cyclotella meneghiana*	Pentachloro-benzene	↑ lipid
Thompson and Couture 1991	*Selenastrum capricornutum*	Cadmium	↑ lipid, protein and carbohydrate
Sicko-Goad and Andresen 1993	*Cyclotella meneghiana* *Melosira varians* *Melosira italica* *Synedra filiformis*	1,2,3-Trichloro-benzene	↑polar lipid ↓ polar lipid ↓ polar lipid ↑ polar lipid
Harrison et al. 1990	*Isochrysis galbana* *Chaetoceros calcitrans* *Thalassiosira pseudonana*	Si deficiency N deficiency	no effect (all species) ↓protein, ↑carbohydrate (all species)
El-Dib et al. 1997	*Selenastrum capricornutum*	fuel oil	↓protein, ↓carbohydrates

diatoms in effluent, as would occur for algae eaten by these animals in receiving waters. Results of this research can be used to support the interpretation of bioassays in which this algae was used as food.

4.2 Methods

The experiment consisted of exposing *Navicula* cultures to a range (0-7%) of BKME concentrations. The effect of effluent on algal growth rate was measured by comparing biomass over time, with biomass measured as ash free dry mass (AFDM) and chlorophyll *a* (Chl*a*). The carbon:nitrogen ratio, total lipid content, and elemental composition were measured as indicators of food quality (Cummins and Klug 1979). Measures of food quality were made when cultures were 15 days old, as cultures are normally fed to mayflies after 2 weeks development (Scrimgeour et al. 1991, Himbeault 1995).

4.2.1 Laboratory Methods

A non-axenic unialgal culture of *Navicula* sp. was obtained from Carolina Biological Supply Company. Cultures were immediately transferred to fresh WC media (a modified Chu no. 10, Guillard and Lorenzen 1972) by transferring 0.1 mL of stock culture into 25 mL of media in 50 mL culture tubes. All glassware and other materials used for culturing were acid-washed and autoclaved at 121°C before use, and culture media was autoclaved at 121°C. Inoculated culture tubes were placed in a Percival® controlled environment chamber at 16°C, with a 16:8h light:dark cycle. Light was provided by Durotest®, Vitalite broad spectrum bulbs. After 16 days, cultures were transferred into 250 mL Erlenmeyer flasks (2 mL into 150 mL fresh media) and were allowed to grow for 11 days. The purpose of culturing diatoms in tubes, and then in flasks, was to increase the quantity of algae until there was sufficient biomass available to initiate the experiment. After 11 days, the entire contents of each Erlenmeyer flask was added to 3 L Pyrex® trays containing 0.5 L media, 1 Erlenmeyer flask per tray. The bottom of each tray was covered with a single layer of unglazed ceramic tiles (2.3 x 2.3 cm), and the top was covered with an acrylic sheet to limit evaporation.

While standard culture media was used for *Navicula* grown in tubes and flasks, media used to fill trays also contained secondary-treated effluent from Weldwood of Canada's bleached kraft pulp mill in Hinton, Alberta. Detailed information regarding the mill's furnish, bleach sequence and effluent treatment

are in Chapter 2. Effluent for this experiment was collected on a single day from the mill's effluent stream just before discharge, and was stored in polyethylene containers at -40°C until used. Pulp mill effluents are often frozen before use in toxicity tests (Ahtiainen et al. 1996; Eklund et al. 1996; Priha 1996; Verta et al. 1996). Effluent replaced distilled water in media to create treatments (n=4) of 0, 1, 3, 5, and 7% on a volume to volume basis. Thawed effluent was added to cooled, autoclaved media to avoid any heat-related changes in chemical composition. The effluent was not filter-sterilized as it was felt that this would also constitute a change in composition. Trays were completely drained and media replaced every 48 h.

Every 3 days, 3 tiles from each tray were sampled by scraping algae from the top surface of tiles with a scalpel and samples were immediately frozen at -40°C. Each sample was later homogenized, split into two, and each portion filtered through a precombusted GF/C filter. Chlorophyll *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. On days 3 and 6 there was insufficient material to measure both Chl*a* and AFDM from each sample. For these days 1 tile was used for each response variable. On remaining days, Chla and AFDM were measured from 3 tiles in each tray and the means of the values were used for statistical analysis.

On day 15, all remaining tiles were scraped and the algae frozen. These samples were used to measure total lipid content, carbon:nitrogen ratios, and elemental composition. Only the 0, 1, 3, and 7% treatments were analyzed for lipids, C:N and elemental composition because these concentrations had, by this time, been chosen for mayfly bioassays. Samples for lipid analysis were frozen at -75°C until analyzed for total lipid content using microgravimetric methods of Gardner et al. (1985). Samples for CHN analysis were freeze-dried and analyzed by a Control Equipment Corporation 440 Elemental Analyzer at

the University of Alberta, Limnology Laboratory. Samples for elemental composition were freeze-dried and sent to Saskatchewan Research Council Analytical Services for analysis. Samples were digested with 5ml of 50% nitric acid in a closed Teflon vessel using a microwave digester, CEM Model MDS 2000. The digest was diluted with de-ionized water to a final volume of 25 mL, and analyzed by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) using a Jarrell-Ash TJA ICAP-61E spectrometer.

4.2.2 Statistical Analysis

The effect of treatment on the slopes of the Chla and AFDM accrual curves was tested by analysis of covariance (ANCOVA) with time as the covariate. For Chla, both dependent and independent variables required log10 transformation. A significant difference in slopes, indicated by a significant Treatment x Time interaction, would indicate differences in the rate of biomass accrual. Individual regressions for each treatment were determined if the ANCOVA indicated that BKME concentration affected the rate of biomass increase. Residual plots were examined to verify that models were appropriate. Relationships between treatment and total lipid content were also investigated by regression. Treatment effect on carbon:nitrogen ratios of *Navicula* was determined by using ANCOVA to test the effects of treatment on adjusted mean nitrogen content, using carbon as the covariate (Atchley 1978, Green 1979). The ICP-AES scan measured the concentration of 24 elements, and principal component analysis was used to identify patterns in the variation in this data set. Analysis of covariance and comparisons of adjusted means were performed using SAS (SAS Institute 1988), and regressions and PCA were performed with Minitab (release 11.0, Minitab Inc. 1996).

4.3 Results

A chemical profile of the effluent, after freezing, is found in Table 4.2. Concentrations of nitrogen and phosphorus were typical for this mill. Only 2 resin acids, pimaric and dehydroabietic acid, were detected in the effluent and these were in low concentrations (Costle et al. 1980). Very few PAHs were

detected, and those were also in low concentrations. The analysis of chlorophenolic compounds was limited by the high number of compounds that did not meet ratio criteria (17 out of 24). Although concentrations for these compounds are given, the identity and/or amounts are of questionable accuracy.

Chl*a* and AFDM increased over time for the 5 treatments (Fig. 4.1), with Chl*a* accrual leveling off after 12 days and AFDM still increasing at day 15. Although there was no effect of treatment on either the slopes or intercepts of Chl*a* accumulation (Table 4.3), the *p*-value for slope differences is suggestive of a difference, and final Chl*a* concentrations were greater in the 5 and 7% BKME treatments (Fig. 4.2). Effluent concentration had a significant effect on the rate of AFDM accumulation (Table 4.5)., with day 15 ADFM significantly higher in 5, and 7% treatments than in 0, 1, and 3% BKME (Fig. 4.2; Table 4.4). Regression equations describing AFDM increase over time in the 5 treatments are in Table 4.6.

Nutritional quality of algae was assessed by measuring total lipid content and carbon:nitrogen ratios. Total lipids ranged from an average ($\pm$ 1SE) of 11.7% ($\pm$0.95) for control *Navicula*, to a high of 15.9% ($\pm$0.82) for the 7% treatment. The regression of percent lipid on the log of effluent concentration was significant (Fig. 4.3) but had a low coefficient of determination (lipid% = 11.4 + $4.33\log_{10}$(%BKME + 1), r^2 = 36.9, p = 0.01). Mean carbon:nitrogen ratios ranged from 1.37 to 1.51 for 7% and 3% BKME, respectively, while *Navicula* from 0 and 1% BKME treatments had C:N ratios of 1.40 and 1.48, respectively. The effect of treatment on carbon:nitrogen ratios was tested by using analysis of covariance to compare the mean nitrogen content for each treatment, adjusted for carbon content. *Navicula* grown in 7% effluent had a significantly lower adjusted nitrogen content than other treatments, and the 3% treatment had a significantly lower adjusted nitrogen than the 1% treatment (Table 4.7), indicating either increased lipid or carbohydrate content in these treatments.

Table 4.2. Chemical composition of BKME after freezing (* failed to meet ratio criteria; values reported for those compounds are maximum concentrations (µg/L) that may be present if the identity is correct).

Compound	(µg/L)	Compound	(µg/L)
$N\text{-}NO_3$	278.01	Pimaric acid	150
$N\text{-}NH_3$	915.94	Dehydroabietic acid	110
TDN	1721.20	Capric acid	280
TKN	3137.10	Myristic acid	1800
Particulate N	1036.40	Palmitic acid	6900
SRP	179.60	Linolenic acid	550
TP	386.40	Linoleic acid	700
TDP	248.40	Oleic acid	1400
DOC	56.70	Behenic acid	180
Particulate C	8370.40	4-chlorophenol	6.3*
Si	1795.00	2,8-dichlorophenol	18.0*
Cl	154.35	2,4/2,5-dichlorophenol	20.7
SO_4	471.76	3,5-dichlorophenol	5.3*
Na	310.00	6-chloroguaiacol	1.2*
K	6.23	4-chloroguaiacol	98.0*
Ca	79.40	5-chloroguacicol	280.0
Mg	18.10	2,4,6-trichlorophenol	6.6
Fe	0.08	3-chlorocatechol	5.5*
Conductivity	1722.00	4-chlorocatechol	7.1*
TDS	132.10	4,6-dichloroguaiacol	3.1*
NFR	48.80	3,4-dichloroguaiacol	19.0*
pH	8.62	4,5-dichloroguaiacol	33.7*
Alkalinity	218.75	3-chlorosyringol	8.4*
HCO_3	237.44	3,6-dichlorocatechol	6.8*
CO_3	14.39	4,5-dichlorocatechol	15.0*
Colour	430.20	5-chlorovanillin	7.1*
Naphthalene	27.50	6-chlorovanillin	330.0
Acenapthene	1.00*	3,4,5-trichloroguaiacol	2.9*
Fluorene	0.69	3,4,6-trichlorocatecho	3.7*
Phenanthrene	2.00	5,6-dichlorovanillin	3.9*
Fluoranthene	1.90	3,4,5,6-tetrachloroguaiacol	1.7*
Pyrene	1.40*	tetrachlorocatechol	3.2*
C1 napthalenes	8.50	3,4,5-tetrachloroveratrole	7.2*

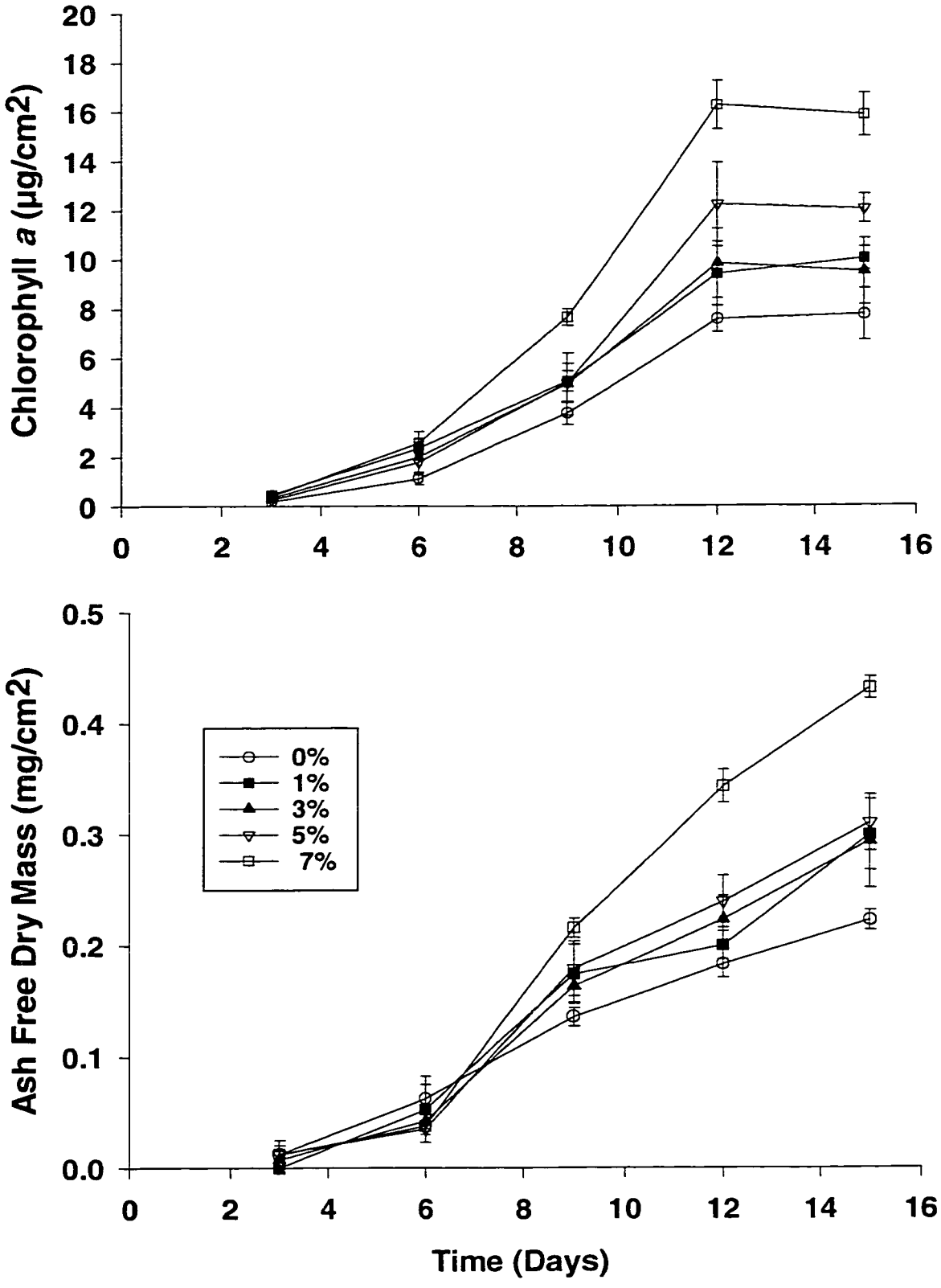


Figure 4.1. Increase in Chl*a* and AFDM of *Navicula* cultured in 0,1,3,5, and 7% effluent for 15 days; values are the mean ± 1SE.

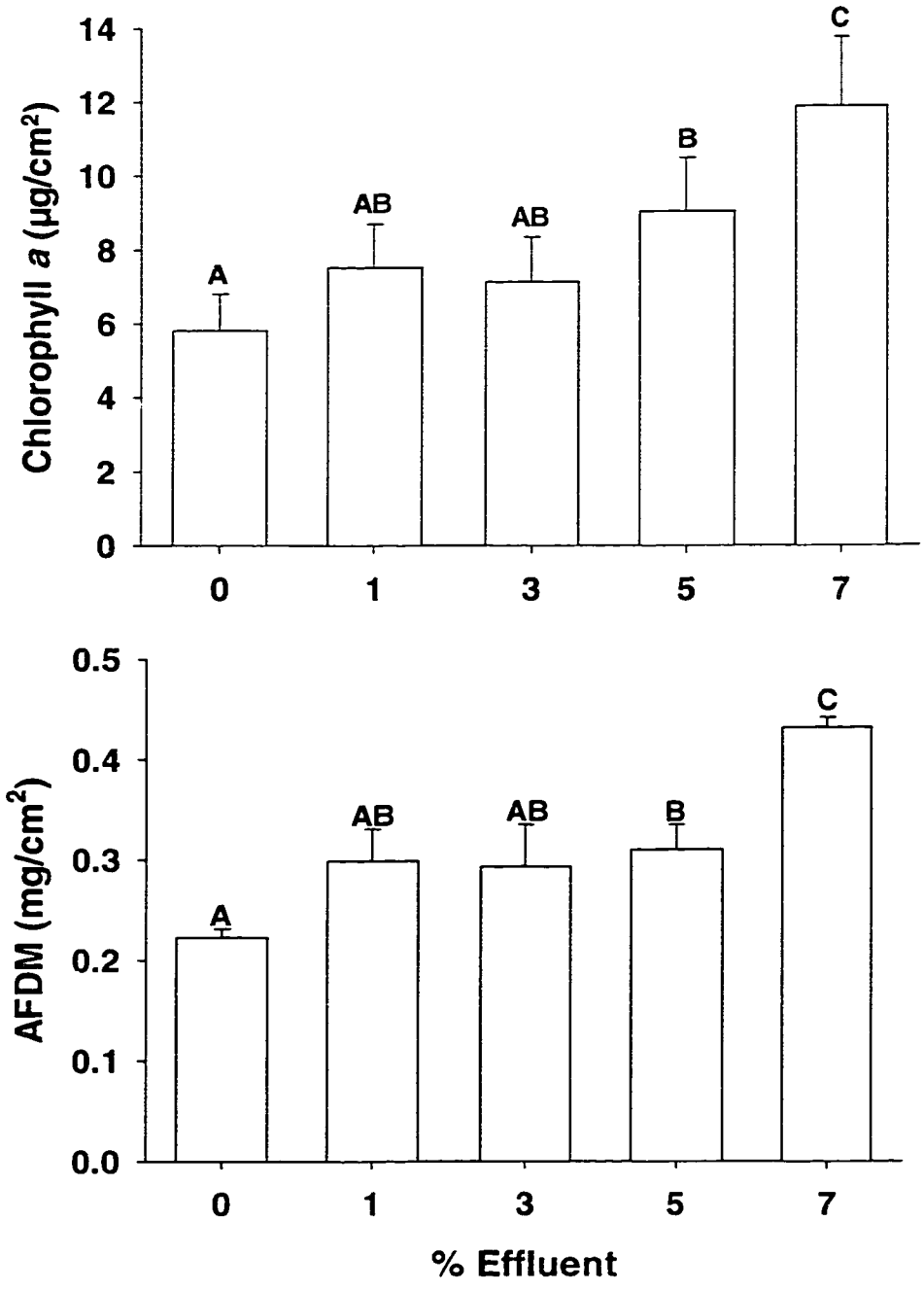


Figure 4.2. Mean (± SE) Chl*a* (µg/cm^2) and AFDM (mg/cm^2) of *Navicula* cultures after 15 days in 0, 1, 3, 5, and 7% BKME; means with shared letters are not significantly different at p=0.05.

Table 4.3. Results of analysis of covariance (time as covariate) of the effect of BKME treatment on *Navicula* biomass on Day 15. Biomass was measured as Chl*a* ($\mu g/cm^2$).

Source	DF	SS	*F*	*p*
Treatment	4	3.344	1.08	0.373
log (day+1)	1	68.6044	956.8	0.000
Treatment x log(day+1)	4	0.6855	2.39	0.057
Error	90	6.4532		
Total	99	79.0871		

Table 4.4. ANOVA of effect of BKME on Day 15 Chla and AFDM content of *Navicula* cultures.

Chla Day15					
Source	DF	SS	MS	*F*	*p*
Treatment	4	153.52	38.38	11.44	0.000
Error	15	50.31	3.35		
Total	19	203.83			
Fisher's protected $LSD_{\alpha=0.05}$=2.758					
AFDM Day 15					
Source	DF	SS	MS	*F*	*p*
Treatment	4	0.09139	0.02285	8.04	0.001
Error	15	0.04261	0.00284		
Total	19	0.13400			
Fisher's protected $LSD_{\alpha=0.05}$=0.080					

Table 4.5. Results of analysis of covariance of the effect of BKME treatment on AFDM over time (time as covariate).

Source	DF	SS	*F*	*p*
Treatment	4	1.26881	809.19	0.000
day	1	0.08013	2.72	0.030
Treatment x day	4	0.07630	12.17	0.000
Error	90	0.14112		
Total	99	1.56636		

Table 4.6. Regression equations for AFDM accrual by *Navicula* cultures in 0, 1, 3, 5, and 7% BKME. All equations had significant constants ($p<0.05$).

Treatment(%)	Regression Equation	*p*<	r^2
0	AFDM= -0.0389 + 0.0180Day	0.001	91.5
1	AFDM= -0.0786 + 0.0249Day	0.001	85.7
3	AFDM= -0.0797 + 0.0251Day	0.001	85.8
5	AFDM= -0.0846 + 0.0266Day	0.001	87.7
7	AFDM= -0.1350 + 0.0381Day	0.001	94.8

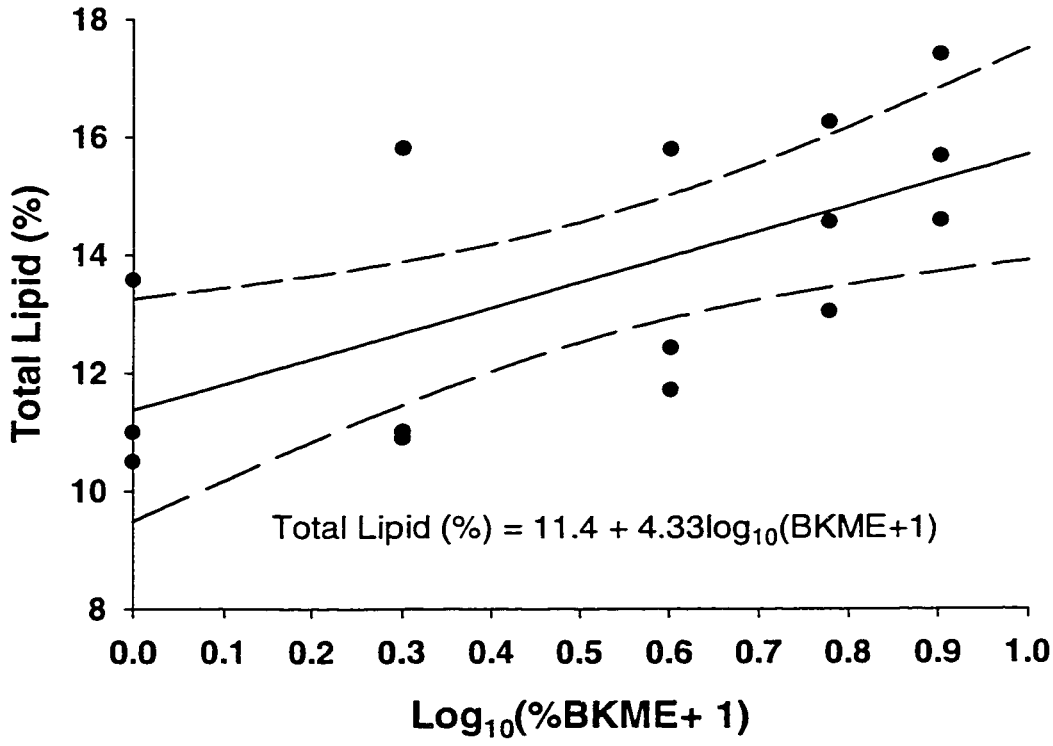


Figure 4.3. Effects of BKME concentration on total lipid (as % of dry wt.) of *Navicula*; regression line, and 95% confidence belts around predicted Y values are shown.

Table 4.7. Analysis of covariance testing the effects of %BKME on adjusted mean nitrogen content of 15d old *Navicula* cultures. Adjusted mean nitrogen concentration, SE of the adjusted means, and means comparisons are given.

Source	DF	SS	MS	*F*	*p*
Model	4	1.66869	0.4171	17.05	0.001
Error	7	0.1713	0.0245		
Total	11	1.8398			

Source	DF	SS	MS	*F*	*p*
Treatment	3	0.8336	0.2779	11.36	0.0044
Carbon	1	0.1390	1.1390	46.55	0.0002

TREATMENT	LSMean	N	SE
0	5.67		0.136
1	5.69		0.090
3	6.03		0.094
7	4.91		0.152

p Ho: $X_1=X_2$				
TRT	0	1	3	7
0	-			
1	0.1035	-		
3	0.8247	0.0341	-	
7	0.004	0.0030	0.007	-

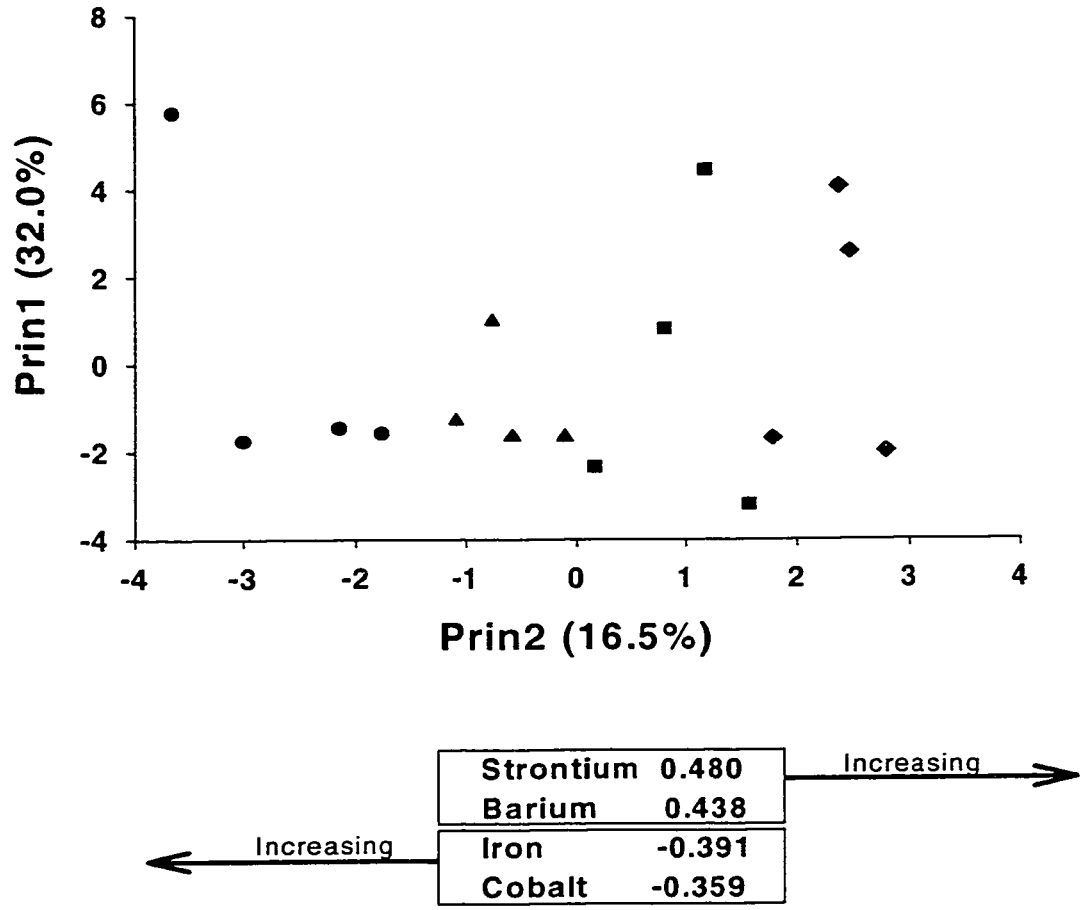


Figure 4.4. Principal components analysis of elemental composition of *Navicula*; ●= 0, ▲= 1, ■=3, ◆=7%. Proportion of total variance explained by each axis and elements with eigenvectors >0.25 are indicated.

Elemental composition of *Navicula* was analyzed to measure any effluent-related changes in concentrations of essential elements (Table 4.8). Principal component analysis of the correlation matrix of the elemental composition data separated treatments along the second axis, which was responsible for 16.5% of the variation (Fig. 4.4). An examination of eigenvectors indicated that barium, strontium, cobalt, and iron contributed to this variation. As effluent concentration increased, *Navicula* contained greater quantities of barium and strontium, and decreasing quantities of cobalt and iron.

Table 4.8. Elemental composition of *Navicula sp.* exposed to 0, 1, 3, and 7% effluent; * indicates essential or beneficial element (Sterner 1995).

ELEMENT	CONCENTRATION (SE) (µg/g)			
	Effluent %			
	0	1	3	7
Aluminum	997.5(37.9)	1030.0(59.7)	1055.060.8)	947.5(21.4)
Barium	11.3(1.89)	19.0(2.65)	26.5(3.07)	27.8(0.946)
Beryllium	0.6(0.125)	0.6(0.125)	0.6(0.125)	0.8(0.125)
Boron*	10.3 (3.09)	8.8(2.90)	10.0(3.34)	8.5(3.28)
Calcium*	7325.0(719)	8850.0(166)	8000.0(414)	8225.0(520)
Cadmium	0.6(0.125)	0.6(0.125)	0.6(0.125)	0.8(0.144)
Chromium	8.3(0.620)	21.0(14.7)	9.6(2.13)	4.1(0.392)
Cobalt*	14.0(1.08)	11.1(1.39)	10.0(2.04)	6.7(0.550)
Copper*	12.5(0.645)	14.3(1.25)	21.8(5.20)	15.0(0.401)
Iron*	12425.0(1141)	9950.0(633)	8575.0(1468)	5900.0319)
Lead	2.0(0.000)	1.5(0.289)	1.8(0.250)	2.0(0.000)
Magnesium*	4750.0(323)	4450.0(218)	4150.0(253)	4875.0(307)
Manganese*	322.5(32.8)	337.5(13.1)	290.0(49.2)	310.0(21.2)
Molybdenum*	0.6(0.125)	6.5(5.83)	0.6(0.125)	0.8(0.144)
Nickel*	117.5(49.2)	101.2(23.9)	87.3(30.2)	33.8(9.66)
Phosphorus*	10575.0(397)	12300.0(548)	10775.0(642)	10575.0(496)
Potassium	2250.0(263)	2200.0(252)	1975.0(345)	2175.0(609)
Silver	0.6(0.125)	0.6(0.125)	0.6(0.215)	0.8(144)
Sodium*	808.0(148)	827.0(102)	747.5(26.9)	1018.0(170)
Strontium	2.4(0.485)	9.6(0.232)	15.8(1.03)	22.5(0.645)
Titanium	36.2(16.6)	8.4(0.584)	12.0(3.54)	10.2(3.30)
Vanadium	4.0(1.00)	4.0(1.00)	4.0(1.00)	4.0(1.00)
Zinc*	25.3(6.17)	26.3(1.55)	38.8(9.97)	30.0(1.08)
Zirconium	7.2(1.24)	14.6(2.91)	12.0(3.65)	8.8(1.65)

4.4 Discussion

Most chronic bioassays do not take into account an interaction between food organisms and the toxicant. However, results of the present experiment demonstrate that food quality, growth rate, and elemental composition of algae were affected by exposure to bleached kraft mill effluent. In the natural environment, algae and herbivores would be exposed to the toxicant. Therefore, allowing toxicant effects on food algae to occur in bioassays may increase realism and, thus, the capacity of simple laboratory bioassays to predict field effects.

4.4.1 Effect of Effluent on Algal Quality

Total lipid content of *Navicula* increased with effluent concentration, a response also reported for other algal species exposed to nutrient or toxicant stress (MacCarthy and Patterson 1974, Soto et al. 1977, Sicko-Goad et al. 1989a,b). Increased lipid content of food algae has the potential to affect bioassay results because changes in food quality can modify toxicity. Indeed, increased dietary lipid reduces toxicity (Hickie and Dixon 1987, Hickie et al. 1989). Lipids also are phagostimulants to some aquatic insects (Cargill et al. 1985), thus, algae that increase in lipid content in response to toxicants may become more palatable or attractive to grazers. Furthermore, algae with greater lipid content may also bioconcentrate greater quantities of hydrophobic contaminants (Barron 1990), thereby increasing intake of potentially deleterious compounds by herbivores.

Diatoms in the 3% and 7% treatments had significantly higher C:N ratios than the other treatments, as indicated by the lower adjusted nitrogen content. Increased carbon content indicates the presence of more lipids or carbohydrates, corroborating my direct measurement of lipid content. Although a higher C:N ratio is normally viewed as an indicator of poorer quality food in aquatic environments (Iverson 1974, Cummins and Klug 1979, Newman et al. 1996), this general assumption is made because ambient sources of C, such as natural detritus, are refractory carbohydrates, such as poorly digestible cellulose, rather than lipids. In this experiment, higher C:N ratios for cultured

algae were indicative of increased lipid content, and therefore of higher food quality. Because C:N ratios cannot distinguish between changes in carbohydrate versus lipid content, I recommend that direct measurement of lipid, carbohydrate, and protein content be used in future studies. Direct measurement is particularly important given that dietary lipid may decrease toxicity, while increased dietary carbohydrates may increase toxicity (Hilton and Hodson 1983, Dixon and Hilton 1985).

Exposure to effluent changed biochemical composition of the algae and bacterial content of the cultures. Micrographs, generated by a confocal laser scanning microscope, of *Navicula* exposed to 0, 1, and 7% treatments (Plate 4.1) revealed that the bacterial population was more abundant in *Navicula* exposed to effluent. Bacteria are abundant in pulp mill effluent (Fulthorpe et al. 1992, Mohammed 1997), are a natural component of periphyton (Wetzel 1983, Newman and McIntosh 1989, Stevenson 1996) and are nutritionally important to various groups of aquatic insects (Cummins and Klug 1979). An increased microbial population may, therefore, represent an additional change in nutritional value of the biofilm.

It is surprising that *Navicula* exposed to effluent grew faster than controls, because Chu no. 10 culture media is designed to contain all required nutrients for algal growth (Nichols 1973), and media was completely replaced every 48h so that nutrient limitation was unlikely. In addition, effluent presence did not change the media N:P ratios to any great extent, as they ranged from 20:1 to 19.5:1 for 0% and 7% treatments, respectively. Possible explanations for increased growth are: (1) the effluent contained some unrecognized micronutrients; (2) diatoms were able to utilize organic carbon sources in the effluent, as stimulation of algal growth by dissolved organic matter has been reported by Geisy (1976), Prakash and Rashid (1968), Tulonen et al. (1992), Vraná and Votruba (1995), and Steinberg and Bach (1996); (3) bacterial respiration resulted in increased C availability, (4) hormesis, the stimulation resulting from exposure to low levels of toxicants (Calabrese 1987); or (5) rapid nutrient cycling occurred within the mat as a function of bacterial abundance.

Navicula exposed to pulp mill effluent had more strontium and barium, and lower concentrations of iron and cobalt than control diatoms. Pulp mill effluents contain many metals, as metals can be accumulated by trees from the soil (McCubbin and Folke 1993). Cobalt (Krishnamurthy and Bharatai 1995, Reddy and Venkateswarlu 1985), strontium (Krosshavn et al. 1996), barium, and iron (Michigan Dept. of Public Health 1990) have all been associated with pulp mill effluents. The predictive relationship between strontium content and effluent exposure was very strong (strontium (µg/g) = 2.41 + 9.7 ln(%BKME + 1), r^2=97.0, df_{error}=14, n=1), suggesting the potential use of this metal as an effluent marker for kraft mills. The reason why concentrations of iron and cobalt were reduced in algae exposed to effluent is not clear, but this observation may be due to dissolved organic material in BKME. Lignins in pulp mill effluent (Lalvani et al. 1997) and other dissolved organic molecules bind to and, thus, affect the availability of iron and other metals (Shapiro 1966, 1969, Suzuki et al. 1992). Metal-metal interactions may also affect accumulation and toxicity of metals in animals and plants (Hewitt 1948, Harrison and Morel 1983, Tomasik et al. 1995). Of the 4 metals, cobalt and iron are considered essential nutrients (Sterner 1995), although the actual requirements for these substances by either diatoms or mayflies is not known.

4.4.2 Potential for Trophic Transfer of Contaminants

Navicula cultured in effluent would likely contain greater quantities of contaminants such as PAHs, resin acid derivatives, and phenolics (Headley et al. 1995; Cash and Culp 1996) that would be available for transfer to higher trophic levels. Although laboratory bioassays frequently assume that uptake from water is the only important exposure route, dietary exposure can be important for substances that sorb to particulate matter (Schrap 1991). Bleached kraft mill effluent contaminants, such as resin acids (Carlberg and Stuthridge 1996) and PAHs (Dobroski and Epifanio 1980, Barron 1990, Harkey et al. 1994), as well as some metals (Kooijman 1991, Allen et al. 1995, Taylor et al. 1998), are likely candidates. Furthermore, dietary uptake is believed

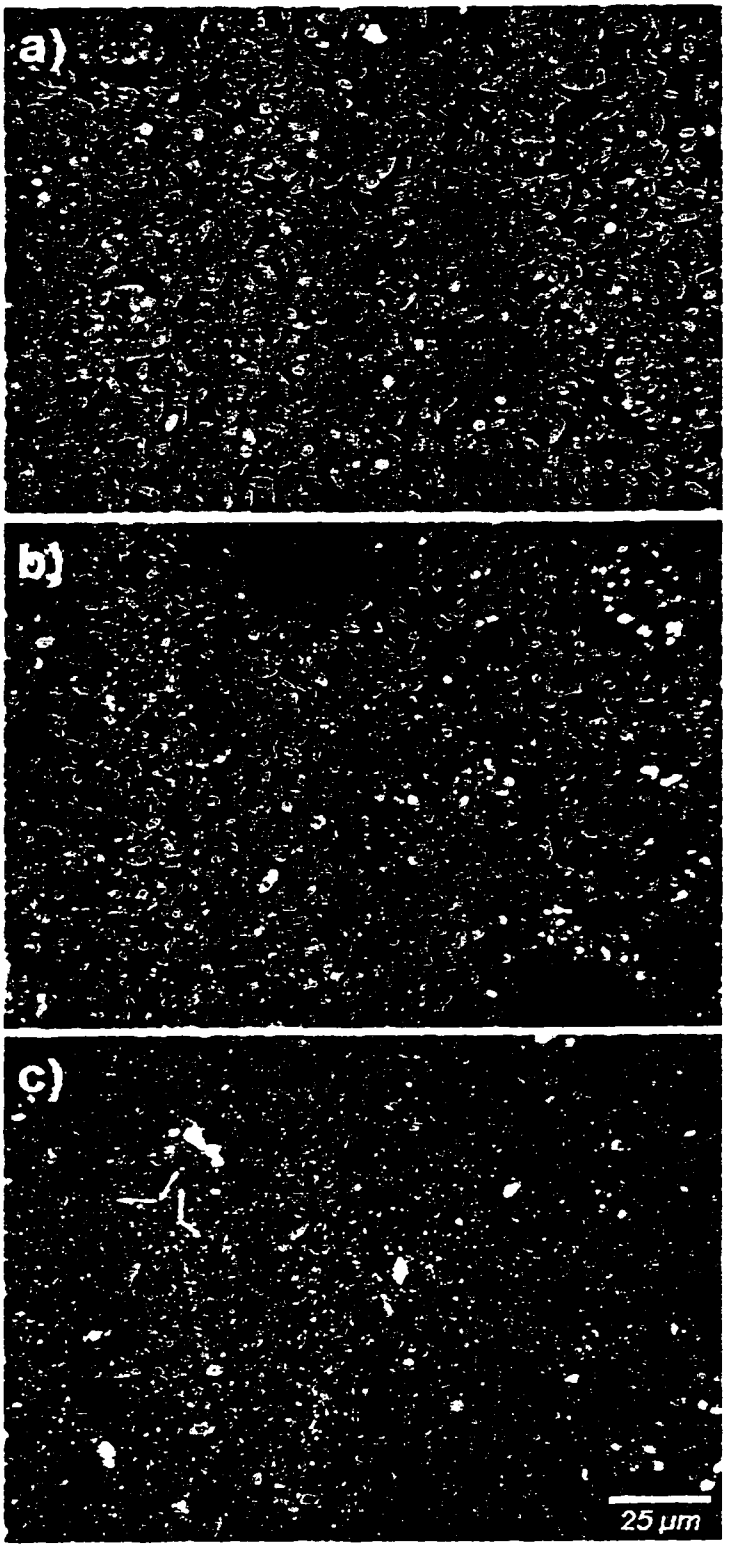


Plate 4.1. Confocal image of *Navicula* mats cultured in (a) 0, (b) 1, and (c) 7% BKME. The red colour is the autofluorescence of diatom chloroplasts, while bacteria are stained green with a nucleic acid stain.

important for lipophilic compounds with log n-octanol/water partition coefficients > 5 (Lanno 1989, Boese 1990), such as tetrachlorophenol, pentachlorophenol, PAHs such as fluoranthene and pyrene, PCBs and a number of pesticides (Jarvinen and Tyo 1978, Thomann and Connolly 1984, Oliver and Nimi 1985).

The route of uptake (bioconcentration or ingestion) can modify exposure to a contaminant, as absorption efficiencies from food are usually lower than absorption efficiencies associated with bioconcentration (Jarvinen and Tyo 1978, Cleveland et al. 1993, Köck and Bucher 1997). However, while exposure via ingestion may contribute only a small amount to body burdens, it has an additive effect (Fisher et al. 1986), and may have a significant impact on the effects of exposure. For example, dietary exposure to the organochlorine pesticide, Endrin, increased mortality rates of fathead minnows (*Pimephales promelas*) by 80% relative to water exposure alone (Jarvinen and Tyo 1978) even though uptake via food was minimal compared to bioconcentration. In addition, although bioconcentration was the most important source of cadmium and chromium to *Daphnia* (Kungolos and Aoyama 1993), the addition of uptake via food significantly increased toxicity. Dietary exposure may be particularly important for toxicants that produce effects by inhibiting feeding or digestion.

4.4.3 Implications for Bioassays with Exposure at 2 Trophic Levels

When live organisms are provided as food to the subjects of toxicity tests, any interaction between food organisms and the toxicant is usually ignored. The present research demonstrated that exposure to pulp mill effluent changed the growth rate and chemical composition of algae. This interaction could affect results of bioassays, as changes in food abundance and dietary lipid, carbohydrate, and protein content modify toxicity (Mehrle et al. 1974, Sosnowski et al. 1979, Phillips and Buhler 1979, Hilton and Hodson 1983, Dixon and Hilton 1985, Holdway and Dixon 1985, Hickie and Dixon 1987, Dahlgren 1988, Hickie et al. 1989, Farkas et al. 1996). As algae would be exposed to the toxicant in field situations, allowing this interaction to occur in bioassays represents an increase in realism rather than a confounding factor. Increasing test realism should be a priority when the purpose of the bioassay is

to predict field effects rather than to simply measure potential toxicity of samples. The effects on algae in receiving waters should be verified, if possible, by field sampling, and because algal species do not exhibit consistent responses to stress (Table 4.1), it would also be advisable to use species found in the natural environment of the test herbivore. Exposing both food and test organisms to the toxicant would also allow more natural partitioning and uptake through bioconcentration and ingestion.

In a typical bioassay on a sample of pulp mill effluent, test animals are exposed to effluent only in the water (Environment Canada 1992). In the natural environment, some effluent components may bind to dissolved organic matter, reducing uptake by living cells or sorption to particulate matter (Carlberg and Stuthridge 1996). Other components may sorb to particulate matter and settle to be eaten, degraded, mineralized, or released into interstitial and overlying water. Compounds in effluent may also bioconcentrate in plants and animals and be transferred via trophic interactions. By considering only bioconcentration in bioassays, we ignore all of these possibilities and their potential modifying effects on toxicity, thus reducing the likelihood that bioassay data will be useful for predicting field effects. While it may be difficult to replicate all of these processes, the cumulative effects of direct toxicity and indirect effects, via secondary poisoning and changes in food quality, might be assessed by exposing both test organisms and their food supplies to the toxicant. This approach, while more complicated, may serve to enhance the predictive ability of simple bioassays.

4.5 Literature Cited

Ahtiainen, J., T. Nakari, and J. Silvonen. 1996. Toxicity of TCE and ECF pulp bleaching effluents assessed by biological toxicity tests. Pages 33-40 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Allen, Y., P. Calow, and D. J. Baird. 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. Environmental Toxicology and Chemistry 14: 1625-1630.

Atchley, W. R. 1978. Ratios, regression intercepts, and the scaling of data. Systematic Zoology 27: 78-83.

Baker, J. H., and L. A. Bradnam. 1976. The role of bacteria in the nutrition of aquatic detritivores. Oecologia (Berl.) 24: 95-104.

Barron, M. G. 1990. Bioconcentration. Environmental Science and Technology 24: 1612-1618.

Ben-Amotz, A., T. G. Tornabene, and W .H. Thomas. 1985. Chemical profile of selected species of microalgae with emphasis on lipids. Journal of Phycology 21: 72-81.

Blanck, H. 1985. A simple, community level, ecotoxicological test system using samples of periphyton. Hydrobiologia 124: 251-161.

Blanck, H., and S. Wängberg. 1988. Validity of an ecotoxicological test system: short-term and long-term effects of arsenate on marine periphyton communities in laboratory systems. Canadian Journal of Fisheries and Aquatic Sciences 45: 1807-1815.

Boese, B. L., H. Lee III, D. T. Sprecht, and R. C. Randall. 1990. Comparison of aqueous and solid-phase uptake for hexachlorobenzene in the tellinid clam *Macoma nasuta* (Conrad): A mass balance approach. Environmental Toxicology and Chemistry 9: 221-231.

Cairns., J. 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100: 47-57.

Calabrese, E.J., M.E. McCarthy, and E. Kenyon. 1987. The occurrence of chemically induced hormesis. Health Physics 52: 531-541.

Cargill II, A. S., K. W. Cummins, B. J. Hanson, and R. R. Lowry. 1985. The role of lipids as feeding stimulants for shredding aquatic insects. Freshwater Biology 15: 455-464.

Carlberg, G. E., and T. R. Stuthridge. 1996. Environmental fate and distribution of substances. Pages 169-178 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental Fate and Effects of Pulp and Paper Mill Effluents*. Florida, Delray Beach.

Cash, K. J., and J. M. Culp. 1996. The effects of bleached kraft pulp-mull effluent on periphyton tissue. DOE FRAP Report 1996-08. Environment Canada, Vancouver, B.C., Canada.

Cleveland, L., E.E. Little, D.R. Buckler, and R.H. Weidmeyer. 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). Aquatic Toxicology 27: 265-280.

Costle, D. M., J. D. Denit, and R. W. Dellinger. 1980. Development document for effluent limitations guidelines and standards for the pulp, paper and paperboard and the builders' paper and board mills - point source categories. United States Environmental Protection Agency, Washington, D.C.

Cowgill, U. M. 1987. Critical analysis of factors affecting the sensitivity of zooplankton and the reproducibility if toxicity results. Water Research 21: 1453-1462.

Cowgill, U. M., D. P. Milazzo, and C. K. Meagher. 1988. New diet for *Ceriodaphnia dubia*. Bulletin of Environmental Contamination and Toxicology 41: 304-309.

Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics 10: 147-172.

Dahlgren, J. 1988. Variation in diet composition: a hazard to the reliability of the LD_{50}-test. Environmental Pollution 49: 177-181.

Dixon, D. G., and J. W. Hilton. 1985. Effects of available dietary carbohydrates and water temperature on the chronic toxicity of waterborne copper to rainbow trout (*Salmo gaidneri*). Canadian Journal of Fisheries and Aquatic Sciences 42: 1007-1013.

Dobroski , C. J., and C .E. Epifanio. 1980. Accumulation of benzo[*a*]pyrene in a larval bivalve via trophic transfer. Canadian Journal of Fisheries and Aquatic Science 37: 2318-2322.

Eklund, B., M. Linde, and M. Tarkepa. 1996. Comparative assessment of the toxic effects from pulp mill effluents to marine and brackish water organisms. Pages 95-105 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

El Jay, A. 1996. Effects of organic solvents and solvent-Atrazine interactions on two algae, *Chlorella vulgaris* and *Selenastrum capricornutum*. Archives of Environmental Contamination and Toxicology 31: 84-90.

El-Dib, M. A., H. F. Abou-Waly, and A. M .H. el-Naby. 1997. Impact of fuel oil on the freshwater alga *Selenastrum capricornutum*. Bulletin of Environmental Contamination and Toxicology 59: 438-444.

Enserink, L., W. Luttmer, and H. Maas-Diepeveen. 1990. Reproductive strategy of *Daphnia magna* affects the sensitivity of its progeny in acute toxicity tests. Aquatic Toxicology 17: 15-26.

Environment Canada. 1992. Biological test method: test of reproduction and survival using the cladoceran *Ceriodaphnia dubia*.

Farkas, S., E. Hornung, and E. Fischer. 1996. Toxicity of copper to *Porcellio scaber* Latr. (Isopoda) under different nutrition status. Bulletin of Environmental Contamination and Toxicology 57: 582-588.

Fisher, D. J., J. R. Clarke, M. H. Roberts, J. P. Connolly, and L. H. Mueller. 1986. Bioaccumulation of Kepone by spot (*Leiostomus xanthrus*): importance of dietary accumulation and ingestion rate. Aquatic Toxicology 9: 161-178.

Forbes, V. E., and T. L. Forbes. 1994. *Ecotoxicology in theory and practice.* Chapman & Hall, London.

Fulthorpe, R. A., S. N. Liss, and D. G. Allen. 1992. Characterization of bacteria isolated from a bleached kraft pulp mill wastewater treatment system. Canadian Journal of Microbiology 39: 13-24.

Gardner, W.S., W.A. Frez, E.A. Cichocki and C.C. Parrish. 1985. Micromethod for lipids in aquatic invertebrates. Limnology and Oceanography 30:1099-1105.

Geisy, J. P. 1976. Stimulation of growth in *Scenedesmus obliquus* (Chlorophyceae) by humic acids under iron limited conditions. Journal of Phycology 12: 172-179.

Genter, R. B. 1996. Ecotoxicology of inorganic toxic stress to algae. Pages 404-458 *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, eds. *Algal Ecology.* Academic Press.

Green, R. H. 1979. *Sampling design and experimental methods for environmental biologists.* John Wiley & Sons.

Groeger, A. W., M. D. Schram, and G. Richard. 1991. Influence of food quality on growth and reproduction in *Daphnia.* Freshwater Biology 26: 11-19.

Guillard, R. R. L., and C. J. Lorenzen. 1972. Yellow-green algae with chlorophyllide C. Journal of Phycology 8: 10-14.

Harkey, G. A., M. J. Lydy, J. Kukkonen, and P. F. Landrum. 1994. Feeding selectivity and assimilation of PAH and PCB in *Diporeia* spp. Environmental Toxicology and Chemistry 13: 1445-1455.

Harrison, G. I., and F. M. M. Morel. 1983. Antagonism between cadmium and iron in the marine diatom *Thalassiosira weissflogii.* Journal of Phycology 19: 495-507.

Harrison, P. J., P. A. Thompson, and G. S. Calderwood. 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. Journal of Applied Phycology 2: 45-56.

Headley, J. V., P. A. Chambers, J. M. Culp, and K. M. Peru. 1995. Evaluation of small volume techniques for broad spectrum analysis of biofilm materials and bleached kraft mill effluents. Northern River Basins Study, Edmonton, Alberta.

Hewitt, E. J. 1948. Relation of manganese and some other metals to the iron status of plants. Nature (London) 161: 489-490.

Hickie, B. E., and D. G. Dixon. 1987. The influence of diet and preexposure on the tolerance of sodium pentachlorophenate by rainbow trout *(Salmo gairdneri).* Aquatic Toxicology 9: 343-353.

Hickie, B. E., D. G. Dixon, and J. F. Leatherland. 1989. The influence of the dietary carbohydrate:lipid ratio on the chronic toxicity of sodium pentachlorophenate to rainbow trout. Fish Physiology and Biochemistry 6: 175-185.

Hilton, J. W., and P. V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). Journal of Nutrition 113: 1241-1248.

Himbeault, K. T. 1995. Alternative approaches for monitoring aquatic ecosystems: a study on the effects of municipal sewage effluent on biota in the South Saskatchewan River. M.Sc. Thesis, Department of Toxicology. University of Saskatchewan, Saskatoon. 144p.

Hoaglund, K. D., J. P. Carder, and R. L. Spawn. 1996. Effects of organic toxic substances. *In* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, eds. *Algal ecology*. Academic Press.

Holdway, D. A., and D. G. Dixon. 1985. Acute toxicity of pulse-dosed methoxychlor to juvenile american flagfish (*Jordanella floridae* Goode and Bean) as modified by age and food availability. Aquatic Toxicology 6: 243-250.

Iverson, T. M. 1974. Ingestion and growth in *Sericostoma personatum* (Trichoptera) in relation to the nitrogen content of ingested leaves. Oikos 25: 278-282.

Jarvinen, A. W., and R. M. Tyo. 1978. Toxicity to fathead minnows of Endrin in food and water. Archives of Environmental Contamination and Toxicology 7: 409-421.

Kimball, K. D., and S. A. Levin. 1985. Limitations of laboratory bioassays: the need for ecosystem-level testing. BioScience 35: 165-171.

Kiørboe, T. 1989. Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. Marine Ecology Progress Series 55: 229-234.

Köck, G., and F. Bucher. 1997. Accumulation of zinc in rainbow trout (*Oncorhynchus mykiss*) after waterborne and dietary exposure. Bulletin of Environmental Contamination and Toxicology 58: 305-310.

Kooijman, S. A. L. M. 1991. Effects of feeding conditions on toxicity for purpose of extrapolation. Comparative Biochemistry and Physiology 100C: 305-310.

Krishnamurthy, S. R., and S. G. Bharati. 1995. Distribution of cobalt in the surface waters of the polluted river Kali Dandeli, North Kanara, Karnataka, India. Environment & Ecology 13: 698-700.

Krosshavn, M., L. Skipperud, H. N. Lien, and B. Salbu. 1996. Transfer, pathways, enrichment, and discharge of ^{137}Cs and ^{90}Sr in the pulp industry. Health Physics 71: 326-334.

Kungolos, A., and I. Aoyama. 1993. Interaction effect, food effect, and bioaccumulation of cadmium and chromium for the system *Daphnia magna* - *Chlorella ellipsoidea*. Environmental Toxicology and Water Quality 8: 351-369.

Lalvani, S. B., T. S. Wiltowski, D. Murphy, and L. S. Lalvani. 1997. Metal removal from process water by lignin. Environmental Technology 18: 1163-1168.

Lanno, R.P., B. E. Hickie, and D .G. Dixon. 1989. Feeding and nutritional considerations in aquatic toxicology. Hydrobiologia 188/189: 525-531.

LaRocca, C. A., D. E. Francisco, and F. A. DiGiano. 1994. Effects of diet on survival, reproduction and sensitivity of *Ceriodaphnia dubia*. Water Research 66: 905-911.

Lewis, M. A. 1995. Algae and vascular plant tests. Pages 135-169 *In* G. M. Rand, ed. *Fundamentals of aquatic toxicology*. Taylor & Francis, Washington D.C.

Lowell, R. B. and Culp, J. M. 1996. Combined effects of dissolved oxygen level and bleached kraft pulp mill effluent and municipal sewage on a mayfly (*Baetis tricaudatus*): assessments using artificial streams, Northern River Basins Study Project Report No. 98, Edmonton, Alberta.

MacCarthy, J. J., and G. W. Patterson. 1974. Effects of cation level of the nutrient medium on the biochemistry of *Chlorella*. Plant Physiology 54: 129-132.

Malins, D. C. 1989. The use of environmental assays for impact assessment. Hydrobiologia 188/189: 87-91.

Maltby, L., and P. Calow. 1989. The application of bioassays in the resolution of environmental problems; past, present and future. Hydrobiologia 188/189: 65-76.

McCubbin, N., and J. Folke. 1993. A review of literature on pulp and paper mill effluent characteristics in the Peace and Athabasca River basins. Project Report No. 15, Northern River Basin Study, Edmonton, Alberta.

Mehrle, P. M., W. W. Johnson, and F. L. Mayer 1974. Nutritional effects on chlordane toxicity in rainbow trout. Bulletin of Environmental Contamination and Toxicology 12: 513-517.

Michigan Dept. of Public Health. 1990. Health Assessment for Packaging Corporation of America, Filer City, Manistee County, Michigan, Region 5. CERCLIS No. MID980794747.

Mohamed, M. M. 1997. Phosphorus limitation of sestonic and sessile bacteria in the Fraser River and the effect of pulp mill effluent. M.Sc. Thesis, Department of Applied Microbiology and Food Science. University of Saskatchewan, Saskatoon.121p.

Newman, M. C., and A. W. McIntosh. 1989. Appropriateness of *Aufwuchs* as a monitor of bioaccumulation. Environmental Pollution 60: 83-100.

Newman, R. M., W. C. Kerfoot, and Z. Hanscom III. 1996. Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. Ecology 77: 2312-2323.

Nichols, H. W. 1973. Growth media - freshwater. Pages 7-24 *In* J. R. Stein, ed. *Handbook of phycological methods.* Cambridge University Press, London.

Oliver, B. G., and A. J. Niimi. 1985. Bioconcentration factors of some halogenated organics for rainbow trout: limitations in their use for prediction of environmental residues. Environmental Science and Technology 19: 842-849.

Parrish, P. R. 1995. Acute toxicity tests. Appendix A pages 947-973 *In* G. M. Rand, ed. *Fundamentals of aquatic toxicology.* Taylor & Frances, Washington, D.C.

Phillips, G. R., and D. R. Buhler. 1979. Influences of Dieldrin on the growth and body composition of fingerling trout (*Salmo gairdneri*) fed Oregon moist pellets or tubificid worms (*Tubifex* sp.). Journal of the Fisheries Research Board of Canada 36: 77-80.

Piorreck, M., K. Baasch, and P. Pohl. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry 23: 207-216.

Prakash, A., and M. A. Rashid. 1968. Influence of humic acids on the growth of marine phytoplankton: dinoflagellates. Limnology and Oceanography 13: 598-606.

Priha, M. H. 1996. Ecotoxicological impacts of pulp mill effluents in Finland. Pages 637-650 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Reddy, P. M., and V. Venkateswarlu. 1985. Ecological studies in the paper mill effluents and their impact on the river Tungabhadra: heavy metals and algae. Proceedings of the Indian Academy of Science (Plant Science) 95: 139-146.

Schrap, S. M. 1991. Bioavailability of organic chemicals in the aquatic environment. Comparative Biochemistry and Physiology 100C: 13-16.

Scrimgeour, G. J., J. M. Culp, M. L. Bothwell, F.J. Wrona, and M.H. McKee. 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly-diatom systems. Oecologia (Berlin) 85: 343-348.

Shapiro, J. 1966. The relation of color to iron in natural waters. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 16: 477-484.

Shapiro, J. 1969. Iron in natural waters - its characteristics and biological availability as determined with the ferrigram. Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie 17: 456-466.

Sicko-Goad, L., and N.A. Andresen. 1993. Effects of lipid composition on the toxicity of trichlorobenzene isomers to diatoms, I. Short-term effects of 1,3,5-

trichlorobenzene. Archives of Environmental Contamination and Toxicology 24: 236-242.

Sicko-Goad, L., M. S. Evans, D. Lazinsky, J. Hall, and M. S. Simmons. 1989a. Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. IV. Pentachlorobenzene and comparison with trichlorobenzene isomers. Archives of Environmental Contamination and Toxicology 18: 656-668.

Sicko-Goad, L., J. Hall, D. Lazinsky, and M. S. Simmons. 1989b. Effects of chlorinated benzenes on diatom fatty acids composition and quantitative morphology. III. 1,2,3-trichlorobenzene. Archives of Environmental Contamination and Toxicology 18: 647-655.

Sosnowski, S. L., D. . Germond, and J. H. Gentile. 1979. The effect of nutrition on the response of field populations of the calanoid copepod *Acartia tonsa* to copper. Water Research 13: 449-452.

Soto, C., J. A. Hellebust, and T. C. Hutchinson. 1977. Effect of naphthalene and aqueous crude oil extracts on the green flagellate *Chlamydomonas angulosa*. III. Changes in cellular composition. Canadian Journal of Botany 55: 2765-2777.

Steinberg, C. E. W., and S. Bach. 1996. Growth promotion by a groundwater fulvic acid in a bacterial/algae system. Acta Hydrochimica et Hydrobiologica 24: 98-100.

Sterner, R. W. 1993. Daphnia growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. Ecology 74: 2351-2360.

Sterner, R. W. 1995. Elemental stoichiometry of species in ecosystems. Pages 387 *In* C. G. Jones and J. H. Lawton, eds. *Linking species and ecosystems*. Champan & Hall, New York.

Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25: 1-9.

Stevenson, R.J. 1996. Patterns of benthic algae in aquatic ecosystems in R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, eds. *Algal ecology*. Academic Press.

Suzuki, T., F. M. Clydesdale, and T. Pandolf. 1992. Solubility of iron in model systems containing organic acids and lignin. Journal of Food Protection 55: 893-898.

Taylor, G., D. J. Baird, and A. M. V. M. Soares. 1998. Surface binding of contaminants by algae: consequences for lethal toxicity and feeding to *Daphnia magna* Straus. Environmental Toxicology and Chemistry 17: 412-419.

Thomann, R. V., and J. P. Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environmental Science and Technology 18: 65-71.

Thompson, P., and P. Couture. 1991. Aspects of carbon metabolism in the recovery of *Selenastrum capricornutum* population exposed to cadmium. Aquatic Toxicology 17: 1-14.

Tomasik, P., C. M. Magadza, S. Mhizha, A. Chirume, M. F. Zaranyika, and S. Muchiriri. 1995. Metal-metal interactions in biological systems. Part IV. Freshwater snail *Bulinus globosus*. Water, Air and Soil Pollution 83: 123-145.

Torres, A. M. R., and L. M. O'Flaherty. 1976. Influence of pesticides on *Chlorella, Chlorococcum, Stigeoclonium* (Chlorophyceae), *Tribonema, Vauceria* (Xanthophyceae) and *Oscillatoria* (Cyanophyceae). Phycologia 15: 25-36.

Tulonen, T., K. Salonen, and L. Arvola. 1992. Effects of different molecular weight fractions of dissolved organic matter on growth on bacteria, algae and protozoa from a highly humic lake. Hydrobiologia 229: 239-252.

Verta, M., J. Ahtiainen, T. Nakari, A. Langi, and E. Talka. 1996. The effect of waste constituents on the toxicity of TCF and ECF pulp bleaching effluents. Pages 41-51 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Visviki, I., and J. W. Rachlin. 1994. Acute and chronic exposure of *Dunaliella salina* and *Chlamydomonas bullosa* to copper and cadmium: Effects on growth. Archives of Environmental Contamination and Toxicology 26: 149-153.

Vraná, D., and J. Votruba. 1995. Influence of soluble humic acids on the growth of algae and blue-green algae. Folia Microbiologia 40: 207-208.

Wetzel, R.G. 1983. *Limnology*. CBS College Publishing, New York.858p.

Chapter 5

But Does it Taste Better? Effects of Bleached Kraft Pulp Mill Effluent on Feeding Behavior of the Mayfly *Ameletus subnotatus*

5.1 Introduction

Feeding inhibition is one of the most frequently measured behavioral responses to toxicants, with effects documented for a variety of organisms including protozoa (Doucet and Maly 1990), rotifers (Juchelka and Snell 1994 1995), flatworms (Ham et al. 1995), molluscs (Laskowski and Hopkin 1996, Marigomez et al. 1986), cladocerans (Allen et al. 1995, Bitton et al. 1995,1996), amphipods (Weeks 1993, Blockwell et al. 1998), amphibians (Schuytema et al. 1993, Brady and Griffiths 1995), fish (Foster et al. 1966, Mathers et al. 1985, Little et al. 1990, Weber 1996), and rodents (Linder and Richmond 1990). Causes of feeding inhibition are varied, including damage to sensory apparatus (Foster et al. 1966, Lemly and Smith 1985), taste aversion (Linder and Richmond 1990), and damage to digestive tissues (Laskowski and Hopkin 1996). Feeding inhibition is seen in response to a wide variety of compounds, and often provides a more sensitive endpoint than acute toxicity (Jones et al. 1991) (Table 5.1). When the altered behavior has the potential to affect fitness (e.g. a reduction in feeding), the effective concentration for behavioral changes may indicate the concentration at which chronic effects will occur.

Feeding inhibition can occur with exposure to pulp and paper mill effluents (McLeese 1973, Cooley 1977, Crane and Maltby 1991, Bitton et al. 1995), and this reduction in feeding may contribute to chronic growth and reproductive effects of these effluents. Pulp mill effluent is a complex mixture of hundreds of compounds (Suntio et al. 1988), including some that are, or are structurally very similar to plant allelochemicals (e.g. diterpene resin acids and

Table 5.1. Literature survey of feeding inhibition studies (see literature cited for a list of surveyed literature). The sensitivity of feeding endpoints (subscript F) is compared with the median lethal concentration (LC_{50}); (<) indicates increased sensitivity, (=) indicates equal sensitivity, and (>) indicates decreased sensitivity. Table values are the number of published accounts meeting the indicated criteria. Studies in which more than 1 taxa or effects of more than 1 toxicant were measured are counted once for each toxicant/taxa combination.

Toxicant class	EC_{F50}[1] *vs.* LC_{50}[2]			$LOEC_F$[3] *vs.* LC_{50}			$NOEC_F$[4] *vs.* LC_{50}		
	<	=	>	<	=	>	<	=	>
Metals	3	1	7	2	-	1	9	-	2
Phenolics	1	-	3	3	-	0	0	-	1
Pesticides	7	-	3	4	1	0	13	-	1
Petroleum products	-	-	-	-	-	-	0	-	1
Complex effluents	5	2	2	-	-	-	-	-	-
PAHs	-	-	-	-	-	-	2	-	0
Miscellaneous	6	-	0	1	-	0	3	-	0

[1]EC_{F50}=concentration that inhibits feeding by 50%; [2]LC_{50}= median lethal concentration;
[3]LOEC= lowest observable effect concentration; [4]NOEC = no observable effect concentration

phenolics). A primary function of allelochemicals is to reduce herbivore damage through mechanisms including general toxicity, hormonal disruption, and feeding inhibition or deterrency (Sláma 1971, Jacobson et al. 1975, Panda and Kush 1995). For example, terpenes are a widespread group of secondary compounds, found in effluents from mills pulping softwoods (Leach and Thakore 1973,1976, Leach et al. 1975; McKague et al. 1977; Rogers et al. 1979; O'Connor et al. 1992; Zang et al. 1997), which may act as feeding deterrents to taxa including insects, snails, and fish (Mabry and Gill 1979, Bell and Harestad 1987, Hay et al. 1990, Harbone 1988, Chan et al. 1990, Young 1993, Xie et al. 1993, Gonzales-Coloma et al. 1995, Panda and Kush 1995, Carotenuto et al. 1996, Hägele et al. 1996). Resin acids reduce feeding rates and food assimilation efficiencies, and decrease growth and fecundity of terrestrial insects (Wagner et al. 1983, Schuh and Benjamin 1984, Elliger et al. 1976,

Saikkonen et al. 1995). Phenolics, another common component of PME (Suntio et al. 1988, McCubbin and Folke 1993), are also a common group of secondary compounds (Panda and Kush 1995) that are mildly toxic to a wide spectrum of organisms (Singleton and Kratzer 1969). Phenolics can damage membranes, disrupt metabolic processes, act as feeding deterrents (Slansky 1992), and interfere with nutrition by binding to proteins (Reese and Beck 1976, Reese 1979) decreasing growth. It is likely that these plant extractives are an important contributor to any feeding inhibition resulting from exposure to pulp mill effluents.

The objective of this research was to measure the effect of biologically-treated BKME on the feeding behavior of a grazing insect found in the receiving water of a mill. The test species chosen, the mayfly *Ameletus subnotatus* (Ameletidae), is a univoltine mayfly found in lotic waters of the foothills of Western Canada and United States (Merritt and Cummins 1996, Zloty and Pritchard 1997). This insect feeds on diatoms and detritus in both erosional and depositional areas (Muttkowski and Smith 1929, Gilpin and Brusven 1970, Merritt and Cummins 1996). As many effluent components sorb to particulate matter (including algae), *Ameletus* living in water receiving mill effluent may be exposed to effluent-related toxicants in their diet as well as the surrounding water. Three experiments were conducted to determine whether BKME caused feeding inhibition in *A. subnotatus*. The first experiment measured the effect of effluent in the water on food consumption by larvae; this is the most common route of exposure for feeding inhibition bioassays. In contrast, the second experiment measured the effect on consumption when food and water were contaminated. Because insects are generally exposed to feeding deterrents in their food supply (Slansky 1992), I hypothesized that larvae would be more responsive to this route of exposure. Exposure via food and water also represents a more realistic situation, as in the field, both algae and mayfly would be exposed to effluent. If exposure to effluent causes feeding inhibition, then the expected result is a decrease in consumption in the presence of effluent, and this inhibition would likely be greater when water and food were

contaminated. Finally, an experiment measuring the effect of effluent on food choice of *A. subnotatus* was conducted. Surprisingly, choice assays are uncommon in behavioral ecotoxicology. However, in experiments specifically designed to test the feeding deterrency of allelochemicals, choice tests are often used because they are more sensitive than no-choice assays (Schoonhoven 1982). The rationale is that insects may eat substances that they would otherwise avoid if the only alternative is starvation.

5.2 Methods

Ameletus subnotatus were collected from the Athabasca River, upstream of the pulp mill's effluent discharge. Nymphs were brought back to the laboratory, where they were held in plastic tubs containing aerated water from the South Saskatchewan River, collected just upstream of Saskatoon. South Saskatchewan River water is similar in quality (conductivity, alkalinity, pH, soluble reactive phosphorus, ammonia, nitrate/nitrites) to the Athabasca (Chambers and Prepas 1994, Lowell and Culp 1996). Animals were held at 10 C under a 18:6 light dark cycle, and were fed cultured *Navicula* for at least 2 weeks before being used in a bioassay. Three experiments were conducted: (1) test animals were exposed to 0, 1, and 7% effluent in water and were fed uncontaminated food; (2) test animals were exposed to 0, 1, 3, and 7% effluent in food and water; and (3) test animals in uncontaminated water were offered a choice of *Navicula* that had been grown in 0, 1, 3, or 7% effluent. The general methods for each experiment are the same and follow.

Feeding trials were conducted at 10°C, in recirculating, artificial streams in a controlled environment room at the National Water Research Institute in Saskatoon, Saskatchewan. The streams (Fig. 5.1) were 250 mL circular, plexiglas containers with a central standpipe and water jets on either side (Walde and Davies 1984, Scrimgeour1992 Scrimgeour et al. 1991, Himbeault 1995, Lowell et al. 1995a,b, Dubé and Culp 1996). All streams were washed with soap, acid-washed, and soaked in distilled water before use, and all tubing

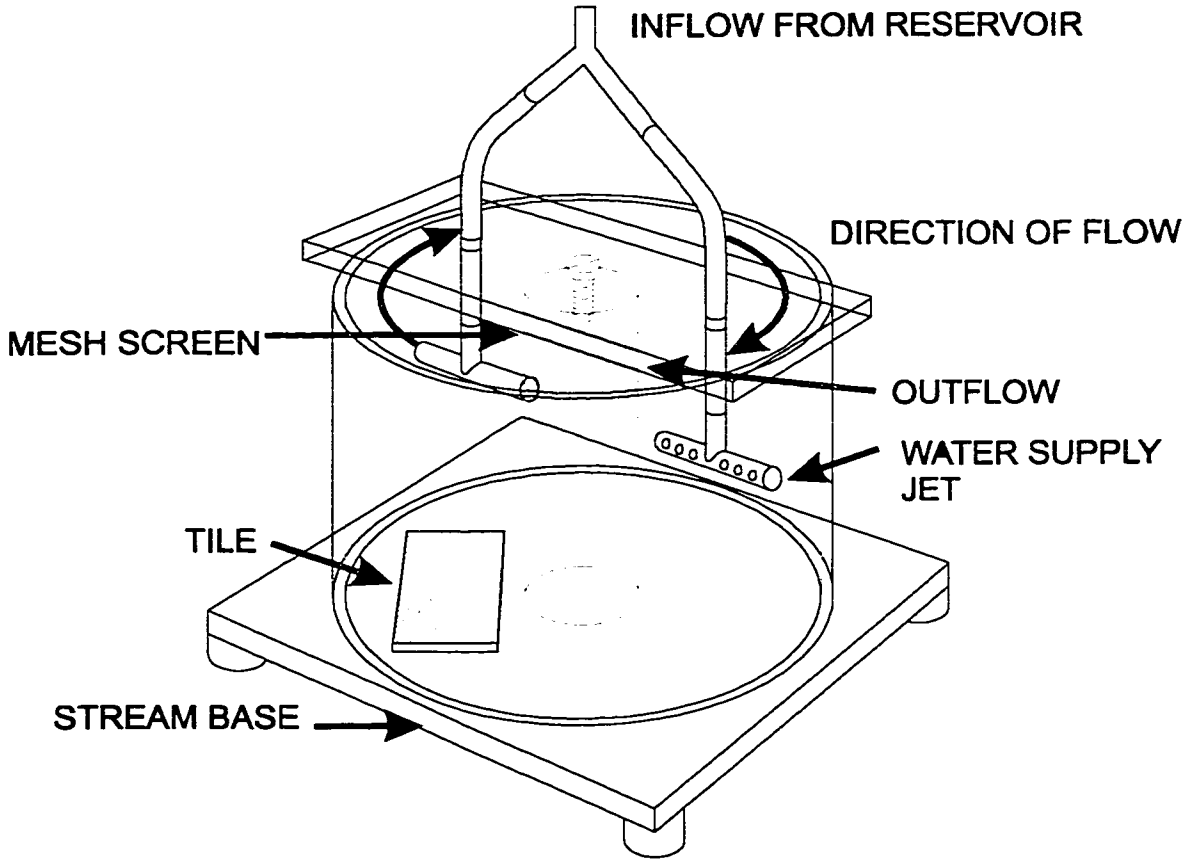


Figure 5.1. Plexiglass artificial stream used for feeding trials. Modified from Himbeault (1995).

used in the system was food grade PVC. Filtered (20 µm), UV sterilized, and aerated South Saskatchewan River water was pumped to the streams from 100 L, food grade, low density polyethylene reservoirs by magnetically driven impeller pumps (Hagen Fluval® 403). Water current in each stream was generated by water jets placed approximately 3 cm above the stream bottom; the jets produced a mean velocity of 5.2 cm/sec (SE=0.24, n=40). Three animals were placed in each stream approximately 16 hours in advance of a trial, and they were not fed during this period. Three animals were used because preliminary experiments indicated that the use of a single animal produced too much within-treatment variance. Animals were supplied with a clean ceramic tile during the acclimation period, and most animals were found on this tile by the end of this period.

Feeding trials took place during the dark cycle, as mayflies often restrict foraging activity to periods of darkness (Harker 1953, Culp et al. 1991, Scrimgeour 1992). To start a feeding trial, clean tiles were removed from each stream and were replaced by a tile upon which a layer of *Navicula* had been cultured. In the case of experiments 2 and 3, *Navicula* had been cultured in the appropriate concentration of effluent (see Chapter 4 for methods), and culturing times for the various treatments had been adjusted to produce tiles with similar biomass to avoid confounding by functional feeding responses. When placing tiles in the experimental streams, water pumps were turned off and care was taken to touch only the sides of the tile. Where applicable, effluent was added to the water reservoir to make up the appropriate dilution, and the water pumps were then turned on again. This process was carried out by 2 people and took approximately 15 minutes to complete. The animals were then left in the dark for 1 h for experiments 1 and 2, and 4.5 h for experiment 3. Videotaped observations of feeding trials indicated that mayflies did not eat continuously during feeding trails; they fed in short bouts separated by periods of movement around the stream or inactivity. At the end of a trial the lights were turned on, and the animals were removed from each stream and preserved in 10% formalin. Tiles were then carefully removed from the streams and all algae on the top surface was removed with a scalpel blade. In addition, algae was collected from tiles that had not been grazed, 5 tiles per treatment. Dry mass was measured by filtering the algae through precombusted, preweighed GFC filters and drying at 40°C for 48 h. For experiment 1, the initial starting biomass was the same for all streams because the tiles were all taken from the same culture, therefore for this experiment the dry mass remaining on the tiles at the end of the feeding trial was compared. In experiments 2 and 3, the initial starting biomass varied slightly between treatments because the algae had been cultured under different treatment conditions. For these experiments, the dry mean mass remaining after the feeding trial was subtracted from the mass on ungrazed tiles from the same treatment, and in this way, the amount consumed was estimated. Data were checked to ensure that assumptions of

normality and equality of variances were met and ANOVA was used to test for treatment effect in both feeding rate and choice experiments. Statistical analysis was performed with Minitab (release 11, Minitab Inc., 1996).

5.3 Results

Experiment 1: Effluent present in water only

In the first experiment, nymphs (mean total length 8.14 mm ± 0.20 SE) were exposed to treated pulp mill effluent in water at concentrations of 0, 1, and 7%, and were presented with uncontaminated food. The initial biomass on the tiles was 2.93 mg per tile (± 0.63 SE, n=5). There was no difference in the amount of biomass grazed (Fig. 5.2) ANOVA, $F_{\alpha=0.05,\ 2,20}$= 2.96, p= 0.075, although there is some suggestion of higher feeding in the effluent-exposed groups. Data from 7 tiles, 3 from the control and 2 from both effluent concentrations, were omitted from the analysis because these tiles showed evidence of loss of algae due to sloughing.

Experiment 2: Effluent in water and contaminated food

In the second experiment, nymphs (mean total length 8.58 mm ± 0.23 SE) were exposed to effluent in food and water. Despite efforts to adjust culturing times among treatments, control tiles and tiles cultured in 7% effluent had more dry mass than tiles cultured in 1% and 3% effluent, and 7% tiles had significantly more dry mass than the control tiles (Table 5.2). Mayflies consumed significantly less algae in all of the effluent treatments than in controls (Fig. 5.3, ANOVA $F_{\alpha=0.05,3,34}$=3.75, p=0.019), and there was no difference between the 3 effluent concentrations.

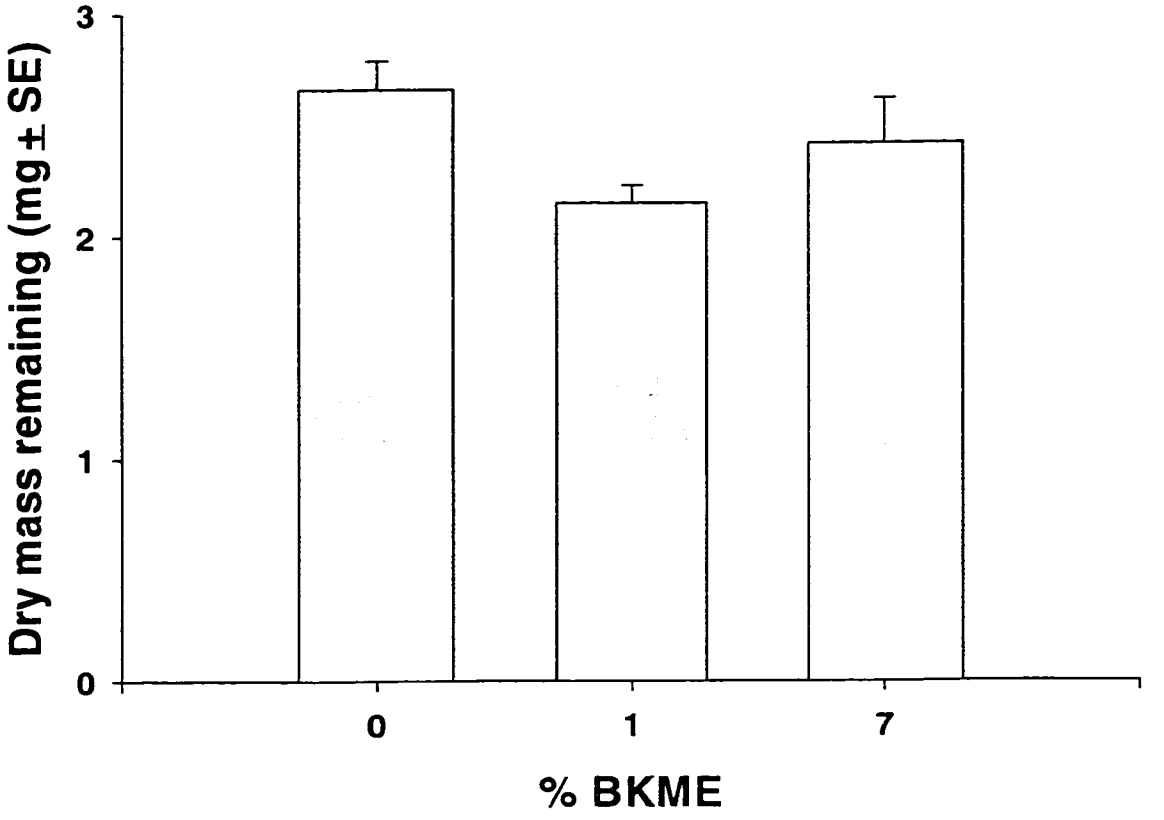


Figure 5.2. Mean dry mass (mg ± SE) of *Navicula* remaining on tiles after 1h feeding trial. Effluent was present in water only, and treatment (%BKME) did not have a significant effect on biomass consumed by *Ameletus* (ANOVA, $F_{\alpha=0.05, 2,20}$= 2.96, p= 0.075).

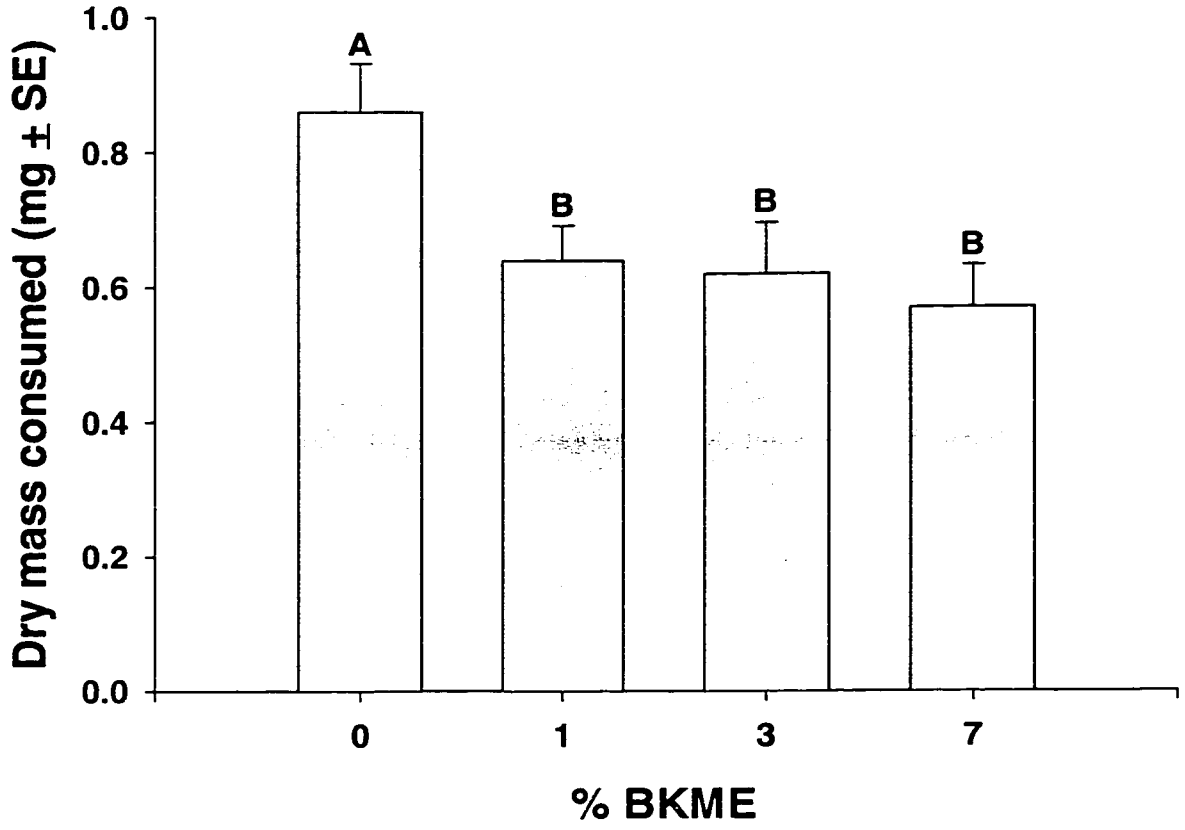


Figure 5.3. Mean dry mass (± SE) of *Navicula* consumed in 1 h by *A. subnotatus* when both food and water were contaminated with 0, 1, 3, and 7% BKME (experiment 2); means with common letters do not differ significantly (Fisher's protected $LSD_{\alpha=0.05}=0.19$).

Table 5.2. Initial dry mass of *Navicula,* and amount consumed by *Ameletus* nymphs when algae and water were contaminated with 0, 1, 3, and 7% BKME.

Treatment	Mean (g/tile)	SE			
Initial dry mass					
0	0.00182	0.00003			
1	0.00164	0.00004			
3	0.00162	0.00006			
7	0.00204	0.00006			
Source	df	SS	MS	*F*	*p*
Treatment	3	0.000006	0.0000002	17.24	<0.01
Error	16	0.0000002	0.0000000		
Total	19	0.0000008			
(Fisher's protected $LSD_{\alpha=0.05/2}$ = 0.0001499)					
Amount consumed					
Source	df	SS	MS	*F*	*p*
Treatment	3	0.0000005	0.0000002	3.75	0.019
Error	36	0.0000016	0.000000044		
Total	39	0.0000021			
(Fisher's protected $LSD_{\alpha=0.05/2}$ = 0.0001902)					

Experiment 3: Contaminated food clean water, choice

The third experiment was designed to test whether mayflies showed a preference for control algae and, thus, evidence of feeding deterrency by effluent. In this experiment, each stream contained control water, three mayflies, and 4 tiles, 1 tile from each treatment. The position of tiles was such that, between replicate streams, a specific treatment was not always found in the same location with respect to the water jets. Initial biomass measurements are in Table 5.3; no significant differences existed between the various treatments. *A. subnotatus* nymphs (mean total length 9.03 mm ± 0.16 SE) consumed a greater biomass (dry mass per tile) from tiles in the 3 and 7% BKME treatments (Table 5.3, Fig. 5.4).

Table 5.3. Initial biomass (dry mass) of *Navicula* and amount consumed by *Ameletus* in 4.5h. Nymphs were given a choice of *Navicula* cultured in 0, 1, 3, and 7% BKME, and there was no effluent in water.

	Treatment	Mean dry mass (g)	SE		
Initial dry mass					
	0	0.00189	0.00006		
	1	0.00170	0.00004		
	3	0.00174	0.00004		
	7	0.00167	0.00013		
Source	df	SS	MS	*F*	*p*
Treatment	3	0.0000001	0.0000000	1.63	0.258
Error	8	0.0000001	0.0000000		
Total	11	0.0000002			
Dry mass consumed					
	Treatment	Mean dry mass (g) consumed	SE		
	0	0.00037	0.00008		
	1	0.00076	0.00024		
	3	0.00114	0.00028		
	7	0.00131	0.00017		
Source	df	SS	MS	*F*	*p*
Treatment	3	0.0000032	0.0000011	4.06	0.021
Error	20	0.0000052	0.0000003		
Total	23	0.0000083			
Fisher's Protected $LSD_{\alpha=0.05/2}$ = 0.000511					

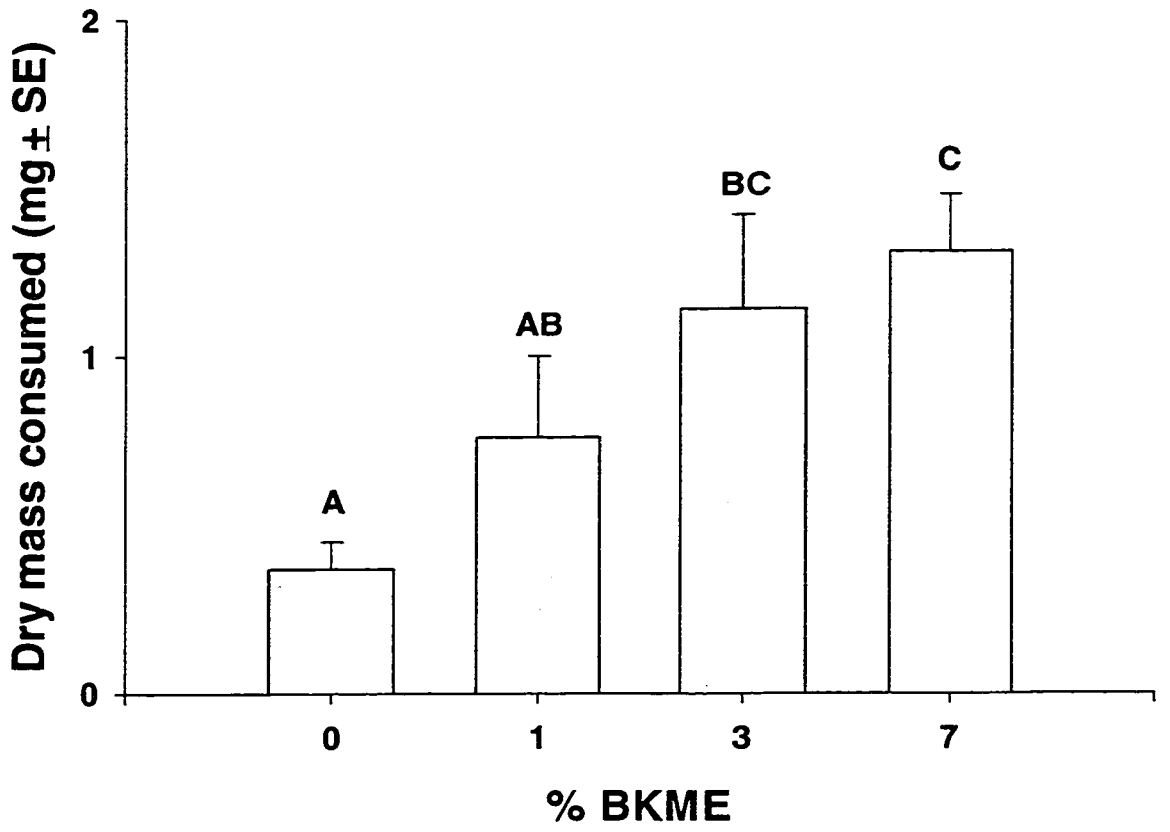


Figure 5.4. Mean dry mass (mg ±SE) consumed in 4.5 h by *A. subnotatus* in experiment 3; means with common letters are not significantly different (Fisher's protected $LSD_{\alpha=0.05} = 0.511$).

5.4 Discussion

This research compared the effect of exposure to pulp mill effluent in water with a more realistic exposure via contamination of both water and food. Feeding of *A. subnotatus* was not affected by exposure to effluent in water, up to a 7% dilution, indicating either that exposure to effluent in water does not affect feeding, or simply that a 7% concentration was not sufficient to inhibit feeding. Results should be interpreted with caution, however, because of the reduced statistical power resulting from the loss of replicates. Pulp mill effluent affects feeding of *Daphnia retrocurvata* (5%, Cooley 1977), and lobster (*Homarus americanus*) (2.5%, McLeese 1973), and in both cases, the test animals were exposed to effluent in water. It is impossible to quantitatively

compare the present study with these other examples, however, as the older studies almost certainly used effluent that was not biologically treated and was, consequently, more toxic. In contrast to the apparent lack of feeding inhibition of larvae exposed to contaminated water, *A. subnotatus* exposed to effluent in water and food consumed significantly less than control larvae. This reduced feeding occurred equally at all concentrations indicating there was no dose response.

Although no-choice bioassays are typically used in studies of feeding inhibition, these tests are understood to be less sensitive (Schoonhoven 1982), and may fail to elucidate mechanisms responsible for altered feeding behavior. Had a choice bioassay not been performed in this study, I would have concluded that effluent contaminants sorbed to algae caused reduced consumption. Furthermore, BKME concentrations as low as 1% would have been interpreted to induce chronic growth and reproductive effects as a result of this reduction in feeding. However, when a choice assay was conducted, *A. subnotatus* unexpectedly exhibited a clear preference for feeding on *Navicula* cultured in 3 and 7% effluent. Mayflies should not have exhibited a preference for consuming contaminated algae if this algae caused feeding inhibition. Findings indicate that there was some qualitative difference between control and contaminated algae that the mayflies were able to detect, and that the observed decrease in consumption in the no-choice assay (experiment 2) was not feeding inhibition.

The apparently contradictory results of the choice *vs.* no-choice bioassays may be explained through the mechanisms of dietary self-selection and compensatory feeding. Dietary self selection is a behavioral phenomena observed when animals are presented with more than 1 food type, all deficient in some dietary component (e.g. protein). The animals consume varying quantities of the food types such that their overall consumption more closely resembles their dietary requirements (Cohen et al. 1987). Compensatory feeding, observed in taxa ranging from slugs to rats (Booth 1974, Rueda 1991), is an increase in ingestion that occurs in response to a decrease in food quality.

The increased ingestion usually partially compensates for the reduced nutritional value of the food, and this response is sometimes accompanied by increased assimilation efficiency (Slansky and Wheeler 1989). Stress, either nutrient or toxicant, can affect macromolecule content (i.e., lipid, carbohydrate, protein) of algal cells (MacCarthy and Patterson 1974, Soto et al. 1977, El Dib et al. 1997, Thompson and Couture 1991, Sicko-Goad et al. 1989a,b). *Navicula* mats grown in pulp mill effluent have greater lipid content, the same N content, more bacteria, and grow more quickly than control algae (Chapter 4). Insects and other herbivores are capable of assessing food quality (Sterner 1993, Slansky 1992,1993, Schoonhoven 1982), and selectively feeding to maximize quality of ingested material (Starkweather 1980, DeMott 1989, Simpson et al. 1988, Slansky 1993). This selection typically takes the form of a preference for plants exhibiting faster growth or higher nitrogen content (Mullin 1963, Sterner 1993, Cowles et al. 1988, Kiørboe 1989), however lipids may also be attractive to some aquatic insects (Cargill et al. 1985). Thus, a preference for algae grown in effluent is consistent with nutritional ecology. Compensatory feeding occurs in the absence of choice, and consumption and food quality are inversely correlated (House 1965, Phillips 1984, Slansky 1993). This mechanism has been reported in insects and other animals including cladocerans, copepods, snails, slugs, and mammals (Arnold 1971; Booth 1974, Calow 1975, Webster and Peters 1978 Rollo and Hawryluk 1988, Slansky and Feeny 1977, Slansky and Wheeler 1989, 1992a, Rueda et al. 1991). Increased consumption of control algae in the absence of choice, and a preference for contaminated algae when presented with a choice suggests a difference in the nutritional quality of these food sources. Effluent-related changes in the nutritional content of algae may prove an overriding influence on feeding behavior, even if PME contains feeding inhibitors, as suggested by the literature (e.g., McLeese 1973, Cooley 1977, Crane and Maltby 1991, Bitton et al. 1995). Insects may ingest damaging or even lethal doses of allelochemicals when feeding responses attempt to compensate for nutritionally dilute food (Stamp

1990, Slansky and Wheeler 1992b, Slansky 1992), suggesting some precedence of nutrition over toxicity.

An increase in the nutritional quality of algae may help to explain reported increased growth rates resulting from effluent exposure. Lowell et al. (1995a) and Dubé and Culp (1996, 1997) reported that mayflies and chironomids respectively, exhibited increased growth when exposed to low concentrations of pulp mill effluent. Dubé and Culp (1996, 1997), suggested that increased growth of chironomids resulted from increased periphyton biomass and the possible utilization of effluent biosolids as a higher quality food source. Lowell et al. (1995a), reported that growth of *Baetis tricaudatus* exposed to 1 and 10% effluent was greater than controls, even when controls experienced unlimited food-availability. Explanations offered included increased palatability of algae leading to increased food consumption, increased nutritive value of algae, stimulation of growth by plant extractives in the effluent, or hormesis. The research presented in this chapter supports the suggestion that the nutritional value of algae is increased by exposure to effluent, and that this increased nutritional value may be responsible for increased growth of grazers.

Laboratory toxicity tests are often criticized for being oversimplified and failing to adequately predict field effects (Cairns 1983, Kimball and Levin 1985, Malins 1989, Maltby and Calow 1989, Brouwer et al. 1990, Forbes and Forbes 1994). In this research, it is apparent that the use of a common test procedure i.e., exposure of animals in water alone, would have failed to identify the effects on feeding behavior and the casual mechanisms responsible for those effects. Simple feeding bioassays may have the potential to act as inexpensive, quick surrogates for other laboratory tests (Bitton 1995, Bitton et al. 1996), but care should be taken to avoid confounding exposure to toxicants with differences in nutritional quality. However, if the intention is to make inferences about field effects, then toxicant influences on nutritional quality of food represents not so much a confounding factor as an increase in environmental realism. Our understanding of toxicant effects on feeding behavior may be improved through

the use of experimental designs that include the following four components: (1) a realistic exposure of both test species and food to toxicants; (2) a measurement of toxicant effects on feeding rate in a no-choice situation; (3) preference testing of contaminated and clean food, and; (4) an assessment of the nutritional quality of control and exposed food.

5.5 Literature Cited

Allen, Y., P. Calow, and D. J. Baird. 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. Environmental Toxicology and Chemistry 14: 1625-1630.

Arnold, D. E. 1971. Ingestion, assimilation, survival and reproduction by *Daphnia pulex* fed seven species of blue-green algae. Limnology and Oceanography 16: 906-920.

Bell, C. M., and A. S. Harestad. 1987. Efficacy of pine oil as a repellent to wildlife. Journal of Chemical Ecology 13: 1409-1417.

Bitton, G., K. Rhodes, B. Koopmen, and M. Cornejo. 1995. Short-term toxicity assay based on daphnid feeding behavior. Water Environmental Research 67: 290-293.

Bitton, G., K. Rhodes, and B. Koopmen. 1996. Ceriofast®: an acute toxicity test based on *Ceriodaphnia dubia* feeding behavior. Environmental Toxicology and Chemistry 15: 123-125.

Blockwell, S. J., E. J. Taylor, I. Jones, and D. Pascoe. 1998. The influence of fresh water pollutants and interaction with *Asellus aquaticus* (L.) on the feeding activity of *Gammarus pulex* (L.). Archives of Environmental Contamination and Toxicology 34: 41-47.

Booth, D. A. 1974. Food intake compensation for increase or decrease in the protein content of the diet. Behavioral Biology 12: 31-40.

Brady, L. D., and R. A. Griffiths. 1995. Effects of pH and aluminum on the growth and feeding behaviour of smooth and palmate newt larvae. Ecotoxicology 4: 299-306.

Brouwer, A., A. J. Murk, and J. H. Koeman. 1990. Biochemical and physiological approaches in ecotoxicology. Functional Ecology 4: 275-281.

Cairns, J. 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100: 47-57.

Calow, P. 1975. The feeding strategies of two freshwater gastropods, *Ancylus fluviatilis* Müll. and *Planorbis contortus* Linn. (Pulmonata), in terms of ingestion rates and absorption efficiencies. Oecologia (Berl.) 20: 33-49.

Cargill II, A. S., K. W. Cummins, B. J. Hanson, and R. R. Lowry. 1985. The role of lipids as feeding stimulants for shredding aquatic insects. Freshwater Biology 15: 455-464.

Carotenuto, A., E. Fattorusso, V. Lanzotti, S. Magno, and L. Mayol. 1996. Lintenolides C-E: unusual antifeedant sesquiterpenes isolated from the marine sponge *Cacospongia* cf. *linteiformis*. Liebegs Annalen der Chemie 1996: 77-81.

Chambers, P. A., and E. E. Prepas. 1994. Nutrient dynamics in riverbeds: the impact of sewage effluent and aquatic macrophytes. Water Research 28: 453-464.

Chan, T. H., K. R. Guertin, V. C. Prasad, A. W. Thomas, G. M. Strunz, and A. Salonius. 1990. Spruce budworm (*Choristoneura fumiferana*) antifeedants. 2. Structure-activity relationship among some functionalized decalin compounds. Canadian Journal of Chemistry 68: 1170-1177.

Cohen, R. W., G. P. Waldbauer, S. Friedman, and N. M. Schiff. 1987. Nutrient self-selection by *Heliothis zea* larvae: a time-lapse film study. Entomologia Experimentalis et Applicata 44: 65-73.

Cooley, J. M. 1977. Filtering performance of *Daphnia retrocurva* in pulp mill effluent. Journal of the Fisheries Research Board of Canada 34: 863-868.

Cowles, T. J., R. J. Olson, and S. W. Chisholm. 1988. Food selection by copepods: discrimination on the basis of food quality. Marine Biology 100: 41-49.

Crane, M., and L. Maltby. 1991. The lethal and sublethal responses of *Gammarus pulex* to stress: sensitivity and sources of variation in an in situ bioassay. Environmental Toxicology and Chemistry 10: 1331-1339.

Culp, J. M., N. E. Glozier, and G. J. Scrimgeour. 1991. Reduction of predation risk under the cover of darkness: avoidance response of mayfly larvae to a benthic fish. Oecologia (Berlin) 86: 163-169.

DeMott, W. R. 1989. Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. Limnology and Oceanography 34: 140-154.

Doucet, C. M., and E. J. Maly. 1990. Effect of copper on the interaction between the predator *Didinium nasutum* and its prey *Paramecium caudatum*. Canadian Journal of Fisheries and Aquatic Sciences 47: 1122-1127.

Dubé, M. G., and J. M. Culp. 1996. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Environmental Toxicology and Chemistry 15: 2019-2027.

Dubé, M. G., and J. M. Culp. 1997. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Water Science and Technology 35: 339-345.

El-Dib, M. A., H. F. Abou-Waly, and A. M. H. El-Naby. 1997. Impact of fuel oil on the freshwater alga *Selenastrum capricornutum*. Bulletin of Environmental Contamination and Toxicology 59: 438-444.

Elliger, C. A., D. F. Zinkel, B. G. Chan, and A. C. Waiss. 1976. Diterpene acids as larval growth inhibitors. Experientia 32: 1364-1366.

Forbes, V. E., and T. L. Forbes. 1994. *Ecotoxicology in theory and practice*. Chapman & Hall, London.

Foster, N. R., A. Scheier, and J. Cairns. 1966. Effect of ABS on feeding behavior of flagfish, *Jordanella floridae*. Transactions of the American Fisheries Society 95: 109-110.

Gilpin, B. R., and M. A. Brusven. 1970. Flood habits and ecology of mayflies of the St. Mary's River in Idaho. Melanderia 4: 19-40.

Gonzalez-Coloma, A., M. Reina, R. Cabrera, P. Castanera, and C. Gutierez. 1995. Antifeedant and toxic effects of sesquiterpenes from *Senecio palmensis* to Colorado Potato Beetle. Journal of Chemical Ecology 21: 1255-1270.

Hägele, B., J. Harmatha, M. Pavlík, and M. Rowell-Rahier. 1996. Sesquiterpenes from the *Senecioneae* and their effect on food choice of the specialized leaf beetles *Oreina cacaliae*, *Oreina speciosissima* and the generalist snail *Arianta arbustorum*. Entomologia Experimentalis et Applicata 80: 169-172.

Ham, L., R. Quin, and D. Pascoe. 1995. Effects of cadmium on the predator-prey interaction between the turbellarian *Dendrocoelum lacteum* (Müller, 1974) and the Isopod crustacean *Asellus aquaticus* (L.). Archives of Environmental Contamination and Toxicology 29: 358-365.

Harborne, J. B. 1988. Insect feeding preferences. *Introduction to ecological biochemistry*. Academic Press Limited, London.

Harker, J. E. 1953. The diurnal rhythm of activity of mayfly nymphs. Journal of Experimental Biology 30: 525-533.

House, H. L. 1965. Effects of low levels of the nutrient content of a food and of nutrient imbalance on the feeding and the nutrition of a phytophagous larva, *Celerio euphorbiae* (Linnaeus) (Lepidoptera: Sphingidae). The Canadian Entomologist 97: 62-68.

Jacobson, M., R. E. Redfern, and G. D. Mills. 1975. Naturally occurring insect growth regulators. II. Screening of insect and plant extracts as insect juvenile hormone mimics. Lloydia 38: 455-472.

Jones, M., C. Folt, and S. Guarda. 1991. Characterizing individual, population and community effects of sublethal levels of aquatic toxicants: an experimental case study using *Daphnia*. Freshwater Biology 26: 35-44.

Juchelka, C. M., and T. W. Snell. 1994. Rapid toxicity assessment using rotifer ingestion rate. Archives of Environmental Contamination and Toxicology 26: 549-554.

Juchelka, C. M., and T. W. Snell. 1995. Rapid toxicity assessment using ingestion rate of cladocerans and ciliates. Archives of Environmental Contamination and Toxicology 28: 508-512.

Kimball, K. D., and S. A. Levin. 1985. Limitations of laboratory bioassays: the need for ecosystem-level testing. BioScience 35: 165-171.

Kiørboe, T. 1989. Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. Marine Ecology Progress Series 55: 229-234.

Laskowski, R., and S. P. Hopkin. 1996. Effect of Zn, Cu, Pb, Cd on fitness in snails (*Helix aspersa*). Ecotoxicology and Environmental Safety 34: 59-69.

Leach, J. M., A. N. Thakore, and J. F. Manville. 1975. Acute toxicity to juvenile rainbow trout (*Salmo gairdneri*) of naturally occurring insect juvenile hormone analogues. Canadian Journal of Fisheries and Aquatic Sciences 32: 2556-2559.

Leach, J. M., and A. N. Thakore. 1973. Identification of the constituents of kraft pulping effluent that are toxic to juvenile coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 30: 479-484.

Leach, J. M., and A. N. Thakore. 1976. Toxic constituents in mechanical pulping effluents. Tappi Journal 59: 129-132.

Lemly, A. D., and R. J. F. Smith. 1985. Effects of acute exposure to acidified water on the behavioral response of fathead minnows, *Pimephales promelas*, to chemical feeding stimuli. Aquatic Toxicology 6: 25-36.

Linder, G., and M. E. Richmond. 1990. Feed aversion in small mammals as a potential source of hazard reduction for environmental chemicals: agrichemical case studies. Environmental Toxicology and Chemistry 9: 95-105.

Little, E. E., R. D. Archeski, B. A. Flerov, and V. I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Archives of Environmental Contamination and Toxicology 19: 380-385.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995a. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environmental Toxicology and Chemistry 14: 1529-1541.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995b. Toxicity testing with artificial streams: effects of differences in current velocity. Environmental Toxicology and Chemistry 14: 1209-1217.

Lowell, R. B. and J. M Culp. 1996. Combined effects of dissolved oxygen level and bleached kraft pulp mill effluent and municipal sewage on a mayfly (*Baetis*

tricaudata): assessments using artificial streams. Project Report No. 98, Northern River Basins Study, Edmonton, Alberta.

Mabry, T. J., and J. E. Gill. 1979. Sesquiterpene lactones and other terpenoids. Pages 501-537 *In* G. A. Rosenthal and D. H. Janzen, eds. *Herbivores: their interaction with secondary plant metabolites.* Academic Press, Inc., New York.

MacCarthy, J. J., and G. W. Patterson. 1974. Effects of cation level of the nutrient medium on the biochemistry of *Chlorella*. Plant Physiology 54: 129-132.

Malins, D. C. 1989. The use of environmental assays for impact assessment. Hydrobiologia 188/189: 87-91.

Maltby, L., and P. Calow. 1989. The application of bioassays in the resolution of environmental problems; past, present and future. Hydrobiologia 188/189: 65-76.

Marigomez, J. A., E. Angulo, and V. Saez. 1986. Feeding and growth responses to copper, zinc, mercury and lead in the terrestrial gastropod *Arion ater* (Linne). The Journal of Molluscan Studies 52: 68-78.

Mathers, R. A., J. A. Brown, and P. H. Johansen. 1985. The growth and feeding behavior responses of largemouth bass (Micropterus salmoides) exposed to PCP. Aquatic Toxicology 6: 157-164.

McCubbin, N., and J. Folke. 1993. A review of literature on pulp and paper mill effluent characteristics in the Peace and Athabasca river basins. Northern River Basin Study, Edmonton, Alberta.

McKague, A. B., J. M. Leach, R. N. Soniassay, and A. N. Thakore. 1977. Toxic constituents in woodroom effluent. Tappi Transactions of the Technical Section 3: TR75-TR81.

McLeese, D. W. 1973. Response of lobsters *Homarus americanus* to odor solution in the presence of bleached kraft mill effluent. Journal of the Fisheries Research Board of Canada 30: 279-282.

Merritt, R. W., and K. W. Cummins. 1996. *Aquatic insects of North America.* Kendall/Hunt Publishing Company, Dubuque, Iowa.

Mullin, M. M. 1963. Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnology and Oceanography 8: 239-250.

Muttkowski, R. A., and G. M. Smith. 1929. The food of trout stream insects in Yellowstone National Park. Roosevelt Wild Life Annals 2: 241-263.

O'Connor, B. I., T. G. Kovacs, and R. H. Voss. 1992. The effect of wood species composition on the toxicity of simulated mechanical pulping effluents. Environmental Toxicology and Chemistry 11: 1259-1270.

Panda, N., and G. S. Kush. 1995. *Host plant resistance to insects.* CAB International.

Phillips, N. W. 1984. Compensatory intake can be consistent with an optimal foraging model. The American Naturalist 123: 867-872.

Reese, J. C., and S. D. Beck. 1976. Effects of allelochemicals on the black cutworm, *Agrotis ipsilon*: effects of catechol, l-dopa, dopamine, and chlorogenic acid on larval growth, development, and utilization of food. Annals of the Entomological Society of America 69: 68-72.

Reese, J. C. 1979. Interactions of allelochemicals with nutrients in herbivore food. Pages 309-330 *In* G. A. Rosenthal and D. H. Janzen, eds. *Herbivores: their interaction with secondary plant metabolites*. Academic Press, Inc., New York.

Rogers, I., H. Mahood, J. Servici, and R. Gordon. 1979. Identifying extractives toxic to aquatic life. Pulp & Paper Canada 80: T286-T290.

Rollo, C. D. 1988. The feeding of terrestrial slugs in relation to food characteristics, starvation, maturation and life history. Malacologia 28: 29-39.

Rollo, C. D., and M. D. Hawryluk. 1988. Compensatory scope and resource allocation in two species of aquatic snails. Ecology 69: 146-156.

Rueda, A. R., F. Slansky, and G.S. Wheeler. 1991. Compensatory feeding response of the slug *Sarasinula plebia* to dietary dilution. Oecologia (Berlin) 1991: 88-181.

Saikkonen, K., S. Neuvonen, and P. Kainulainen. 1995. Oviposition and larval performance of European sawfly in relation to irrigation, simulated acid rain and resin acid concentration of Scots pine. Oikos 74: 273-282.

Schoonhoven, L. M. 1982. Biological aspects of antifeedants. Entomologia Experimentalis et Applicata 31: 57-69.

Schuh, B. A., and D. M. Benjamin. 1984. The chemical feeding ecology of *Neodiprion dubiosus* Schedl, *N. rugifrons* Midd, and *N. lecontei* (Fitch) on jack pine (*Pinus banksiana* Lamb.). Journal of Chemical Ecology 10: 1071-1079.

Schuytema, G. S., A. V. Nebeker, J. A. Peterson, and W. L. Griffis. 1993. Effects of pentachlorophenol-contaminated food organisms on toxicity and bioaccumulation in the frog *Xenopus laevis*. Archives of Environmental Contamination and Toxicology 24: 359-364.

Scrimgeour, G. J. 1992. Foraging patch selection by the lotic mayfly *Baetis tricaudatus*. *Department of Biological Sciences*. University of Calgary, Calgary, AB.

Scrimgeour, G. J., J. M. Culp, M. L. Bothwell, F. J. Wrona, and M. H. McKee. 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly-diatom systems. Oecologia (Berlin) 85: 343-348.

Sicko-Goad, L., M. S. Evans, D. Lazinsky, J. Hall, and M. S. Simmons. 1989a. Effects of chlorinated benzenes on diatom fatty acid composition and

quantitative morphology. IV. Pentachlorobenzene and comparison with trichlorobenzene isomers. Archives of Environmental Contamination and Toxicology 18: 656-668.

Sicko-Goad, L., J. Hall, D. Lazinsky, and M. S. Simmons. 1989b. Effects of chlorinated benzenes on diatom fatty acids composition and quantitative morphology. III. 1,2,3-trichlorobenzene. Archives of Environmental Contamination and Toxicology 18: 647-655.

Simpson, S. J., M. S. J. Simmonds, and W. M. Blaney. 1988. A comparison of dietary selection behavior in larval *Locusta migratoria* and *Spodoptera littoralis*. Physiological Entomology 13: 225-238.

Singleton, V. L., and F. H. Kratzer. 1969. Toxicity and related physiological activity of phenolic substances of plant origin. Journal of Agricultural Food Chemistry 17: 497-512.

Sláma, K. 1971. Insect juvenile hormone analogues. Annual Review of Biochemistry 40: 1079-1102.

Slansky, F. 1992. Allelochemical-nutrient interactions in herbivore nutritional ecology. Pages 135-173. *Herbivores: their interactions with secondary plant metabolites*. Academic Press.

Slansky, F. 1993. Nutritional ecology: the fundamental quest for nutrients. Pages 29-91 *In* N. E. Stamp and T. M. Casey, eds. *Caterpillars. Ecological and evolutionary constraints on foraging*. Chapman & Hall, New York, London.

Slansky, F., and G. S. Wheeler. 1989. Compensatory increases in food consumption and utilization efficiencies by velvetbean caterpillars mitigate impact of diluted diets on growth. Entomologia Experimentalis et Applicata 51: 175-187.

Slansky, F., and G. S. Wheeler. 1992a. Caterpillar's compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. Entomologia Experimentalis et Applicata 65: 171-186.

Slansky, F., and G. S. Wheeler. 1992b. Feeding and growth responses of laboratory and field strains of velvetbean caterpillars (Lepidoptera: Noctuidae) to food nutrient level and allelochemicals. Journal of Economic Entomology 85: 1717-1730.

Slansky, F., and P. Feeny. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecological Monographs 47: 209-228.

Soto, C., J. A. Hellebust, and T. C. Hutchinson. 1977. Effect of naphthalene and aqueous crude oil extracts on the green flagellate *Chlamydomonas angulosa*. III. Changes in cellular composition. Canadian Journal of Botany 55: 2765-2777.

Stamp, N. E. 1990. Growth versus molting time of caterpillars as a function of temperature, nutrient concentration and the phenolic rutin. Oecologia (Berl.) 82: 107-113.

Starkweather, P. L., and J. G. Gilbert. 1977. Feeding in the rotifer *Brachionus calyciflorus*. Oecologia (Berlin) 28: 133-139.

Starkweather, P. L. 1980. Aspects of the feeding behavior and trophic ecology of suspension-feeding rotifers. Hydrobiologia 73: 63-72.

Sterner, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. Ecology 74: 2351-2360.

Suntio, L. R., W. Y. Shiu, and D. Mackay. 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere 17: 1249-1290.

Thompson, P., and P. Couture. 1991. Aspects of carbon metabolism in the recovery of *Selenastrum capricornutum* population exposed to cadmium. Aquatic Toxicology 17: 1-14.

Wagner, M. R., D. M. Benjamin, K. M. Clancy, and B. A. Schun. 1983. Influence of diterpene resin acids on feeding and growth of larch sawfly, *Pristiphora erichsonii* (Hartig). Journal of Chemical Ecology 9: 119-127.

Walde, S. J., and R. W. Davies. 1984. The effect of intraspecific interference on *Kogotus nonus* (Plecoptera) foraging behaviour. Canadian Journal of Zoology 62: 2221-2226.

Weber, D. N. 1996. Lead-induced metabolic imbalances and feeding alterations in juvenile fathead minnows (*Pimephales promelas*). Environmental Toxicology and Water Quality 11: 45-51.

Webster, K. E., and R. H. Peters. 1978. Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. Limnology and Oceanography 23: 1238-1245.

Weeks, J. M. 1993. Effects of dietary copper and zinc concentrations on feeding rates of two species of talitrid amphipods (Crustacea). Bulletin of Environmental Contamination and Toxicology 50: 883-890.

Xie, Y., M. B. Isman, Y. Feng, and A. Wong. 1993. Diterpene resin acids: major active principles in tall oil against variegated cutworm, *Peridroma saucia* (Lepidoptera: Noctuidae). Journal of Chemical Ecology 19: 1075-1084.

Young, J. 1993. Value-added tall oil products aid production, environmental goals. Pulp and Paper : 81-82.

Zang, Y., P. A. Bicho, C. Breuil, J. N. Saddler, and S. N. Liss. 1997. Resin acid degradation by bacterial strains grown on CMTP effluent. Water Science and Technology 35: 33-39.

Zloty, J., and G. Pritchard. 1997. Larvae and adults of *Ameletus* mayflies (Ephemeroptera: Ameletidae) from Alberta. The Canadian Entomologist 129: 251-289.

Literature reviewed for Table 5.1

Allen, Y., P. Calow, and D. J. Baird. 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna.* Environmental Toxicology and Chemistry 14: 1625-1630.

Atema, J., and L. S. Stein. 1974. Effects of crude oil in the feeding behaviour of the lobster *Homarus americanus*. Environmental Pollution 6: 77-86.

Berman, M. S., and D. R. Heinle. 1980. Modification of the feeding behavior of marine copepods by sublethal concentrations of water-accommodated fuel oil. Marine Biology 56: 59-64.

Bitten, G., K. Rhodes, and B. Koopmen. 1996. Ceriofast®: an acute toxicity test based on *Ceriodaphnia dubia* feeding behavior. Environmental Toxicology and Chemistry 15: 123-125.

Bitton, G., K. Rhodes, B. Koopmen, and M. Cornejo. 1995. Short-term toxicity assay based on daphnid feeding behavior. Water Environmental Research 67: 290-293.

Blockwell, S. J., E. J. Taylor, I. Jones, and D. Pascoe. 1998. The influence of fresh water pollutants and interaction with *Asellus aquaticus* (L.) on the feeding activity of *Gammarus pulex* (L.). Archives of Environmental Contamination and Toxicology 34: 41-47.

Bodar, C. W. M., I. Van der Sluis, and P. A. Voogt. 1988. Effects of cadmium on consumption, assimilation and biochemical parameters of *Daphnia magna*: possible implications for reproduction. Comparative Biochemistry and Physiology, Series C 90: 341-346.

Borgmann, U., and K. M. Ralph. 1986. Effects of cadmium, 2,4-dichlorophenol, and pentachlorophenol on feeding, growth, and particle-size-conversion efficiency of white sucker larvae and young common shiners. Archives of Environmental Contamination and Toxicology 15: 473-480.

Brady, L. D., and R. A. Griffiths. 1995. Effects of pH and aluminum on the growth and feeding behaviour of smooth and palmate newt larvae. Ecotoxicology 4: 299-306.

Brown, J. A., P. H. Johansen, P. W. Colgan, and R. A. Mathers. 1987. Impairment of early feeding behavior of largemouth bass by pentachlorophenol exposure: a preliminary assessment. Transactions of the American Fisheries Society 116: 71-78.

Capuzzo, J. M. 1979. The effects of halogen toxicants on survival, feeding and egg production of the rotifer *Brachionus plicatilis*. Estuarine and Coastal Marine Science 8: 307-316.

Cleveland, L., E. E. Little, S. J. Hamilton, D. R. Buckler, and J. B. Hunn. 1986. Interactive toxicity of aluminum and acidity to early life stages of brook trout. Transactions of the American Fisheries Society 115: 610-620.

Collvin, L. 1985. The effect of copper on growth, food consumption and food conversion of perch *Perca fluviatilis* L. offered maximal food rations. Aquatic Toxicology 6: 105-113.

Cooley, J. M. 1977. Filtering performance of *Daphnia retrocurva* in pulp mill effluent. Journal of the Fisheries Research Board of Canada 34: 863-868.

Day, K., and N. K. Kaushik. 1987a. The adsorption of fenvalerate to laboratory glassware and the alga *Chlamydomonas reinhardii*, and its effect on uptake of the pesticide by *Daphnia galeata mendotae*. Aquatic Toxicology 10: 131-142.

Day, K. E., and N. K. Kaushik. 1987b. An assessment of the chronic toxicity of the synthetic pyrethroid, fenvalerate, to *Daphnia galeata mendotae*, using life tables. Environmental Pollution 44: 13-26.

Day, K. E., and N. K. Kaushik. 1987c. Short-term exposure of zooplankton to the synthetic pyrethroid, fenvalerate, and its effect of rates of filtration and assimilation of the alga, *Chlamydomonas reinhardii*. Archives of Environmental Contamination and Toxicology 16: 423-432.

Day, K. E., N. K. Kaushik, and K. R. Solomon. 1987. Impact of fenvalerate on enclosed freshwater planktonic communities and on in situ rates of filtration of zooplankton. Canadian Journal of Fisheries and Aquatic Sciences 44: 1714-1728.

Doucet, C. M., and E. J. Maly. 1990. Effect of copper on the interaction between the predator *Didinium nasutum* and its prey *Paramecium caudatum*. Canadian Journal of Fisheries and Aquatic Sciences 47: 1122-1127.

Evans, G. W., M. Lyes, and A. P. M. Lockwood. 1977. Some effects of oil dispersants on the feeding behaviour of the brown shrimp, *Cragnon cragnon*. Marine Behavior and Physiology 4: 171-181.

Fernández-Casalderrey, A., M. D. Ferrando, and E. Andreu-Moliner. 1992. Filtration and ingestion rates for *Brachionus calycifloris* after exposure to endosulfan and diazinon. Comparative Biochemistry and Physiology 103C: 357-361.

Fernández-Casalderrey, A., M. D. Ferrando, and E. Andreu-Moliner. 1994. Effects of sublethal concentrations of pesticides on the feeding behavior of *Daphnia magna*. Ecotoxicology and Environmental Safety 27: 82-89.

Ferrando, M. D., and E. Andreu. 1993. Feeding behavior as an index of copper stress in *Daphnia magna* and *Brachionus calyciflorus*. Comparative Biochemistry and Physiology, Series C 106: 327-331.

Ferrando, M. D., C. R. Janssen, E. Andreu, and G. Persoone. 1993. Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus*. III. The effects of chemicals on the feeding behavior. Ecotoxicology and Environmental Safety 26: 1-9.

Flickinger, A. L., R. J. F. Bruins, R.W. Winner, and J. H. Skillings. 1982. Filtration and phototactic behavior as indices of chronic copper stress in *Daphnia magna* Straus. Archives of Environmental Contamination and Toxicology 11: 457-463.

Folmar, L. C., D. R. Craddock, J. W. Blackwell, G. Joyce, and H. O. Hodgins. 1981. Effects of petroleum exposure on predatory behavior of coho salmon (*Oncorhynchus kisutch*). Bulletin of Environmental Contamination and Toxicology 27: 458-462.

Folmar, L. C., and H. O. Hodgins. 1982. Effects of Aroclor 1254 and No. 2 fuel oil, singly and in combination, on predator-prey interactions in coho salmon (*Oncorhynchus kisutch*). Bulletin of Environmental Contamination and Toxicology 29: 24-28.

Foster, N. R., A. Scheier, and J. Cairns. 1966. Effect of ABS on feeding behavior of flagfish, *Jordanella floridae*. Transactions of the American Fisheries Society 95: 109-110.

Geiger, J. G., and A. L. Buikema. 1981. Oxygen consumption and filtering rate of *Daphnia pulex* after exposure to water-soluble fractions of naphthalene, phenanthrene, No. 2 fuel oil, and coal-tar creosote. Bulletin of Environmental Contamination and Toxicology 27: 783-789.

Gliwicz, M. Z., and A. Sieniawska. 1986. Filtering activity of *Daphnia* in low concentrations of a pesticide. Limnology and Oceanography 31: 1132-1138.

Gómez, A., G. Cecchine, and T. W. Snell. 1997. Effect of pentachlorophenol on predator-prey interaction of two rotifers. Aquatic Toxicology 37: 271-282.

Gulati, R. D., C. W. M. Bodar, A. L. G. Schuurmans, J. A. J. Faber, and D. I. Zandee. 1988. Effects of cadmium exposure on feeding of freshwater planktonic crustaceans. Comparative Biochemistry and Physiology 90C: 335-340.

Ham, L., R. Quin, and D. Pascoe. 1995. Effects of cadmium on the predator-prey interaction between the turbellarian *Dendrocoelum lacteum* (Müller, 1974) and the Isopod crustacean *Asellus aquaticus* (L.). Archives of Environmental Contamination and Toxicology 29: 358-365.

Juchelka, C. M., and T. W. Snell. 1994. Rapid toxicity assessment using rotifer ingestion rate. Archives of Environmental Contamination and Toxicology 26: 549-554.

Juchelka, C. M., and T. W. Snell. 1995. Rapid toxicity assessment using ingestion rate of cladocerans and ciliates. Archives of Environmental Contamination and Toxicology 28: 508-512.

Kersting, K., and H. Van der Honing. 1981. Effect of the herbicide dichlobenil on the feeding and filtering rate of *Daphnia magna*. Verhandlungen der Internationale Vereinigung fur Theoretische und Angewandte Limnologie 21: 1135-1140.

Laskowski, R., and S. P. Hopkin. 1996. Effect of Zn, Cu, Pb, Cd on fitness in snails (*Helix aspersa*). Ecotoxicology and Environmental Safety 34: 59-69.

Lemly, A. D., and R. J. F. Smith. 1985. Effects of acute exposure to acidified water on the behavioral response of fathead minnows, *Pimephales promelas*, to chemical feeding stimuli. Aquatic Toxicology 6: 25-36.

Lett, P. F., G. J. Farmer, and F. W. H. Beamish. 1976. Effect of copper on some aspects of the bioenergetics of Rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 33.

Linder, G., and M. E. Richmond. 1990. Feed aversion in small mammals as a potential source of hazard reduction for environmental chemicals: agrichemical case studies. Environmental Toxicology and Chemistry 9: 95-105.

Lingaraja, T., P. S. B. Rao, and V. K. Venugopalan. 1979. DDT induced ethological changes in estuarine fish. Environmental Biology of Fishes 4: 83-88.

Little, E. E., R. D. Archeski, B. A. Flerov, and V. I. Kozlovskaya. 1990. Behavioral indicators of sublethal toxicity in rainbow trout. Archives of Environmental Contamination and Toxicology 19: 380-385.

Marigomez, J. A., E. Angulo, and V. Saez. 1986. Feeding and growth responses to copper, zinc, mercury and lead in the terrestrial gastropod *Arion ater* (Linne). The Journal of Molluscan Studies 52: 68-78.

McLeese, D. W. 1973. Response of lobsters *Homarus americanus* to odor solution in the presence of bleached kraft mill effluent. Journal of the Fisheries Research Board of Canada 30: 279-282.

McNaught, D. C., S. D. Bridgham, and C. Meadows. 1988. Effects of complex effluents from the River Raisin on zooplankton grazing in Lake Erie. Pages 128-137 *In* J. Cairns. and J. R. Pratt, eds. *Functional testing of aquatic biota for estimating hazards of chemicals*. American Society for Testing Materials, Philadelphia.

Oseid, D. M., and L. L. Smith. 1974. Factors influencing acute toxicity estimates of hydrogen sulfide to freshwater invertebrates. Water Research 8: 739-746.

Reading, J. T., and A. L. Buikema. 1980. Effects of sublethal concentrations of selenium on metabolism and filtering rate of *Daphnia pulex*. Bulletin of Environmental Contamination and Toxicology 24: 929-935.

Reeve, M.R., M. A. Walter, K. Darcy, and T. Ikeda. 1977. Evaluation of potential indicators of sub-lethal toxic stress on marine zooplankton (feeding, fecundity, respiration, and excretion): controlled ecosystem pollution experiment. Bulletin of Marine Science 27: 105-113.

Sandheinrich, M. B., and G. J. Atchison. 1989. Sublethal copper effects on bluegill, *Lepomis macrochirus*, foraging behavior. Canadian Journal of Fisheries and Aquatic Sciences 46: 1977-1985.

Scherer, E., R. E. McNicol, and R. E. Evans. 1997. Impairment of lake trout foraging by chronic exposure to cadmium: a black-box experiment. Aquatic Toxicology 37: 1-7.

Schuytema, G. S., A. V. Nebeker, J. A. Peterson, and W.L. Griffis. 1993. Effects of pentachlorophenol-contaminated food organisms on toxicity and bioaccumulation in the frog *Xenopus laevis*. Archives of Environmental Contamination and Toxicology 24: 359-364.

Steele, C. W., D. W. Owens, and A. D. Scarfe. 1990. Attraction of zebrafish, *Brachydanio rerio*, to alanine and its suppression by copper. Journal of Fish Biology 36: 341-352.

Steele, C. W., S. Strickler-Shaw, and D. H. Taylor. 1992. Attraction of crayfishes *Procambarus clarkii*, *Orconectes rusticus* and *Cambarus bartoni* to a feeding stimulant and its suppression by a blend of metals. Environmental Toxicology and Chemistry 11: 1323-1329.

Waiwood, K. G., and F. W. H. Beamish. 1978. The effect of copper, hardness and pH on the growth of rainbow trout, *Salmo gairdneri*. Journal of Fish Biology 13: 591-598.

Wang, R. T., and J. A. C. Nicol. 1977. Effects of fuel oil on sea catfish: feeding activity and cardiac responses. Bulletin of Environmental Contamination and Toxicology 18: 170-176.

Weber, D. N. 1996. Lead-induced metabolic imbalances and feeding alterations in juvenile fathead minnows (*Pimephales promelas*). Environmental Toxicology and Water Quality 11: 45-51.

Wedemeyer, G. A., N. C. Nelson, and W. T. Yasutake. 1979. Physiological and biochemical aspects of ozone toxicity to rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 36: 605-614.

Weeks, J. M. 1993. Effects of dietary copper and zinc concentrations on feeding rates of two species of talitrid amphipods (Crustacea). Bulletin of Environmental Contamination and Toxicology 50: 883-890.

Woltering, D. M., J. J. Hedtke, and L. J. Weber. 1978. Predator-prey interactions of fishes under the influence of ammonia. Transactions of the American Fisheries Society 107: 500-504.

Wong, C. K., J. R. Strickler, and F. R. Engelhardt. 1983. Feeding behavior of *Daphnia pulex* in crude oil dispersions. Bulletin of Environmental Contamination and Toxicology 31: 152-157.

Chapter 6

Effects of Chronic Exposure to Bleached Kraft Mill Effluent in Two Mayfly Species: *Ameletus subnotatus* (Ameletidae) and *Baetis tricaudatus* (Baetidae).

6.1 Introduction

Bleached kraft pulp mill effluent (BKME) is a complex mixture containing nutrients and potentially toxic compounds, including chlorinated phenolics, resin acids, polycyclic aromatic hydrocarbons (PAHs), and many other substances (Suntio et al. 1988, Government of Canada 1991). Improvements in effluent treatment and reduction or elimination of molecular chlorine in the bleaching process have substantially reduced toxicity of these effluents (Solomon et al. 1993). Effluents from Canadian mills are rarely acutely toxic at full strength (Environment Canada 1997), however, exposure to these effluents may cause chronic growth and reproductive effects. Chronic effects often result from toxicant-related changes in the acquisition or utilization of metabolic resources (Sibley and Calow 1989, Naylor et al. 1989, Baird et al. 1990, Calow and Sibley 1990, Calow 1991, Maltby et al. 1990, Sibley 1996, Wicklum and Davies 1996), and they have the potential to affect population growth and community composition (van Leeuwen et al. 1986, Klok and de Roos 1996, Sibley 1996).

Despite the important role that benthic insects play in riverine communities by making energy from both primary and microbial production available to fish, little is known about the chronic effects of effluent on these organisms. Field-assessments of effluent effects are typically done at the community level, and insects such as mayflies, caddisflies and stoneflies are only occasionally used in laboratory bioassays (Petersen and Petersen 1988, Diamond et al. 1992,). This is despite the fact that sensitivity of these taxa to poor water quality is well established (Wiederholm 1984, Buikema and Voshell 1993, Norris and Georges 1993). Chronic exposure to toxicants can reduce

growth rate, increase time to emergence, and increase mortality of mayflies (Hatakeyama 1989, Diamond et al. 1992). Measurements of the chronic effects of BKME on benthic insects include Lowell et al.'s (1995a) measurement of BKME effects on the mayfly *Baetis tricaudatus*, and Dubé and Culp's (1996, 1997) study of the effects of effluent on chironomid growth. Both studies found that low concentrations (<5%) increased size of animals and suggested that changes in food quality, hormetic effects, or the presence of phytochemicals might be responsible. Dubé and Culp (1996, 1997) reported reduced growth of midges (relative to 1%) at concentrations ≥5%, while Lowell et al. (1995a) saw no evidence of toxicity to mayflies at 10% BKME. Reproductive effects were not measured. A major difference between the two studies was that the food supply for chironomids was grown in effluent, while algae fed to mayflies had been grown in control water, suggesting that contaminants sorbed to algae may have been responsible for the increased toxicity observed in Dubé and Culp's (1996, 1997) experiment.

The objective of this research was to measure the effects of BKME in a realistic exposure to contaminated food and water on the growth, survival, and reproduction of mayflies. Two species were chosen: *Ameletus subnotatus* (Ameletidae) and *Baetis tricaudatus* (Baetidae). *Ameletus subnotatus* was chosen because this species responded to effluent additions in both mesocosm and feeding experiments (Chapters 3 and 5). In this study, the effect of BKME on growth over a 98-day exposure was measured; no attempt was made to measure reproductive effects because of the long life-cycle of this species. *Baetis tricaudatus* was chosen because it is a very widespread species, and because use of this species would allow a comparison to Lowell et al. (1995a) using a different bleached kraft mill. Two experiments measured: (1) the effects of BKME on early life stages; and (2) the effects of BKME on growth, survival, and fecundity of this species.6.2 Methods

Ameletus subnotatus bioassay

Ameletus subnotatus nymphs, less than 4 weeks old, were used for the bioassay. The animals were obtained by in vitro fertilization of eggs from adults

collected as young nymphs in the Athabasca river and reared in the laboratory. Nymphs were collected in late October in the Athabasca River at Hinton, Alberta, upstream of the mill outfall. They were kept in the lab and fed cultured *Navicula* until their emergence in late May. In vitro fertilization of eggs was accomplished by removing the seminal vesicle and testes from males, macerating these tissues in an insect Ringers solution, and mixing this with eggs removed from subimago females. Eggs and sperm were combined for about 75 minutes before being transferred to glass Petri dishes containing 10°C autoclaved river water. Fertilized eggs were held at 10°C with a 16:8 light:dark cycle until hatching and were checked weekly to monitor development. Hatching occurred over the course of a week in mid-August, and during this time nymphs were transferred daily to glass Petri dishes in which *Navicula* had been cultured. Nymphs were held at 10 °C until the start of the bioassay.

The bioassay was conducted in new, Costar© polystryene 12-well plates. Before the bioassay, the well plates were acid-washed and *Navicula* had been cultured in a standard WC 10 media in the wells for approximately 3 weeks to provide a food source for the nymphs during the bioassay. The bioassay was started in mid-September, when nymphs were about 4 weeks old, and continued for 98 days. Culture media in the wells was replaced with test solutions just before adding animals; effluent concentrations of 0 and 1% were used. Five *Ameletus* were randomly allocated to each of 6 replicate wells. The approximate volume of test solutions was 4 mL per cell. Test solutions were made with water from the South Saskatchewan River that had been passed through a 20 µm filter, autoclaved, and cooled to 10°C. Animals were kept at 10°C with a light dark cycle of 12:12 h. Test solutions were replaced weekly by using a clean pipette to remove most (about 80%) of the solution in the well, and new solution was added very slowly. *Navicula* growth in the well plates was sufficient to maintain *ad libitum* food until the last 2 weeks of the bioassay at which time additional *Navicula*, cultured in the appropriate concentration of effluent, was added once. Mean concentrations of dissolved oxygen (DO) in the wells, measured at the end of the dark cycle (i.e., at the lowest point during

the day), were 6.08 mg/L in the controls and 6.78 mg/L in the 1% treatment (t-test, DF=10, t=-1.62, p=0.14). During the light cycle, oxygen concentrations averaged 7.38 mg/L in the controls and 7.97 mg/L in the effluent treatment wells (t-Test, DF=10, t=-2.23, p=0.057).

The chronic bioassay ran for 98 days, at which point surviving *Ameletus* were killed and fixed in 10% formalin. Thorax length was measured at 500x magnification using a stereomicroscope with a *camera lucida*, and digitizing pad. Thorax length was measured as the distance from the anterior medial edge of the prothorax to the posterior medial edge of the metathorax.

Baetis tricaudatus Methods

1. Baetis Growth Bioassay

The bioassay took place in an artificial stream system similar to that used by other investigators (Walde and Davies 1984, Scrimgeour 1992, Scrimgeour et al. 1991, Himbeault 1995, Lowell et al. 1995 a,b, and Dubé and Culp 1996, 1997). The stream system was modified to reduce operating difficulties experienced by previous users, in particular, frequent clogging of water jets by algae (Himbeault pers comm, Dubé pers comm), and to ensure that all components were constructed with food-grade materials (Plate 6.1). Treatment solutions were stored beneath the streams in 40 L reservoirs made of food grade, low density polyethylene. Little Giant 3-SC-MD magnetic impeller pumps circulated test solutions from the reservoirs through a check valve and a ball valve (preventing backflow and allowing flow adjustment, respectively), tubing, and a manifold to the test streams. The streams were constructed from food grade, low density polyethylene. They were 6.9 cm deep, with a diameter of 10.3 cm, a 3.3 cm center standpipe, and a 435 mL volume. The jet system of Walde and Davies (1984) was replaced with a single water port, a 4 mm (outer

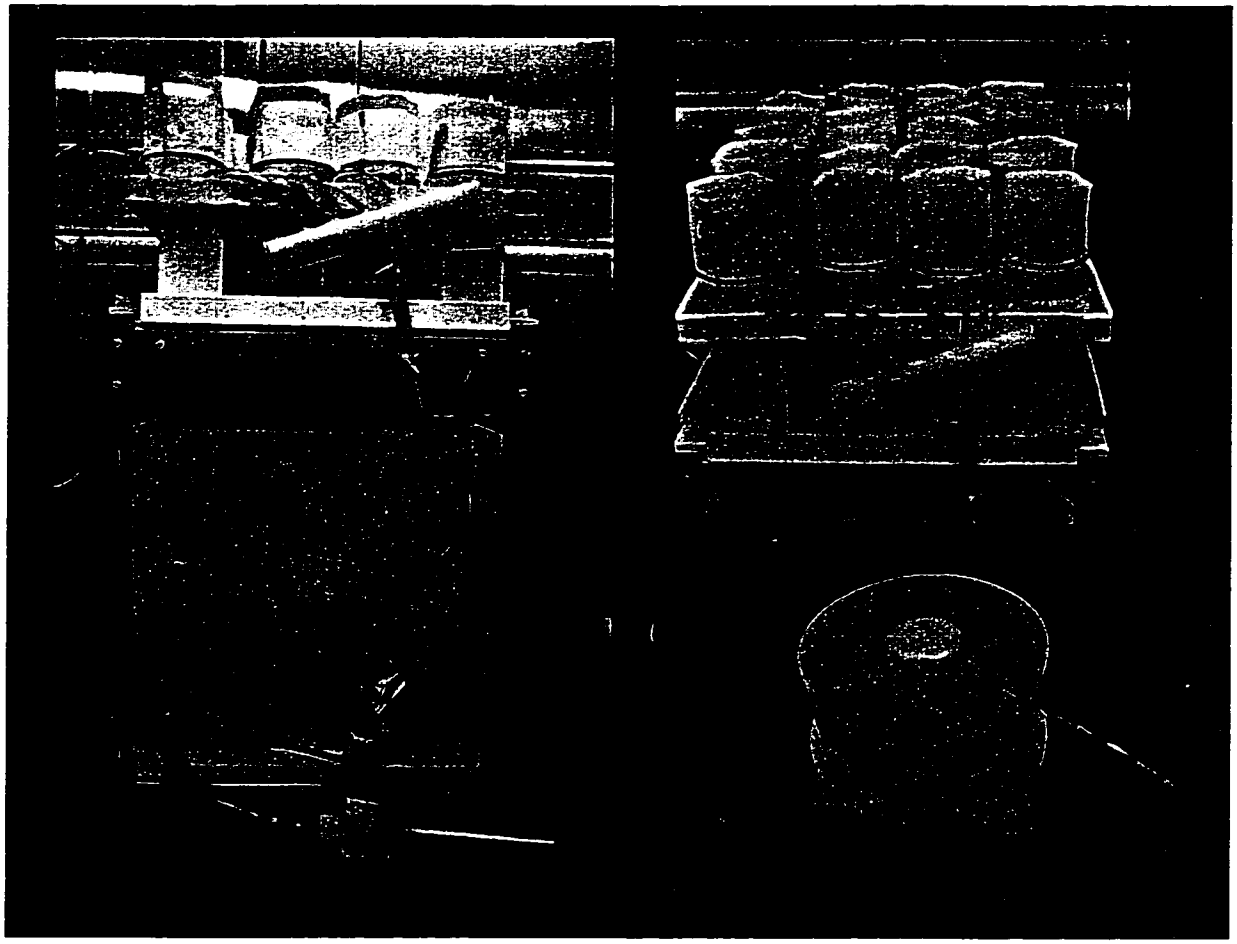


Plate 6.1. Stream system used for the chronic *Baetis tricaudatus* assay.

diameter) polystyrene tube located 3 cm from the bottom. A 210 µm Nitex bag slipped around the outside of the streams and was held in place with aquarium safe silicone and a nitrile rubber O-ring. This bag acted as a screen to prevent animals from leaving the streams and had a band of Velcro along the top edge, allowing the bag to be closed to capture emerging insects. Treatment solutions were allowed to continuously overflow the streams and, by means of a wet table and tubing, were returned to the reservoir. All components of the stream system (except the reservoirs) were new and acid-washed before use. The water reservoirs were soap-washed and soaked in 3 changes of distilled water for 96 h before use. Test solutions were made with water from the South Saskatchewan River that had been filtered though a 20 µm particle filter and then UV sterilized.

Baetis tricaudatus were collected from the South Saskatchewan River, 21 km downstream of Saskatoon, and were kept in cool, aerated water while being transported to the laboratory. The insects were held in aerated tanks and fed cultured *Navicula* for 2 days before starting the bioassay. Twenty-five insects were randomly allocated (in groups of 5) to each of 60 streams (n=20 per treatment). All animals were examined under a dissecting microscope to ensure that mechanically damaged individuals were not used. A subset of these animals was randomly chosen and preserved in 10% formalin to allow body size at the beginning of the experiment to be measured. After all animals were added to the streams, effluent was added to water reservoirs to create the desired effluent concentrations of 0, 1, or 7%. Animals that died overnight the first day were presumed to have died due to handling stress and were replaced. The streams were held at 17°C with a light:dark cycle of 16:8 h, with 30 min dawn/dusk periods. Light was provided by 4', 2 tube, electronic ballast, fluorescent fixtures suspended directly above each stream table. The fixtures were equipped with Duro-Test® Vitalite® tubes which provide a close simulation of the natural spectrum.

Streams were checked daily for dead animals, and *Navicula*, cultured in the same dilution of effluent, was provided *ad libitum*. Every 48 hours, test solutions were replaced with a fresh solution made from UV sterilized, river water and BKME. Effluent composition is given in Chapter 4. Dissolved oxygen concentration in the streams ranged from 7.3-8.45 mg/L, and current velocity was approximately 3 cm/sec. After 11 days, 6 animals (2 from controls, 4 from 1% BKME) had emerged and many others had developed dark wingpads. At that time, all animals from 15 replicates of each treatment were preserved in 10% formalin. The remaining 5 streams were left running to collect emerged individuals. Emerged males were weighed and the eggs from female last nymphal instar, subimagos or imagos (depending upon at which stage they died) were counted.

Nymphs from the 15 replicates collected on day 11 were measured with the aid of a stereomicroscope, *camera lucida* and a digitizing pad. Total body

length was measured as the distance from the anterior edge of the labium to the tip of the abdomen, excluding the cerci. Animals were individually placed in preweighed foil boats, dried at 60°C for 48 hours and weighed on a CAHN C-31 Series microbalance.

2. Early life stage test

Baetis tricaudatus with dark wingpads, indicating that emergence was imminent (Clifford 1970), were collected from the South Saskatchewan River at the Clarkboro ferry crossing, about 21 km downstream of Saskatoon. These animals were held in the laboratory at 18°C until they became imagos (approximately 2 days). *In vitro* fertilization of eggs was accomplished by removing the seminal vesicle and testes from males, macerating these tissues in an insect Ringers, and mixing this with eggs removed from females. The combined eggs and sperm were left for 10 minutes before the eggs were transferred to glass Petri dishes containing 16°C river water (similar to river water temperatures at that time). Small quantities of eggs were then removed using a glass pipette and randomly assigned to treatment wells. Due to the difficult nature of this operation and the desire to minimize handling of the eggs, the number of eggs transferred to wells could not be standardized. The mean number of eggs per well was 40.9 (n=42, s=13.26). Treatment concentrations were 0, 1, 5, 10, 15, 20 and 50% (n=6 per treatment). A preliminary experiment using effluent concentrations ranging from 0-100% indicated that 95% of all hatching that occurs within 45 d from fertilization does so on days 9 and 10. Therefore, the number of hatched eggs and the number of live and dead nymphs were counted on day 10.

6.3 Results

6.3.1 Ameletus growth bioassay

Exposure to effluent had a significant effect on mortality and growth of *A. subnotatus* nymphs. Mortality was significantly higher in the effluent treatment (Kruskal Wallis, H=4.49, DF=1, p=0.034) averaging 53.4% in the controls and

83.3% in 1% BKME. Even though mortality was greatest in the effluent treatment, growth was also increased; the thorax length of animals exposed to 1% BKME was significantly larger (1.2mm ± 0.06) than that of control animals (0.9mm ± 0.03) (t-test, T=-4.30, DF= 8, p=0.0027).

Baetis growth bioassay

During the experiment, total length of nymphs increased from an average of 2.12 mm (s=0.349, n=14) to an average (±SE) of 3.68 (±0.13), 4.17 (±0.10) and 4.37 (±0.11) mm in control, 1, and 7% treatments respectively. Growth of *Baetis* increased with exposure to BKME; there was a significant treatment effect on total length (ANOVA, $F_{2,42}$=9.27, p<0.001), and dry weight (ANOVA, $F_{2,42}$=9.26, p<0.001) (Fig. 6.1). Dry weight had to be transformed to it's reciprocal to correct for a non-normal error distribution. *Baetis* exposed to 1 and 7% effluent were larger and heavier than control insects, and there was no difference between the 2 effluent concentrations (Fisher's protected $LSD_{\alpha=0.05}$ TL= 0.33, 1/Wt =1.92). There was a significant treatment effect on nymph mortality (ANOVA $F_{2,42}$=3.24, p=0.049, Fisher's protected $LSD_{\alpha=0.05}$=2.43, Fig. 6.2) with significantly fewer live *Baetis* recovered from 7% BKME streams. There was no effect of treatment on the dry weight of adult males (ANOVA $F_{2,11}$= 0.12, p=0.891), or fecundity (ANOVA $F_{2,9}$=1.84, p=0.213) (Fig. 6.3). Dry weight of females could not be accurately determined after the dissection necessary to remove and count eggs. The lack of effect on fecundity should be interpreted with caution as female *Baetis* were recovered from only 3 of the control streams.

6.3.2 Baetis Early Life Stage Test

There was a significant treatment effect on the number of eggs hatched, due largely to a decrease in hatching success at 5% BKME (ANOVA $F_{6,35}$=7.06, p<0.001, Fig 6.4). There was also a significant treatment effect on nymph mortality (ANOVA $F_{6,35}$=15.41, p<0.001), with mortality ≥50% in BKME concentrations over 1% (Fig. 6.4).

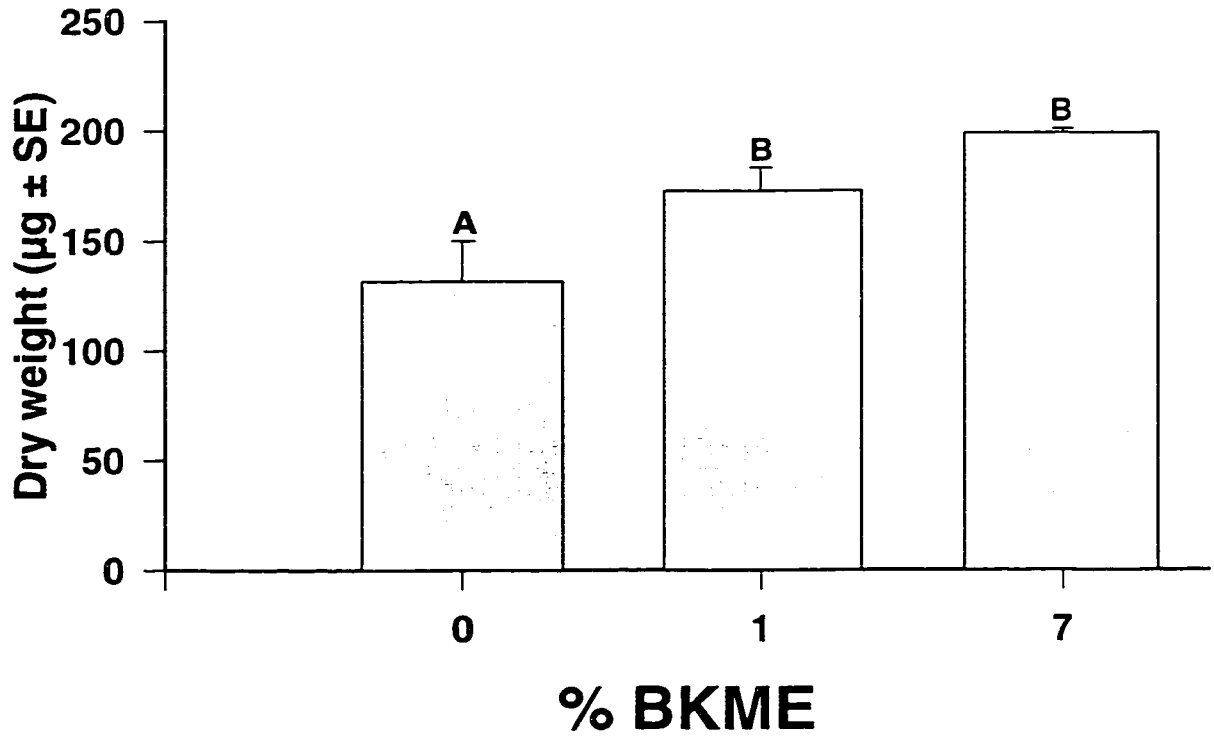


Figure 6.1. Dry weight of *Baetis tricaudatus* nymphs after exposure to 0, 1, and 7% BKME in food and water for 11 days. Means with common letters are not significantly different based upon a Fisher's Protected LSD (α=0.05) of 1.92 for the reciprocal of weight.

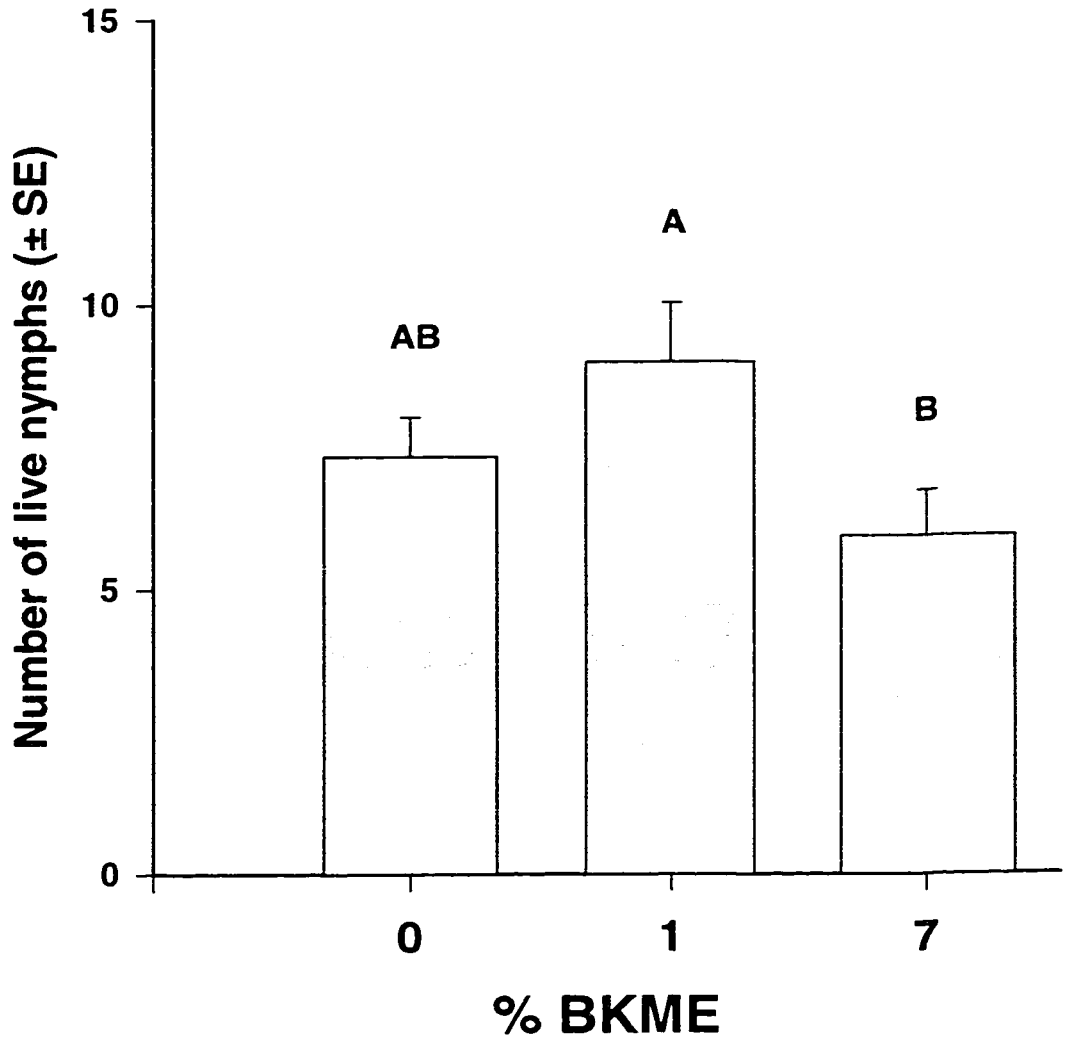


Figure 6.2. Number (±SE) of *B. tricaudatus* surviving to the end of an 11 d exposure to 0, 1, and 7% BKME. Means with a common letter are not significantly different based on Fisher's Protected LSD(α=0.05)=2.43.

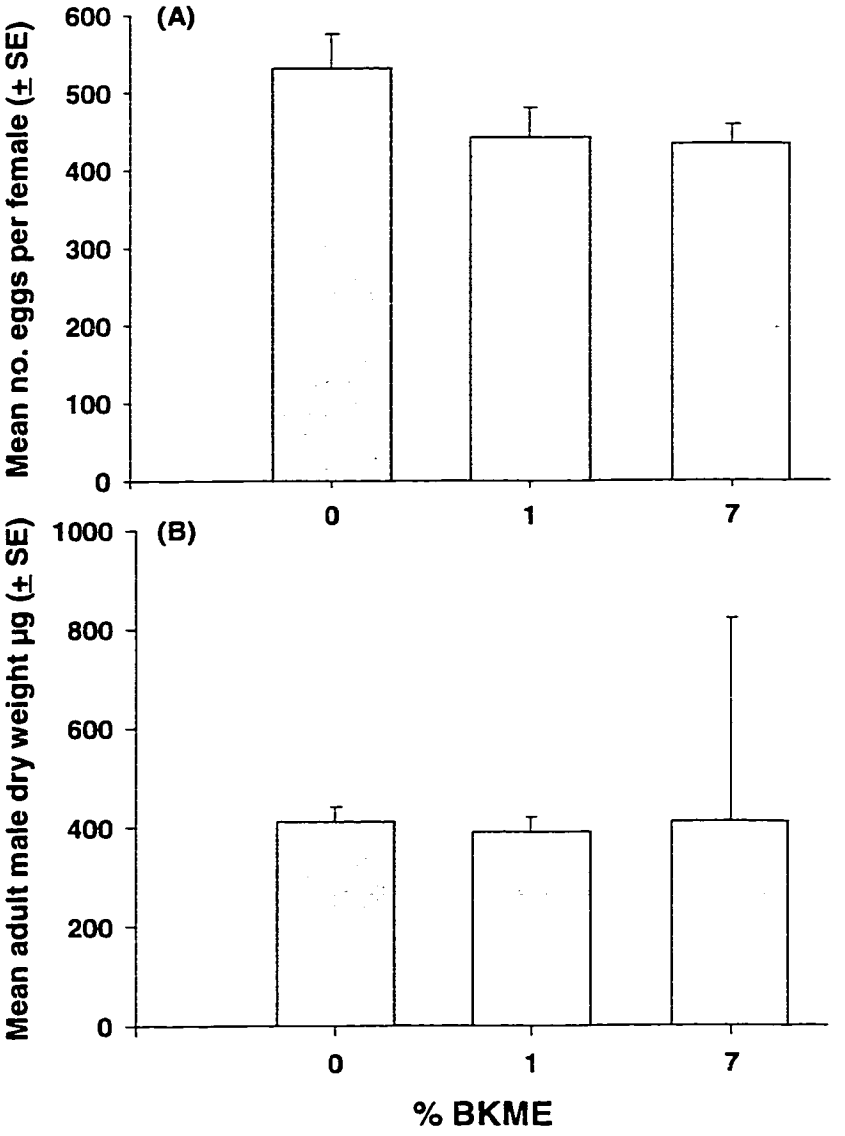


Figure 6.3. (A) Mean (±SE) number of eggs per female, and (B) mean (±SE) adult male dry weight of *Baetis tricaudatus* exposed to 0, 1, and 7% BKME for 11 days.

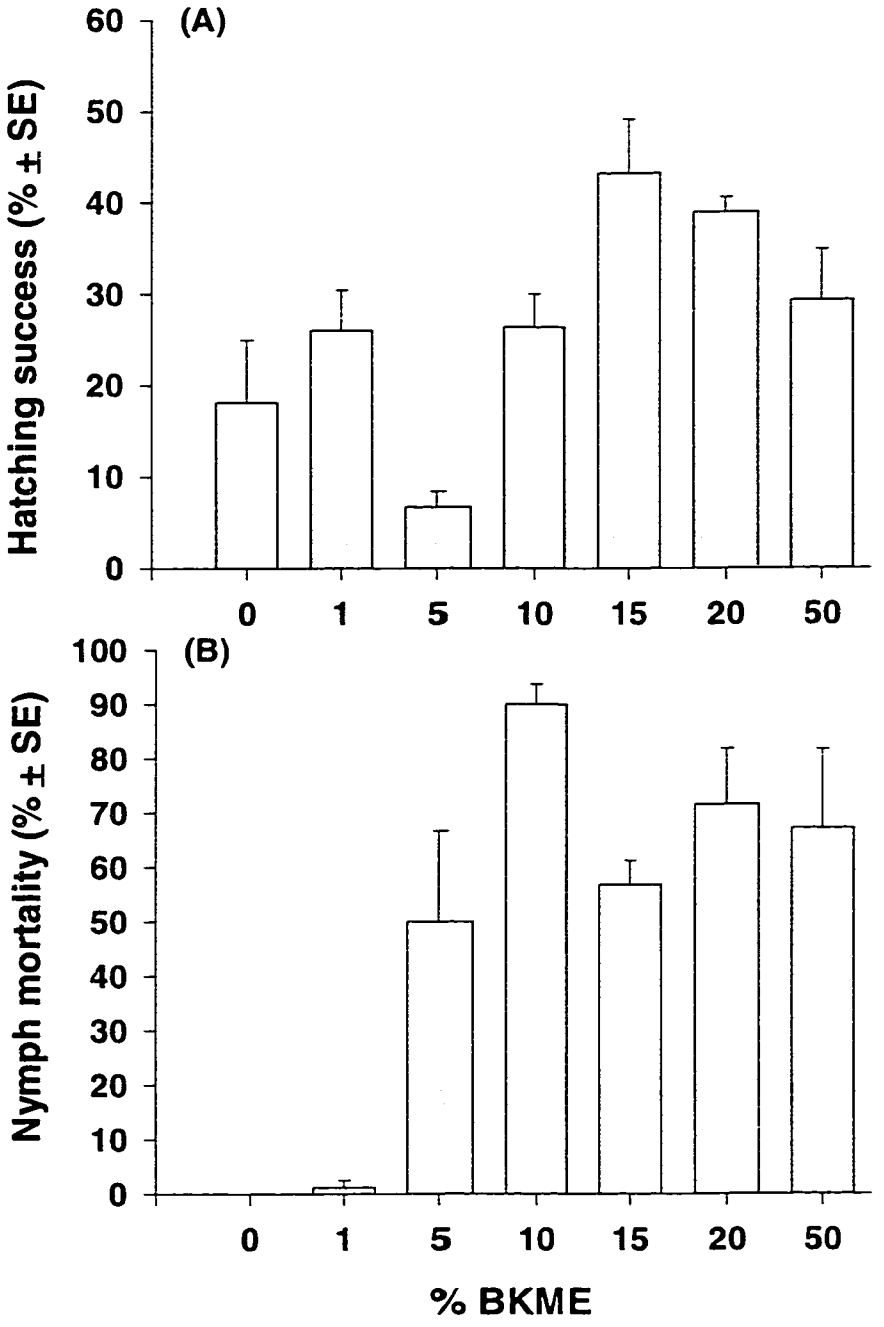


Figure 6.4. (A) Hatching success and (B) nymph mortality of *Baetis tricaudatus* exposed to 0, 1, 5, 10, 15, 20, and 50% BKME throughout embryonic development.

6.4 Discussion

Chronic exposure of both species of mayflies to bleached kraft mill effluent resulted in increased growth, but also increased mortality of *Ameletus subnotatus* at 1% and *Baetis tricaudatus* at 7%. Increased size in response to pulp mill effluent has been reported for *Baetis tricaudatus* by Lowell et al. (1995a), for *Ameletus subnotatus* (Podemski and Culp 1996, Chapter 3), and for chironomids by Dubé and Culp (1996, 1997). In this study, the effluent is a combination of pulp mill and municipal wastes, and Himbeault (1995) reported that *Baetis tricaudatus* size increased in response to exposure to municipal effluent.

Pulp mill effluent is a complex mixture, and mechanisms responsible for the observed growth response are not obvious. In field studies, exposure to pulp mill effluent can increase size and/or density of grazing invertebrates (Hall et al. 1991, Dubé et al. 1997), and this response is typically attributed to increased algal or detrital food resources resulting from nutrients and particulate matter in the effluent. For the single species bioassay mentioned, food was not limited, thus, increased food availability cannot be solely responsible. Alternate explanations include hormesis (Lowell et al. 1995a, Himbeault 1995), an increase in feeding rates caused by some unidentified compound(s) in the effluent (Lowell et al. 1995a, Himbeault 1995, Dubé and Culp 1996), a change in food quality (Lowell et al. 1995a, Dubé and Culp 1996,1997), or the presence of phytochemicals with insect hormonal activity (Lowell et al. 1995a, Dubé and Culp 1996,1997). Hormesis, a biphasic dose-response with stimulation at low and inhibition at high concentrations (Southam and Erlich 1943, Calabrese et al. 1987), cannot be ruled out, and stimulation at low concentrations (typically at 6.5 and 12.5%) has occurred in chronic *Ceriodaphnia* assays of pulp mill effluent (Hatfield Consultants Ltd. 1996a,b; Environment Canada 1997). While increased feeding can occur in fish exposed to pulp mill effluent (McLeay and Brown 1979), it is not likely to be responsible for the observed effects on mayflies, as feeding assays demonstrated a

decrease in consumption by *Ameletus subnotatus* exposed to effluent in food and water (Chapter 5).

The most likely explanation for increased growth is that bleached kraft mill effluent enhances the quality of food available for these insects. Preferential feeding by *Ameletus* on diatom mats cultured in effluent (Chapter 5) supports the idea that food quality is improved by exposure to effluent. This change in quality may be the result of effluent-related biochemical changes in algal composition; a change in the lipid, carbohydrate, or lipid content of algae is a common response to toxicant or nutrient stress (Soto et al. 1977; Piorreck et al. 1984; Ben Amotz et al. 1985; Sicko-Goad et al. 1989a,b; Thompson and Couture 1991). Navicula cultured in effluent exhibited a small but significant increase in lipid content in response to effluent (Chapter 4). Addition of effluent also increased the microbial population of the cultured diatom mat that was fed to mayflies in this study (Chapter 4). Effluent has been reported to increase microbial production in riverine periphyton (Mohammed 1997). Pulp mill effluent contains particulate matter including cellular fragments, protozoa, algae and bacteria (Zanella et al. 1978, NCASI 1978), and Dubé and Culp (1996, 1997) hypothesized that this particulate matter might represent a high quality, leading to increased growth rates of chironomids. Bacteria and particulate organic matter may represent a high quality, more easily digestible food source for insects, like *Baetis* and *Ameletus*, that feed on detritus in addition to diatoms (Muttkowski and Smith 1929, Moore and Love 1977, Gray and Ward 1979, Ciborowski and Clifford 1983, Matthews and Tarter 1989, Rader and Ward 1989, Mihuc and Minshall 1995). Sivaramakrishan and Venkataraman (1987) reported that two *Baetis* species had higher growth rates on a detrital diet than when fed algae. Bacteria may be an even more important food source for mayflies fed laboratory-cultured diatoms because mayflies rely on small sand grains ingested normally with their food to aid in the mechanical damage of diatom frustules necessary for digestion (Arens 1989).

While exposure to effluent increased mayfly size, toxicity was also observed, with increased mortality at 1% in *Ameletus subnotatus* and at 7% in

Baetis tricaudatus (5% for first instars). Toxicity at 5-7% agrees with the observations of Dubé and Culp (1996,1997) who reported decreased growth in chironomids at concentrations ≥5%. Lowell et al. (1995a) did not report any evidence of toxicity at effluent concentrations as high as 10%, however, in that study, *Baetis* were fed algae that had not been pre-exposed to effluent and therefore would have had less exposure to contaminants in their diet. Exposure to contaminants in food in addition to a water-exposure can significantly increase mortality in daphnids (Kungolos and Ayoama 1993). The effluents used in Lowell et al. (1995a) and this study were also from different sources and, thus, may differ in toxicity. Dubé and Culp (1996, 1997), while reporting toxicity effects in the form of decreased growth of chironomids, did not, however, report increased mortality.

Simultaneous observation of increased mortality and increased growth is unexpected because a reduction in growth would typically precede mortality along a toxicity or stress gradient. There was no evidence of an energetic cost associated with effluent exposure in terms of growth of nymphs, adult size, or fecundity of *Baetis tricaudatus*. While the small sample size demands that these results be interpreted with caution, trends suggest that mayflies allocate little energy to defensive mechanisms. Sibley and Calow (1989) predicted that animals subjected to naturally high mortality rates should exhibit short life-cycles and invest little in defense. Estimated mortality rates (i.e., mortality before reproduction) in unimpacted populations of mayflies are high, ranging from 91-99.9% (Werneke and Zwick 1992, Clifford and Boerger 1974).

There were differences in the sensitivity of mayflies to BKME, both between species and between different life stages of a single species. The reduced geographic range of *Ameletus* compared to *Baetis* (Edmunds et al. 1976, Edmunds and Waltz 1978, Zloty 1996) suggests it has a narrower range of environmental tolerances, thus greater sensitivity to chemical stressors is not unexpected. *Baetis* has been reported to be less sensitive than other mayflies to coal-ash (Scullion and Edwards 1980). Sensitivity to BKME was age-dependent; egg hatching was not affected by effluent concentrations as high as

50%, while first instar nymphs were more susceptible than older nymphs, showing high mortality at concentrations above 1%. The developing embryo is well protected from the outside environment by the chorion, and hatching success may be unaffected by quite high concentrations of toxicants (Sweeny et al. 1993). Conversely, young mayfly nymphs tend to be more sensitive to chemical stress than older nymphs (Fiance 1978, Allard and Moreau 1987, Diamond et al. 1992); increased sensitivity of young individuals is a well recognized phenomena in toxicology (Rand et al. 1995, McKim 1995, Green et al. 1986). A concentrated hatching period at days 9-11 with many unhatched eggs remaining is typical for baetids (Elliot 1972, Benech and Vignes 1972). Had the bioassay been allowed to continue it is likely that further hatching would have occurred; typically baetid egg masses show a brief period of hatching followed by delayed, sporadic hatching occurring over several months (Elliot 1972).

The poor performance of control mayflies in this study indicates that the use of *Baetis* for chronic toxicology testing requires further development. In aquatic toxicology, high mortality in controls is believed to indicate that the test animals were in suboptimal condition, which can result in increased susceptibility to toxicants. Poor control performance may suggest that the toxicity of pulp mill effluent was overestimated in this study. However, high mortality is not uncommon in natural populations (Werneke and Zwick 1992, Clifford and Boerger 1974) or laboratory experiments with mayflies. For example, Brittain (1976) reported 90% mortality in laboratory-reared *Leptophlebia vespertina*; Clifford et al. (1979) estimated a daily mortality rate of 2.5% in *Leptophlebia cupid*; Sweeney et al. (1986) reported 50-97% mortality in *Leptophlebia intermedia*; and Gerhadt (1990) had control mortalities ranging from 20% for *Leptophlebia marginata,* to 65% in *Baetis rhodani*. One possible explanation for the poor performance of controls is that the diet, a monoculture of the pennate diatom *Navicula*, did not provide sufficient nutrition. Although diatoms are reported to be a high quality food source for grazing insects (Anderson and Cummins 1979, Sweeney and Vannote 1984), these insects

would not naturally feed on a single-species diet. Single-species diets produce daphnids that are more sensitive to toxicants than those produced on multi-species diet*s* (Cowgill et al. 1987, Knight and Waller 1992), and some unialgal diets have proved unacceptable for rearing copepods (Hart and Santer 1994). The diet of a test species is recognized as an important modifying factor in toxicity testing (Dixon and Hilton 1985, Cowgill 1987, Hickie and Dixon 1987, Dahlgren 1988, Hickie 1989, Hickie et al. 1989, Farkas et al. 1996), and the smaller size and high mortality in controls seen in this study agree with the characteristics of mayflies raised on inadequate diets (Larsen 1978, Sweeney et al. 1986, Söderström 1991). Studies that have not reported high mortality (e.g. Scrimgeour 1992, Sweeney et al. 1993, Lowell et al. 1995a) provided a natural assemblage of periphytic organisms as food. As a defined diet is more preferable for organisms used for toxicity testing, this is an area that will require further research.

Biologically-treated pulp mill effluent can have stimulatory or inhibitory influences on aquatic communities, with the balance between these influences shifting as effluent concentration increases. At low concentrations of biologically-treated kraft mill effluent (i.e., <5%) the dominant effect of effluent is enhanced growth. Nutrient enrichment by effluent and the resultant increase in primary production cannot be entirely responsible for stimulation at higher trophic levels, as enhancement has been observed in grazing insects in food-unlimited situations in this study and that of Lowell et al. (1995a). The most likely explanation, one that is supported by feeding experiments (Chapter 5), is that effluent exposure increases the quality of food available for these insects. However, hormesis or the presence of phytochemicals with insect hormonal activity may also play a role. As effluent concentrations increase to 5% and above, contaminant effects appear in the form of decreased growth (Dubé and Culp 1996, 1997) or increased mortality (this study). Young mayflies and some species, e.g. *Ameletus subnotatus*, may be more sensitive to effluent than others. At concentrations between 5 and 10%, animals were larger than control animals, but were in poorer condition than animals exposed to 1% effluent. It

appears that in this concentration range, the balance of stimulatory and inhibitory effects of effluent begins to shift towards inhibition. However, aside from restricted areas within the mixing zone, effluent concentrations in the receiving waters of Canadian pulp mills are generally below 5%, suggesting that these discharges are unlikely to have deleterious effects on aquatic communities.

The use of stream organisms for effluent toxicity testing is an area requiring further research. The test organisms normally used to monitor effluent toxicity of the mill in this study are the cladoceran *Ceriodaphnia dubia*, the green alga *Selenastrum capricornutum*, and the fathead minnow (*Pimephales promelas*). None of these organisms are indigenous to the Athabasca river headwaters. Chronic toxicity tests from 1993-1995 using these organisms have generated estimates of the LOEC (lowest observed concentration that causes an effect) ranging from 80-100% BKME for *Ceriodaphnia* mortality, 40-100% for *Ceriodaphnia* reproduction, 100% for growth and mortality of fathead minnows, and 12.5-100% for *Selenastrum* growth (Golder Associates 1996). It is apparent that these tests may not adequately protect water quality for indigenous species of mayflies. McKinney and Wade (1996) reported that *Anodonta imbecilis* was more sensitive to pulp and paper mill effluents than *Ceriodaphnia dubia*, and that reliance on the Ceriodaphnia 7d chronic test would be unlikely to protect an already threatened native mussel fauna. Buikema and Voshell (1993) suggested that development of toxicity tests using insects, particularly mayflies and stoneflies, should be a priority to improve our ability to protect the aquatic environment. Baetid mayflies may be an ideal group to focus on, as these insects are sensitive to toxicants (Oseid and Smith 1974, Mayer and Ellersieck 1986), they develop quickly compared to many aquatic insects (Jackson and Sweeney 1995), and perhaps most importantly, are one of the few mayfly genera found in the rivers and lakes of northern Canada (Lehmkuhl 1973, Edmunds et al. 1976), where increasing industrial natural resource exploitation may threaten the quality of aquatic ecosystems.

6.5 Literature Cited

Allard, M., and G. Moreau. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. Hydrobiologia 144: 37-49.

Anderson, N. H., and K. W. Cummins. 1979. Influences of diet on the life histories of aquatic insects. Journal of the Fisheries Research Board of Canada 36: 335-342.

Arens, W. 1989. Comparative functional morphology of the mouthparts of stream animals feeding on epilithic algae. Archive für Hydrobiologie/Suppl. 83: 253-354.

Baird, D. J., I. Barber, and P. Calow. 1990. Clonal variation in general response of *Daphnia magna* Straus to toxic stress. I. Chronic life-history effects. Functional Ecology 4: 399-407.

Ben-Amotz, A., T. G. Tornabene, and W. H. Thomas. 1985. Chemical profile of selected species of microalgae with emphasis on lipids. Journal of Phycology 21: 72-81.

Benech, V., and C. Vignes. 1972. Etude expérimentale de l'incubation des oeufs de *Baetis rhodani* Pictet. Freshwater Biology 2: 243-252.

Brittain, J. E. 1976. Experimental studies on nymphal growth in *Leptophlebia vespertina* (L.) (Ephemeroptera). Freshwater Biology 6: 445-449.

Buikema, A. L., and J. R. Voshell 1993. Toxicity studies using freshwater benthic macroinvertebrates. Pages 344-398 *In* D. M. Rosenberg and V. H. Resh, eds. *Freshwater biomonitoring and benthic macroinvertebrates.* Chapman and Hall, New York.

Calabrese, E.J., M.E. McCarthy and E. Kenyon. 1987. The occurrence of chemically induced hormesis. Heath Physics 52:531-541.

Calow, P. 1991. Physiological costs of combating chemical toxicants: ecological implications. Comparative Biochemistry and Physiology 100C: 3-6.

Calow, P., and R. M. Sibley. 1990. A physiological basis of population processes: ecotoxicological implications. Functional Ecology 4: 283-288.

Ciborowski, J. J. H., and H. F. Clifford. 1983. Life histories, microdistribution and drift of two mayfly (Ephemeroptera) species in the Pembina River, Alberta, Canada. Holarctic Ecology 6: 3-10.

Clifford, H. F. 1970. Analysis of a northern mayfly (Ephemeroptera) population, with reference to allometry of size. Canadian Journal of Zoology 48: 305-316.

Clifford, H. F., and H. Boerger. 1974. Fecundity of mayflies (Ephemeroptera), with special reference to mayflies of a brown-water stream of Alberta, Canada. The Canadian Entomologist 106: 1111-1119.

Clifford, H. F., H. Hamilton, and B. R. Killins. 1979. Biology of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera: Leptophlebiidae). Canadian Journal of Zoology 57: 1026-1045.

Cowgill, U. M. 1987. Critical analysis of factors affecting the sensitivity of zooplankton and the reproducibility if toxicity results. Water Research 21: 1453-1462.

Dahlgren, J. 1988. Variation in diet composition: a hazard to the reliability of the LD_{50}-test. Environmental Pollution 49: 177-181.

Diamond, J. M., E. L. Winchester, D. G. Mackler, and D. Gruber. 1992. Use of the mayfly *Stenonema modestum* (Heptageniidae) in subacute toxicity assessments. Environmental Toxicology and Chemistry 11: 415-425.

Dixon, D. G., and J. W. Hilton. 1985. Effects of available dietary carbohydrates and water temperature on the chronic toxicity of waterborne copper to rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 42: 1007-1013.

Dubé, M. G., and J. M. Culp. 1997a. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Water Science and Technology 35: 339-345.

Dubé, M. G., and J. M. Culp. 1997b. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Environmental Toxicology and Chemistry 15: 2019-2027.

Dubé, M. G., J. M. Culp, and G. J. Scrimgeour. 1997. Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent. Canadian Journal of Fisheries and Aquatic Sciences 54: 2584-2595.

Edmunds, G. F., and R. D. Waltz. 1978. Ephemeroptera. Pages 126-163 in R. W. Merritt and K. W. Cummins, eds. *An Introduction to the aquatic insects of North America*. Kendall/Hunt, Dubuque, Iowa.

Edmunds Jr., G. F., S. L. Jensen, and L. Berner. 1976. *The mayflies of North and Central America*. University of Minnesota Press, Minneapolis, MN.

Elliott, J. M. 1972. Effect of temperature on the time of hatching in *Baetis rhodani* (Ephemeroptera: Baëtidae). Oecologia (Berl.) 9: 47-51.

Environment Canada. 1997. Toxicology Expert Working Group Final Report: recommendations from Cycle 1 review.

Farkas, S., E. Hornung, and E. Fischer. 1996. Toxicity of copper to *Porcellio scaber* Latr. (Isopoda) under different nutrition status. Bulletin of Environmental Contamination and Toxicology 57: 582-588.

Fiance, S. B. 1978. Effects of pH on the biology and distribution of *Ephemerella funeralis* (Ephemeroptera). Oikos 31: 332-339.

Gerhardt, A. 1990. Effects of subacute doses of cadmium on pH-stressed *Leptophlebia marginata* (L.) and *Baetis rhodani* Pictet (Insecta: Ephemeroptera). Environmental Pollution 67: 29-42.

Golder Associates. 1996. Environmental effects monitoring Cycle I final report for Weldwood of Canada Limited, Calgary, AB.

Government of Canada. 1991. Priority Substances List Assessment Report No. 2: effluents from pulp mills using bleaching.

Gray, L. J., and J. V. Ward. 1979. Food habits of stream benthos at sites of differing food availability. The American Midland Naturalist 102: 156-167.

Green, D. W. J., K. A. Williams, and D. Pascoe. 1986. The acute and chronic toxicity of cadmium to different life history stages of the freshwater crustacean *Asellus aquaticus* (L.). Archives of Environmental Contamination and Toxicology 15: 465-471.

Hall, T. J., R. K. Haley, and L. E. LaFleur. 1991. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental channels. Environmental Toxicology and Chemistry 10: 1051-1060.

Hart, R. C., and B. Santer. 1994. Nutritional suitability of some uni-algal diets for freshwater calanoids: unexpected inadequacies of commonly used edible greens and others. Freshwater Biology 31: 109-116.

Hatakeyama, S. 1989. Effect of copper and zinc on the growth and emergence of *Epeorus latifolium* (Ephemeroptera) in an indoor model stream. Hydrobiologia 174: 17-27.

Hatfield Consultants Ltd. 1996a. Fraser River Environmental Effects Monitoring (EEM) Cycle One Interpretive Report. Hatfield Consultants Ltd., West Vancouver.

Hatfield Consultants Ltd. 1996b. Weyerhauser Canada Ltd. Environmental Effects Monitoring (EEM) Cycle One Interpretive Report. Hatfield Consultants Ltd., West Vancouver.

Hickie, B. E. 1989. Nutritional and feeding considerations in toxicity testing with early life stages (ELS) of fish. Pages 16-22 *In* R. P. Lanno, ed. *Nutritional considerations in toxicity testing.* Short course presented at SETAC Tenth Annual Meeting, Toronto, Ontario.

Hickie, B. E., and D. G. Dixon. 1987. The influence of diet and preexposure on the tolerance of sodium pentachlorophenate by rainbow trout *(Salmo gairdneri).* Aquatic Toxicology 9: 343-353.

Hickie, B. E., D. G. Dixon, and J. F. Leatherland. 1989. The influence of the dietary carbohydrate:lipid ratio on the chronic toxicity of sodium pentachlorophenate to rainbow trout. Fish Physiology and Biochemistry 6: 175-185.

Himbeault, K. T. 1995. Alternative approaches for monitoring aquatic ecosystems: a study on the effects of municipal sewage effluent on biota in the South Saskatchewan River. M.Sc. Thesis Department of Toxicology. University of Saskatchewan, Saskatoon. 144p.

Jackson, J. K., and B. W. Sweeney. 1995. Egg and larval development times for 35 species of tropical stream insects from Costa Rica. Journal of the North American Benthological Society 14: 115-130.

Klok, C., and A. M. de Roos. 1996. Population level consequences of toxicological influences on individual growth and reproduction in *Lumbriculus rubellus* (Lumbriculidae, Oligochaeta). Ecotoxicology and Environmental Safety 33: 118-127.

Knight, J. T., and W. T. Waller. 1992. Influence of the addition of Cerophyl® on the *Selenastrum capricornutum* diet of the cladoceran *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry 11: 521-534.

Kungolos, A., and I. Aoyama. 1993. Interaction effect, food effect, and bioaccumulation of cadmium and chromium for the system *Daphnia magna* - *Chlorella ellipsoidea*. Environmental Toxicology and Water Quality 8: 351-369.

Larsen, R. 1978. Fødens kvalitative betydning for veksten til *Baetis rhodani* Pikt. (Ephemeroptera). Norwegian Journal of Entomology 25: 106-108.

Lehmkuhl, D. M. 1973. A new species of *Baetis* (Ephemeroptera) from ponds in the Canadian arctic, with biological notes. The Canadian Entomologist 105.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995a. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environmental Toxicology and Chemistry 14: 1529-1541.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995b. Toxicity testing with artificial streams: effects of differences in current velocity. Environmental Toxicology and Chemistry 14: 1209-1217.

Maltby, L., C. G. Naylor, and P. Calow. 1990. Effect of stress on a freshwater benthic detritivore: scope for growth in *Gammarus pulex*. Ecotoxicology and Environmental Safety 19: 285-291.

Matthews, K. A., and D. C. Tarter. 1989. Ecological life history, including laboratory respiratory investigation of the mayfly, *Ameletus tarteri* (Ephemeroptera: Siphloneuridae). Psyche 96: 21-37.

Mayer Jr., F. L., and M. R. Ellersieck. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. United States Department of the Interior, Fish and Wildlife Service, Washington, DC.

McKim, J. M. 1995. Early life stage toxicity tests, Appendix B. Pages 974-1011 in G. M. Rand, ed. *Fundamentals of aquatic toxicology*. Taylor & Francis, Washington, D.C.

McKinney, A. D., and D. C. Wade. 1996. Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecilis* to pulp and paper mill effluents discharged to the Tennessee River and its tributaries. Environmental Toxicology and Chemistry 15: 514-517.

McLeay, D.J. and D.A. Brown. 1979. Stress and chronic effects of untreated and treated bleached kraft pulp mill effluent on the biochemistry and stamina of juvenile coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 36:1049-1059.

Mihuc, T. B., and G. W. Minshall. 1995. Trophic generalists vs. trophic specialists: implications for food web dynamics in post-fire streams. Ecology 76: 2361-2372.

Mohamed, M. M. 1997. Phosphorus limitation of sestonic and sessile bacteria in the Fraser River and the effect of pulp mill effluent. M.Sc. Thesis, Department of Applied Microbiology and Food Science. University of Saskatchewan, Saskatoon. 121p.

Moore, J.E., and R.J. Love. 1977. Effect of a pulp and paper mill effluent on the productivity of periphyton and phytoplankton. Journal of the Fisheries Research Board of Canada 34: 856-862.

Muttkowski, R. A., and G. M. Smith. 1929. The food of trout stream insects in Yellowstone National Park. Roosevelt Wild Life Annals 2: 241-263.

NCASI. 1978. A study of the fate of biosolids from biologically treated effluent in laboratory and constructed streams, Research Triangle Park, NC.

Naylor, C., L. Maltby, and P. Calow. 1989. Scope for growth in *Gammarus pulex*, a freshwater benthic detritivore. Hydrobiologia 188/189: 517-523.

Norris, R. H., and A. Georges. 1993. Analysis and interpretation of benthic macroinvertebrate surveys. Pages 234-286 *In* D. M. Rosenberg and V. H. Resh, eds. *Freshwater biomonitoring and benthic macroinvertebrates*. Chapman and Hall, New York.

Oseid, D. M., and L. L. Smith Jr. 1974. Factors influencing acute toxicity estimates of hydrogen sulfide to freshwater invertebrates. Water Research 8: 739-746.

Petersen, R C., and L.B.-M. Petersen. 1988. Compensatory mortality in aquatic populations: its importance for interpretation of toxicant effects. Ambio 17: 381-386.

Piorreck, M., K. Baasch, and P. Pohl. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. 23: 207-216.

Podemski, C. L., and J. M. Culp. 1996. Nutrient and contaminant effects of bleached kraft mill effluent on benthic algae and insects of the Athabasca river. Pages 571-580 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van

Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie press, Delray Beach, Florida.

Rader, R. B., and J. V. Ward. 1989. Influence of impoundments on mayfly diets, life histories, and production. Journal of the North American Benthological Society 8: 64-73.

Rand, G. M., P. G. Wells, and L. S. McCarty. 1995. Introduction to aquatic toxicology. Pages 3-66 *In* G. M. Rand, ed. *Fundamentals of aquatic toxicology.* Taylor & Frances, Washington, D.C.

Scrimgeour, G. J. 1992. Foraging patch selection by the lotic mayfly *Baetis tricaudatus.* Ph.D. Thesis, Department of Biological Sciences. University of Calgary, Calgary, AB.

Scrimgeour, G. J., J. M. Culp, M. L. Bothwell, F. J. Wrona, and M. H. McKee. 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly-diatom systems. Oecologia (Berlin) 85: 343-348.

Scullion, J. and R.W. Edwards.1980. The effects of coal industry pollutants on the macroinvertebrate fauna of a small river in the South Wales coalfield. Freshwater Biology 10:141-162.

Sibley, R. M. 1996. Effects of pollutants on individual life histories and population growth rates. Pages 197-223 In M. C. Newman and C. H. Jagoe, eds. *Ecotoxicology: a hierarchical treatment.* Lewis Publishers, Boca Raton, FL.

Sibley, R. M., and P. Calow. 1989. A life-cycle theory of responses to stress. Biological Journal of the Linnean Society 37: 101-116.

Sicko-Goad, L., J. Hall, D. Lazinsky, and M. S. Simmons. 1989a. Effects of chlorinated benzenes on diatom fatty acids composition and quantitative morphology. III. 1,2,3-trichlorobenzene. Archives of Environmental Contamination and Toxicology 18: 647-655.

Sicko-Goad, L., M. S. Evans, D. Lazinsky, J. Hall, and M. S. Simmons. 1989b. Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. IV. Pentachlorobenzene and comparison with trichlorobenzene isomers. Archives of Environmental Contamination and Toxicology 18: 656-668.

Sivaramakrishnan, K. G., and K. Venkataraman. 1987. Observations on feeding propensities, growth rate and fecundity in mayflies (Insecta: Ephemeroptera). Proceedings of the Indian Academy of Science (Animal Science) 96: 305-309.

Söderström, O. 1991. Effects of temperature and food quality on life-history parameters in *Parameletus chelifer* and *P. minor* (Ephemeroptera): a laboratory study. Freshwater Biology 20: 295-303.

Solomon, K., H. Bergman, R. Hugget, D. Mackay, and B. McKague. 1993. A review and assessment of the ecological risks associated with the use of chlorine dioxide for the bleaching of pulp.

Soto, C., J. A. Hellebust, and T. C. Hutchinson. 1977. Effect of naphthalene and aqueous crude oil extracts on the green flagellate *Chlamydomonas angulosa.* III. Changes in cellular composition. Canadian Journal of Botany 55: 2765-2777.

Southam, C. M., and J. Ehrlich. 1943. Effects of extract of western red-cedar heartwood on certain wood-decaying fungi in culture. Phytopathology 33: 517-524.

Suntio, L. R., W. Y. Shiu, and D. Mackay. 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere 17: 1249-1290.

Sweeney, B. W., and R. L. Vannote. 1984. Influence of food quality and temperature on life history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer.* Freshwater Biology 14: 621-630.

Sweeney, B. W., D. H. Funk, and L. J. Standley. 1993. Use of the stream mayfly *Cloeon triangulifer* as a bioassay organism: life history response and body burden following exposure to technical chlordane. Environmental Toxicology and Chemistry 12: 115-125.

Sweeney, B. W., R. L. Vannote, and P. J. Dodds. 1986. Effects of temperature and food quality on growth and development of a mayfly, *Leptophlebia intermedia.* Canadian Journal of Fisheries and Aquatic Sciences 43: 12-18.

Thompson, P., and P. Couture. 1991. Aspects of carbon metabolism in the recovery of Selenastrum capricornutum population exposed to cadmium. Aquatic Toxicology 17: 1-14.

van Leeuwen, C. J., M. Rijkeboer, and N. G. 1986. Population dynamics of *Daphnia magna* as modified by chronic bromide stress. Hydrobiologia 133: 277-285.

Walde, S. J., and R. W. Davies. 1984. The effect of intraspecific interference on *Kogotus nonus* (Plecoptera) foraging behaviour. Canadian Journal of Zoology 62: 2221-2226.

Werneke, U., and P. Zwick. 1992. Mortality of the terrestrial adult and aquatic nymphal life stages of *Baetis vernus* and *Baetis rhodani* in the Breitenbach, Germany (Insecta: Ephemeroptera). Freshwater Biology 28: 249-255.

Wicklum, D., and R. W. Davies. 1996. The effects of chronic cadmium stress on energy acquisition and allocation in a freshwater benthic invertebrate predator. Aquatic Toxicology 35: 237-252.

Wiederholm, T. 1984. Responses of aquatic insects to environmental pollution. Pages 508-557 *In* V. H. Resh and D. M. Rosenberg, eds. *The ecology of aquatic insects*. Praeger Publishers, New York.

Zanella, E., J. Conkey, and M. Tesmer. 1978. Nonsettleable solids. Characteristics and use by two aquatic food chain organisms. Tappi 61: 61-65.

Zloty, J. 1996. A revision of the nearctic *Ameletus* mayflies based on adult males, with descriptions of seven new species (Ephemeroptera: Ameletidae). The Canadian Entomologist 128: 293-346.

Chapter 7
Synthesis and New Horizons

7.1 Introduction

My research focused on disentangling the influence of nutrients and contaminants in a bleached kraft pulp mill effluent (BKME) and determining which of these components contributed most to the impact of this effluent discharge on benthic producers and consumers of the upper Athabasca River at Hinton, Alberta. Enrichment effects of pulp mill effluent have, until recently, been largely overlooked in the pursuit of measures of toxicity. However, nutrient addition can have significant and far-reaching influences on an ecosystem, and should not be ignored simply because responses may be stimulatory rather than inhibitory. Effects of BKME on benthic insects and periphyton of the study river were assessed by using an integrated approach which linked a field experiment, field observations, and behavioral and chronic laboratory bioassays. This approach produced a more complete understanding of the effects of pulp mill effluent on benthic biota than has been gained by more traditional means. Conclusions of this research have been dealt with in the preceding chapters, therefore the following discussion summarizes the various studies and places them in the perspective of what might occur in the Athabasca River.

7.2 Effects of BKME on Benthic Primary Producers

Primary producers showed growth stimulation in response to effluent additions in field and laboratory experiments. This conclusion is, of course, restricted to the effluent concentrations tested as growth inhibition, either through toxicity or colour-related light reduction, could occur at higher concentrations. In the field experiment, periphyton biomass responded quickly

to a 1% effluent treatment, achieving substantially greater biomass than controls by the end of the 28 d experiment. This result was clearly due to nutrient enrichment by the effluent, as the responses of the effluent and nutrient treatments were remarkably similar. In contrast, effluent-stimulation of *Navicula* growth in the laboratory was not likely to have been a nutrient response. The culture media used and the frequency of renewal would have made nutrient limitation highly unlikely. Possible explanations for this laboratory observation include the presence in effluent of unidentified micronutrients, algal utilization of dissolved organic carbon, and stimulation by low levels of toxicants in the effluent. Laboratories conducting toxicity testing with algae may add nutrients to test media in an attempt to remove "interference" by nutrients. My results suggest that, at least for some algae, nitrogen and phosphorus in effluent may not be solely responsible for increased growth.

Diatom community composition is extensively used as an indicator of water quality, and my research demonstrates that it responds to exposure to pulp mill effluent. Exposure to effluents reduced species richness of diatoms and the relative abundance of a few, numerically dominant species changed. Specifically, the relative abundance of *Achnanthes minutissima* decreased while that of *Diatoma tenuis*, *Fragilaria vaucheriae*, and *Cymbella silesiaca* increased in response to BKME-exposure. Nutrient additions caused similar changes in community composition, confirming that nutrients in the effluent were responsible for observed changes. Changes in community composition in the artificial stream study resembled differences between communities in the Athabasca, upstream and downstream of the discharge. Periphyton community composition also showed similar changes when exposed to pulp mill effluent in an artificial stream experiment on the Fraser River (Cash and Culp 1996). Thus, it appears that diatom community composition would be a valuable and inexpensive addition to biomonitoring programs in these rivers. Results of the present studies can also offer guidance for future studies of diatom response to BKME. First, results suggest that artificial stream experiments should be at least 6 wk. in duration. Some differences were noted in the response of diatom

community composition in the artificial stream experiment and the Athabasca River. *Diatoma tenuis* became numerically dominant in treatment streams, but was the dominant species only at the downstream site with the least exposure to effluent, and *Fragilaria vaucheriae* exhibited less of a response to effluent in the artificial streams than in the river. I hypothesize that these differences occurred because 28 d was insufficient for communities in the artificial streams to reach equilibrium. This conclusion was reached because, when the duration of the experiment was increased from 28 to 42 days, these differences did not occur (Podemski and Culp unpublished data). Second, artificial substrates, while beneficial for obtaining reliable data for biomass measures, may not be as useful for examining community responses. In a second artificial stream experiment at the same location, a change in diatom community composition was not observed in response to nutrient additions (Chambers 1996). That study used artificial substrates rather than a transplanted periphytic community. Similarly, in a second, 42 d run of my artificial stream experiment, BKME-induced community composition shifts observed on natural substrates (i.e., rocks) did not occur on artificial substrates (Podemski and Culp unpublished data). Thus, it appears that short-term colonization processes override BKME effects on tiles, while responses of transplanted periphyton appear to be the result of shifts in relative abundance of established taxa.

The key ecological effect of BKME-induced increases in primary production has been assumed to be more abundant food for higher trophic levels (Dubé et al. 1997). However, this research indicates that an additional complication may be a change in food quality. While looking for feeding-deterrent activity of effluent, an unexpected finding was the discovery that the mayfly *Ameletus subnotatus* showed a consistent preference for feeding on diatoms cultured in bleached kraft mill effluent. This result, combined with results of other feeding trials and analysis of the chemical composition of the diatoms, suggested that exposure to effluent increased lipid and bacterial content and, thus, quality of the algae as food for mayflies. Increased food quality may explain observations of increased growth in situations where

insects were exposed to effluent in food unlimited conditions (this research, Lowell *et al.* 1995). A change in periphyton macromolecule content has been observed in response to sewage or industrial effluents (Himbeault 1995, Guckert 1992), but has yet to be measured at a pulp mill. Mill effluent also contains particulate material that may represent high-quality food for collector-gatherers (NCASI 1978, Zanella 1978, Dubé et al. 1996,1997). In the field, altered community composition may also have increased food quality as larger, erect or filamentous (more easily grazed) diatoms became more abundant. Although an increase in "edibility" occurred in the Athabasca River, this would not be the case for all receiving environments because eutrophication can increase the relative abundance of inedible blue-green algae (Wetzel 1983, Elwood et al. 1981, Fairchild et al. 1985).

A potentially useful applied observation from this research was the strong relationship between strontium concentration of diatoms cultured in effluent and concentration of effluent to which the algae was exposed. The kraft process concentrates by 25-200 x, elements from groups 1 and 2 of the periodic table that are contained in the process water and wood (Ravila and Holm 1994). Of the elements that showed a relationship with effluent concentration (Fe, Co, Ba, Sr), the predictive relationship was strongest for strontium. Within the Environmental Effects Monitoring program for pulp mills, the concentration of resin acids (typically dehydroabietic) is used as a marker of effluent exposure. However, resins acids are not present in effluents from mills pulping hardwoods, and in many softwood effluents, resin acids are in such low concentrations after secondary treatment that they are not detectable in the receiving environment and, thus, are poor markers. The relationship between effluent concentration and strontium concentration (or other elements in groups 1 or 2) should be assessed to determine if a predictive relationship exists in the field.

7.3 Effects of BKME on Benthic Consumers

Effects of low concentrations (1-7%) of BKME on benthic insects was largely stimulatory. In the artificial stream experiment, an increase in the abundance of insects and faster growth of certain taxa occurred in streams receiving effluent (1%) additions. The response was not different from that which occurred in streams receiving a comparable nutrient addition, leading to the conclusion that the predominant effect of effluent was that of nutrient enrichment. In contrast to Lowell et al. (1995), there was no evidence of stimulation by effluent beyond that achieved by increasing food availability (i.e., nutrient enrichment). The reason may lie in the differences in the test systems used (single species versus mesocosm) because, in single species tests in streams similar to that used by Lowell et al. (1995), growth stimulation by BKME was observed in food-unlimited situations. Alternatively, the difference may lie in the effluents used; in 2 of 4 chronic *Ceriodaphnia* tests conducted as part of cycle 1 of the EEM program, a 12.5% dilution of effluent from the mill used in the Lowell *et al.* (1995) study, showed 60 and 100% stimulation of reproduction (Hatfield Consultants Ltd. 1996). No such response was observed in the chronic *Ceriodaphnia* tests conducted with Weldwood effluent (Golder Associates Ltd. 1996). Changes in community composition of benthic insects, although relatively minor, were again similar between nutrient and effluent treatments, and resembled changes that occurred in the river downstream of the mill outfall, supporting a conclusion of nutrient enrichment.

In laboratory bioassays, increased growth was observed in 2 species of mayflies exposed to BKME, even though food limitation did not occur. This result further supports the hypothesis that food quality of diatoms was improved by exposure to BKME. However, effluent also increased mortality of mayflies at concentrations where growth was still greater than that of controls. Mortality occurred at 7% BKME in mid-late instar *Baetis tricaudatus*, and at 1% BKME for *Ameletus subnotatus*. Lowell et al. (1995) did not observe any signs of toxicity at concentrations up to 10%, however, they did not pre-expose food algae to

effluent, and thus mayflies would have had less exposure to contaminants than in this study. Although mortality of *Ameletus* at 1% BKME was not evident in the field experiment, the duration of the laboratory experiment was >3 x that of the field experiment, and almost all mortality in the laboratory bioassay occurred in the last month. In what was, to my knowledge, the first use of in vitro fertilized mayfly eggs in a toxicity test, high mortality of first instar *Baetis tricaudatus* was observed at concentrations above 1%. Increased sensitivity of early life stages is well known (e.g., Powlesland and George 1686, Mayer and Ellersieck 1986), however, whether this mortality occurs in the Athabasca or whether this increased mortality would have a significant effect on population dynamics is not clear. Petersen and Petersen (1988) suggested that toxicant-induced mortality of early life stages kills individuals that would have normally been removed by density-dependent mortality. In addition, the impact at the population level of increased mortality is not known; Kammenga et al. (1996) demonstrated that a change in time to first reproduction in an aquatic nematode had a greater effect on fitness (as measured by the intrinsic rate of population increase) than increased mortality. In aquatic insects such as mayflies, alterations in generation times may have significant consequences at the population level if those changes reduce emergence synchrony (and, thus, difficulty finding mates), or result in emergence during seasons that are not conducive to survival in the aerial environment. The impact of changes in growth rates, mortality rates and fecundity on population parameters of riverine insects is clearly an area that requires further research, as is the identification of the effect of chemical stressors on these parameters. An additional, related area requiring attention is the thermal effects of pulp mill effluent on riverine biota. Effluents are generally 20-35°C when released, and may be released in sufficient volume to affect water temperatures (see Chapter 2). Small increases in water temperature can result in earlier emergence and increased separation of male and female emergence times (Nebeker 1971), and this particular aspect of effluent impacts has yet to be addressed.

Figure 7.1 depicts effects that occur along a concentration gradient of Weldwood effluent. The median SRP concentration in effluent during the 1993 experiment was 263 µg/L, and the median in-river SRP concentration upstream of the outfall was 1 µg/L. Using these data, SRP loadings from effluent can therefore increase in-river SRP by a high of 8 µg/L when the in-river effluent concentration is 3%, to lows of 0.4 µg/L during high flows. Cellular growth rate of algae in the Athabasca River saturates at SRP 2-5 µg/L (approximately 2% BKME) and peak biomass is achieved at 14 µg/L (5% BKME) (Dale and Chambers 1995). No further stimulation of periphyton biomass will occur if effluent concentration increases beyond 5%, although increased colour and toxicity may eventually occur as effluent concentrations increase. Note too, that in winter, algal growth is limited by cold temperatures and poor light penetration, while in the summer algal biomass is limited by the scouring effect of high water flows (Chambers 1996). Thus, effluent-stimulation of periphyton biomass is likely limited to the fall (September through November) and in periods of low flow in spring.

At low concentrations of effluent, increased food availability results in increased abundance and size of some invertebrates (Fig. 7.1). This enrichment effect is carried upwards through the food web; Gibbons et al. (1996) reported increased size and condition of an insectivorous fish (spoonhead sculpin) in the Athabasca downstream of the effluent discharge. At 1%, increased mortality begins to occur in young mayflies (this study), above 5% chronic growth effects begin to occur in chironomids (Dubé and Culp 1996, 1997) and at 7%, mortality of older baetid mayflies increases. Note, however, that at all of these concentrations, animals exposed to BKME still grow faster than controls. It is not known at what specific effluent concentrations the growth of these insects would be reduced with respect to controls. Lack of a growth effect while exhibiting increased mortality may indicate that mayflies do not allocate substantial energetic resources to defense and, thus, growth or reproductive effects will not occur. However, the effect, or lack thereof, of BKME-exposure on mayfly fecundity has not yet been convincingly

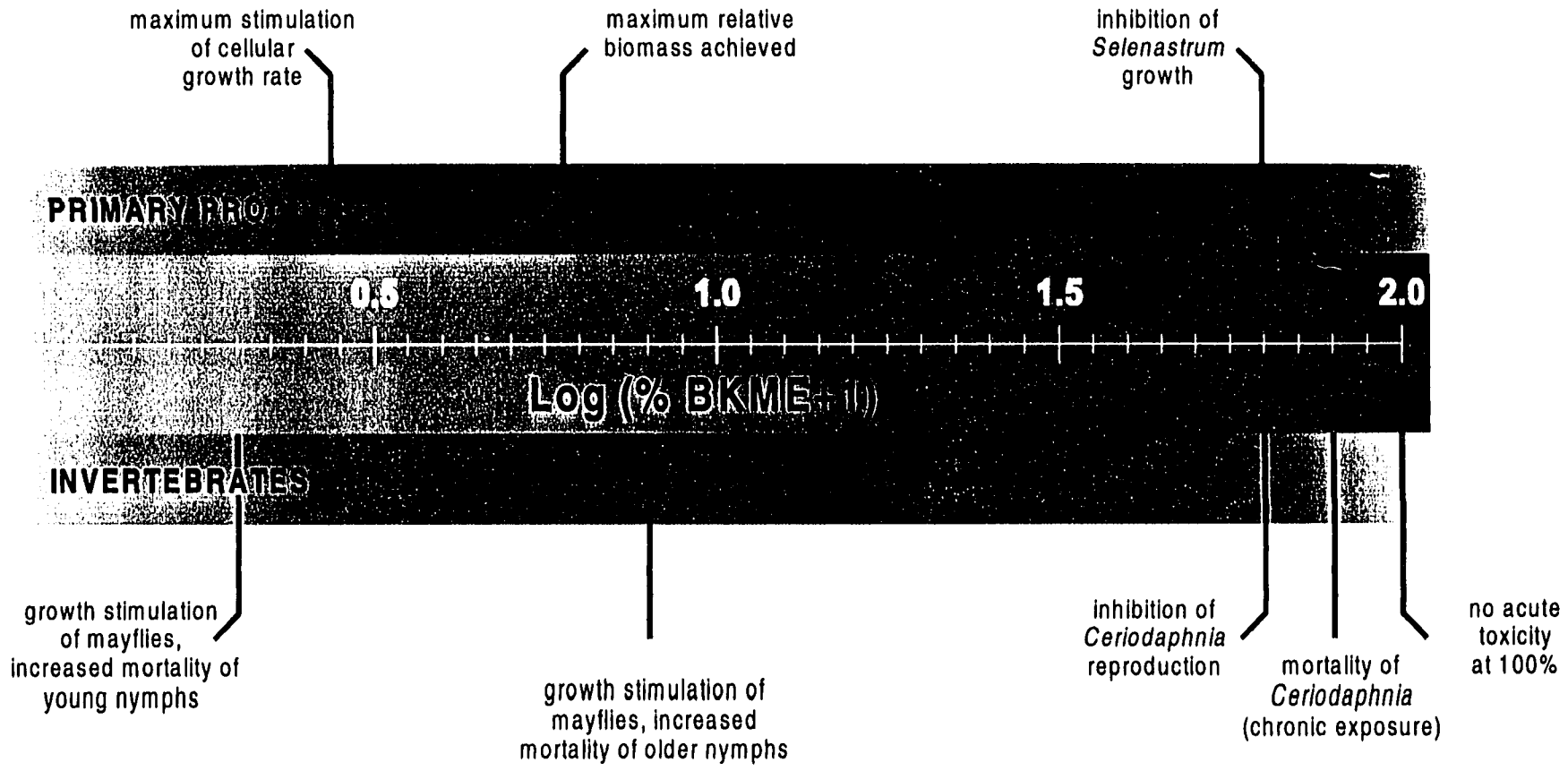


Figure 7.1. Ecological effects on primary producers and invertebrates along a bleached kraft pulp mill effluent gradient.

demonstrated. In chronic toxicity tests, inhibitory effects in *Ceriodaphnia* and *Selenastrum* appear at effluent concentrations of 65-87%, and no acute toxicity of this effluent to *Daphnia magna* or fathead minnows (*Pimephales promelas*) has been detected. Given that effluent concentrations (at complete mix) in the river range from well below 1% to 3%, it is unlikely that inhibitory effects of this effluent occur in receiving waters. These conclusions are limited to periphyton and benthic grazing insects, as biomagnification of contaminants may affect higher trophic levels.

7.4 New Horizons for Riverine Ecotoxicology

My research utilized several novel approaches to assess the effects of BKME on benthic organisms. The artificial stream system developed for this work is a significant addition to the tools available for impact assessment in river systems. Impact assessment through field sampling is often confounded by other discharges or downstream changes in the physicochemical environment. High variability and the inability to apply treatments to true replicates results in the collection of observations that cannot definitively identify the effects of a discharge. Through an experiment in the artificial stream system and concurrent field sampling, effects of effluent discharge were identified and it was shown that these effects were largely attributable to nutrients in the effluent. These accomplishments would not have been possible without the use of the artificial stream system. A mobile version of this system has since been used to assess pulp mill effluent effects on the Fraser River, B.C. (Cash and Culp 1995), and is currently being considered as a technique for the Environmental Effects Monitoring program for pulp mills (Dubé et al. 1997).

Several non-standard bioassays were conducted that may be useful approaches in ecotoxicology. Behavioral bioassays are more sensitive and produce results more quickly than chronic bioassays (Jones et al. 1991), and in the present work, they provided insight into the mechanisms through which effluent effects occur. The use of indigenous organisms rather than standard

bioassay organisms, and preexposure of food algae to toxicants are both methods through which the realism and, thus, accuracy of bioassays may be improved. When the intention of a bioassay is to predict field effects, rather than to simply measure or compare the potential toxicity of different effluents, priority should be placed on improving the realism, not precision, of such bioassays. Standard chronic bioassays currently used to monitor toxicity of effluents from Canadian pulp mills do not include food as an exposure route, nor do they use riverine species such as mayflies or stoneflies, which are sensitive to poor water quality. Chronic effects in *Ceriodaphnia*, *Selenastrum* and *Pimephales promelas* occurred at effluent concentrations ranging from 66% to >100% (Fig. 7.1), while chronic effects occurred in mayflies at lower concentrations. The development of a bioassay using an organism such as baetid mayflies would improve our ability to protect aquatic resources. These animals can be reared to emergence in the laboratory (this study, Scrimgeour 1992) and in vitro fertilization of eggs is possible. Thus, laboratory culturing, which is the first step in the development of a bioassay protocol, seems feasible. Alternatively, side-by-side testing of surrogate and indigenous species could be done to "calibrate" toxicity tests and improve our ability to set regulatory guidelines that protect the environment.

Finally, an integrated approach was used to provide weight-of-evidence, and a better understanding of effluent effects. Integrative monitoring involves the use of several measures, typically chemical analysis, experimental results and field observation, to assess an impact (Chapman 1995, 1996). More information emerges from this linking of components than simply the sum of the various individual components. Integrated studies may be particularly valuable for assessing less severe impacts (Clements and Kiffney 1994). The mesocosm experiment generated definitive cause-effect data, and linking this experiment with field observations allowed me to assign causality for downstream changes in the river to the effluent discharge. Behavioral bioassays and algal culturing helped to identify the cause of mayfly growth-stimulation by effluent, a phenomena observed in both field and laboratory.

Use of realistic laboratory bioassays, i.e., using relevant species exposed through both water and food supply, allowed the determination that BKME concentrations causing chronic effects in these species were lower than the lowest concentrations identified by regulatory testing. Overall, the combination of the varied approaches has provided a new understanding of the effects of BKME discharge on the benthic community of this and perhaps other, northern rivers.

7.5 Literature Cited

Cash, K. J., and J. M. Culp. 1996. The effects of bleached kraft pulp-mill effluent on periphyton tissue. National Hydrology Research Institute, Saskatoon, Saskatchewan.

Chambers P.A. 1996. Nutrient enrichment in the Peace, Athabasca, and Slave rivers: assessment of present conditions and future trends. Northern River Basins Study Synthesis Report No. 4, Edmonton, Alberta.

Chapman, P.M. 1995. Do sediment toxicity tests require field validation? Environmental Toxicology and Chemistry 14: 1451-1453.

Chapman, P.M. 1996. Recent perspectives in integrated monitoring. Pages 651-657 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Clements, W.H., and P.M. Kiffney. 1994. Integrated laboratory and field approach for assessing impacts of heavy metals at the Arkansas River, Colorado. Environmental Toxicology and Chemistry 13: 397-404.

Dale, A.R., and P.A. Chambers. 1995. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, seasonal variation. Northern River Basins Study Report No. 68, Edmonton, Alberta.

Dubé M.G., J.M. Culp and J.A. Smith. 1998. Development of a mesocosm technology for environmental effects monitoring methods application Proceedings, 3rd International Conference on Environmental Fate and Effects of Pulp and Paper Mill Effluents. Rotorua, New Zealand.

Dubé, M.G., and J.M. Culp. 1996. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Environmental Toxicology and Chemistry 15: 2019-2027.

Dubé, M.G., and J.M. Culp. 1997. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Water Science and Technology 35: 339-345.

Dubé, M.G., J.M. Culp, and G.J. Scrimgeour. 1997. Nutrient limitation and herbivory: processes influenced by bleached kraft pulp mill effluent. Canadian Journal of Fisheries and Aquatic Sciences 54: 2584-2595.

Elwood, W. J., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62: 146-158.

Fairchild, G. W., R. L. Lowe, and W. B. Richardson. 1985. Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. Ecology 66: 465-472.

Gibbons W., K. Munkittrick and W. Taylor. 1996. Suitability of small fish species for monitoring the effects of pulp mill effluent on fish populations of the Athabasca River 1994 and 1995. Project Report No. 100, Northern River Basins Study, Edmonton, AB.

Golder Associates Ltd. 1996. Environmental Effects Monitoring Cycle 1 Final Report, Weldwood of Canada Limited.

Guckert, J. B., S. C. Nold, H. L. Boston, and D. C. White. 1992. Periphyton response in an industrial receiving stream: lipid-based physiological stress analysis and pattern recognition of microbial community structure. Canadian Journal of Fisheries and Aquatic Sciences 49: 2579-2587.

Hatfield Consultants Ltd. 1996. Weyerhauser Canada Ltd. Environmental Effects Monitoring (EEM) Cycle One Interpretive Report. Hatfield Consultants Ltd., West Vancouver.

Himbeault, K. T. 1995. Alternative approaches for monitoring aquatic ecosystems: a study on the effects of municipal sewage effluent on biota in the South Saskatchewan River. Pages 144. *Department of Toxicology*. University of Saskatchewan, Saskatoon.

Jones, M., C. Folt, and S. Guarda. 1991. Characterizing individual, population and community effects of sublethal levels of aquatic toxicants: an experimental case study using *Daphnia*. Freshwater Biology 26: 35-44.

Kammenga, J.E., M. Busschers, N.M. Van Straalen, P.C. Jepson, and J. Bakker. 1996. Stress induced fitness reduction is not determined by the most sensitive life-cycle trait. Functional Ecology 10: 106-111.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995. Toxicity testing with artificial streams: effects of differences in current velocity. Environmental Toxicology and Chemistry 14: 1209-1217.

Lowell, R.B., J.M. Culp, and F.J. Wrona. 1994. Stimulation of increased short-term growth and development of the mayfly *Baetis tricaudatus* from the

Thompson River basin following exposure to biologically treated pulp mill effluent. National Hydrology Research Institute, Saskatoon, SK.

Mayer, F.L., and M.R. Ellersieck. 1986. Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. United States Department of the Interior, Fish and Wildlife Service, Washington, DC.

NCASI. 1978. A study of the fate of biosolids from biologically treated effluent in laboratory and constructed streams. National Council of the Paper Industry for Air and Stream Improvement, Inc., Research Triangle Park, NC.

Nebeker, A.V. 1971. Effect of high winter water temperatures on adult emergence of aquatic insects. Water Research 5: 777-783.

Petersen, R C., and L.B.-M. Petersen. 1988. Compensatory mortality in aquatic populations: its importance for interpretation of toxicant effects. Ambio 17: 381-386.

Powlesland, C., and J. George. 1986. Acute and chronic toxicity of nickel to larvae of *Chironomus riparis* (Meigen). Environmental Pollution Series A 42: 47-64.

Ravila, A., and E. Holm. 1994. Radioactive elements in the forest industry. The Science of the Total Environment 157: 339-356.

Scrimgeour, G. J. 1992. Foraging patch selection by the lotic mayfly *Baetis tricaudatus*. Pages 202. *Department of Biological Sciences*. University of Calgary, Calgary, AB.

Wetzel, R. G. 1983. *Limnology*. CBS College Publishing, New York.

Zanella, E., J. Conkey, and M. Tesmer. 1978. Nonsettleable solids. Characteristics and use by two aquatic food chain organisms. Tappi 61: 61-65.

Appendix 1

A1. Pulp Mills, Pulp Mill Effluent, and Methods for Assessing the Impacts of this Industry on the Aquatic Environment.

The purpose of this Appendix is to provide background information for readers not familiar with pulp mills, effluent composition, and/or methods for assessing the impacts of effluent discharge. The chapter is organized into 4 sections, each of which may be read independently of the others. Section A1.1 briefly describes the various methods that are used to assess the environmental effects of pulp mill effluents. Section A1.2 provides a brief literature review of effluent effects in the freshwater environment. Section A1.3 describes the workings of a pulp mill and identifies process contributors to the effluent stream; this knowledge is fundamental to an appreciation of effluent composition and variability. The final section (A1.4) discusses effluent composition and, briefly, the environmental fate and effects of selected effluent components.

A1.1 Current techniques for Assessing the Environmental Effects of Pulping Effluents

A variety of methods have been used to predict or assess the effects of pulp mill effluent on the aquatic environment. The most commonly employed methods are chemical evaluation of effluents, single-species toxicity testing, and field surveys of fish and/or invertebrate communities in the receiving environment. Each method produces a different type of information, and has advantages and disadvantages particular to it.

A1.1.1 Chemical analysis of effluents

Analysis of effluent composition is largely done as part of the regulatory process. In Canada, mill licenses are generally under the jurisdiction of provincial governments, and therefore monitoring requirements may vary from province to province. Mill effluents may be monitored for a variety of parameters including biological oxygen demand (BOD), chemical oxygen demand (COD), quantities of suspended solids, forms of nitrogen and

phosphorus, pH, some metals, chlorinated phenolics, resin and fatty acids, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated dioxins and furans (PCDD and PCDF). The frequency of measurement varies from weekly to yearly, depending upon the parameter and the mill. A knowledge of effluent composition is of limited use for predicting environmental effects because: (1) this method relies upon knowledge of the environmental effects of various compounds, which does not exist for many effluent components; (2) most chemical analyses measure only a target suite of compounds rather than providing a complete analysis of the effluent, thus, the effects of unidentified compounds cannot be considered; and (3) it is very difficult to predict the toxicity of mixtures of toxicants without knowledge of the modes of action and interactions between the various compounds (Suter 1993). This is knowledge that at the current time we simply do not have.

A1.1.2 Laboratory bioassays

The standard bioassays used to assess toxicity of effluents from Canadian mills include acute bioassays using rainbow trout (*Oncorhynchus mykiss*) and the cladoceran *Daphnia magna*; and chronic bioassays using the green alga *Selenastrum capricornutum*, fathead minnows (*Pimephales promelas*), and *Ceriodaphnia dubia*. In Alberta, mills are required to conduct acute trout bioassays monthly, acute *Daphnia* bioassays weekly, and to sample benthic communities in receiving environments at varying intervals (SENTAR Consultants Ltd. 1997). The use of chronic tests has recently been added as a part of the new federal Environmental Effects Monitoring program (Environment Canada 1992), and is required quarterly in 1 of every 3 years.

Single-species toxicity testing has the advantage of providing quick, cost-effective results that are reproducible and, because bioassays are done using standardized methods and species, the results are comparable between mills and other toxicants (Kimball and Levin 1985). There are, however, significant drawbacks to single-species toxicity testing. While the strictly standardized and

controlled conditions reduce variability, an additional consequence is that these tests take place under biotic and abiotic conditions that are far removed from field conditions. Laboratory bioassays fail to include toxicant effects on inter- and intraspecific interactions, and do not assess toxicant effects under ambient physicochemical conditions, resulting in some question as to the relevance of results. In many cases the test species used for bioassays do not occur in the receiving waters. It is assumed that these surrogate species are more sensitive, and, thus, no observable effect concentrations (NOEC) generated from laboratory bioassays will protect the natural community. However, species used for toxicity testing are chosen not only for their believed sensitivity, but largely for their amenability to lab rearing (Cairns et al. 1992). Clearly, the requirement to be lab cultured will rule out more sensitive species. Thus, standard bioassay organisms may underestimate toxicity and as a result fail to protect more sensitive species in the receiving environment (LaPoint et al. 1996). For example, McKinney and Wade (1996) reported that the NOEC for *Anodonta imbecilis* exposed to a pulp and paper mill effluent averaged 38.36% of the NOEC calculated from 7d *Ceriodaphnia* bioassays. The appropriateness of surrogate species is particularly a concern for riverine environments, as there is currently no standard test protocol using an organism typical of flowing water. These shortcomings give rise to the biggest criticism of single-species toxicity testing - that it does not and cannot accurately predict real-world effects (Crow and Taub 1979, Kimball and Levin 1985, Cairns 1983).

A1.1.3Field surveys

Surveying of receiving environments to detect effluent effects is a part of routine monitoring programs; this is also the most common experimental design in published studies of effluent effects on benthic invertebrates. Types of data collected include concentrations of specific compounds in water, sediment, and/or tissues; fish abundance and community composition; various measurements of fish age, size or condition; biomarkers such mixed function

oxygenases; invertebrate community structure and biomass; and algal biomass measured either as chlorophyll *a* or ash free dry mass. Field surveys generally take more time to complete and are more expensive than laboratory bioassays. The data collected are more realistic than laboratory bioassays, but at the same time tend to suffer from high variance due to the spatial and temporal variability typically encountered in field situations, making detection of effects less likely. Field surveys also tend to suffer from pseudoreplication, which makes inferential hypothesis testing impossible without use of complex experimental designs such as BACI (Green 1979, Stewart-Oaten et al. 1986, Stewart-Oaten et al. 1992) which are limited to situations in which some preimpact data exist.

A1.1.4Field experiments and mesocosms

A third approach to assessing effluent effects is the use of *in situ* experiments, and microcosms/mesocosms. These methods provide an intermediary between the high precision-low realism of single-species bioassays, and the realistic but highly variable data from field surveys. Experimental investigations can cover a broad range of complexity, from large artificial stream channels (e.g., NCASI's experimental stream system, Hall et al. 1991) to smaller systems containing simplified food webs, food chains, or single species (e.g., caged fish or mussel studies). A wide range of responses can be measured, including variables normally measured in both lab bioassays and field surveys. An experimental approach allows for the collection of data that are not compromised by pseudoreplication, and allows for some control of confounding variables. The degree of precision and realism obtained in these investigations will depend upon the complexity of the system, the degree of control over environmental conditions, and the number of replicates. Experimental investigations still tend to be restricted to basic research rather than monitoring or impact assessment, as they are perceived to be prohibitively expensive and/or too complex for routine monitoring.

A1.1.5Integrated approaches to impact assessment

Single-species toxicity testing, chemical analysis of effluents, field surveys, and experimental investigations all provide useful data, yet each approach has it's limitations and shortcomings, and none in isolation will completely reveal the effects of an effluent discharge. More recently, it has been suggested that the strongest evidence for effluent-related effects could be obtained through the use of an integrated approach (Hall and Miner 1997). Hall (1996) has applied the term "integrated" to studies in which laboratory bioassays are linked with measures of effects in receiving waters, as a form of validation of laboratory results. One example of an integrated approach is Chapman's (1990) Sediment Quality Triad. In the triad, chemical analysis of contaminated sediments is linked with laboratory bioassays using the sediment and an examination of field effects. Chapman (1996) requires three components in order for a study to be considered to have been integrative: chemical analysis, experimentation, and observation (field monitoring). Integrated approaches, regardless of definition followed, result in a more comprehensive understanding and provide greater weight-of-evidence for assessing effluent effects.

A1.2 Environmental Effects of Pulp Mill Effluent

A literature review of the environmental effects of pulping effluents is necessarily complicated by the fact that regular improvements occur in cooking methods, pulp washing and bleaching techniques, as well as effluent treatment. A consequence of these changes on our scientific understanding of environmental effects is that older published works are of limited relevance today. As pulping technology has changed, so have the problems associated with effluent discharge; pulping effluents today are without a doubt less toxic than those discharged a couple of decades ago. However, the net impact of the industry may indeed be greater due to the increased loading to receiving waters as a result of more mills operating. It is also important to recognize that the furnish, pulping process, bleaching, and effluent treatment must be similar for

any data regarding environmental effects to be comparable. These details have an unfortunate tendency to be absent in many primary literature publications, and this absence lessens the usefulness of such investigations.

Prior to the mid-1960's, most mills released untreated effluent, and the impact of these discharges was associated with high BOD loading, high colour and large quantities of settleable organic materials. Reported effects included the presence of large quantities of settled wood fibres, bark and wood pieces; bacterial slimes ("sewage fungus") coating the substratum; a decrease in dissolved oxygen; and fish kills and a severely affected benthic community (Whitney and Spindler 1959, Hynes 1971, NCASI 1989a,b,c). Mercury contamination was also a concern due to the use of phenyl-mercury acetate as a fungicide, a practice that was discontinued in Canada in the late 1960's (McCubbin and Folke 1993). A few Canadian mills still release effluent that has received only primary treatment; the effects of these effluents are usually associated with low dissolved oxygen concentrations, organic enrichment and deposition of solids (e.g., Robinson et al. 1994). A benthic invertebrate community dominated by pollution tolerant taxa, such as tubificid oligochaetes and chironomids, are common observation in the receiving waters of these mills (Vander Wal 1977, Hilton 1980). In more modern pulping operations, improved fibre recovery during pulp washing and the addition of secondary treatment have greatly reduced these problems.

In the 1970's, research into effluent impacts emphasized toxicity, largely attributed to resin acids in effluent (e.g., Leach et al. 1975, Leach and Thakore 1976, McKague et al. 1977, Holbom and Lehiten 1980). During the 1970's, most mills in the United States added secondary (biological) treatment systems which effectively reduce both BOD and many toxicity related problems (see section A1.3). At present, most but not all mills in Canada utilize secondary treatment. Mills with properly operating biological treatment systems generally do not produce acutely toxic effluent. In the late 1970-80's, the focus of research shifted to the effects of chlorinated organic materials, usually

measured as adsorbable organic halogens (AOX) in effluent. Research during the 1980's focused on the biomagnification and effects of chlorinated organics on aquatic life, and on developing analytical techniques and learning more about AOX formation, composition, and removal in effluent treatment systems. AOX was the subject of regulation in many countries (Cook 1990), and today, the movement towards both elemental chlorine free bleaching (ECF) and totally chlorine free bleaching (TCF) has significantly reduced or eliminated AOX loading to receiving waters. Effluent toxicity is no longer correlated with AOX (McCubbin and Folke 1993), and the focus is now shifting towards the effects of nutrient discharge (e.g., Bothwell 1992, Scrimgeour et al. 1995, Scrimgeour and Chambers 1996, Dale and Chambers 1995 a,b, Podemski and Culp 1996) and to the potential of pulping effluents to cause reproductive dysfunction in fish (e.g., Munkittrick et al. 1992, 1994, 1996; Gagnon et al. 1994,1995; McMaster et al. 1996).

Research on the environmental effects of pulping effluents has focused on fish populations. In general, effluents from modern mills with complete or substantial chlorine dioxide substitution and biological treatment of effluent show little acute toxicity to fish (Robinson et al. 1994). Sublethal effects include induction of liver detoxification enzymes (Swanson et al. 1992, Munkittrick et al. 1994, Hewitt et al. 1996, Martel et al. 1996, Williams et al. 1996); changes in immunological function (Kennedy et al. 1996) and parasite loads (Bagge and Valtonen 1996); and decreased growth and reproduction (Munkittrick et al. 1991, 1992). Reproductive effects such as delayed maturation, decreased ovary/testicular size (Munkittrick et al. 1994), and changes in plasma stress and sex steroid levels (McMaster et al. 1991, Munkittrick et al. 1992,1994) are seen, suggesting that some as yet unidentified component(s) of effluent are disrupting hormone function in these animals.

Effects on freshwater invertebrate communities have received considerably less attention, at least in the primary literature. Scrimgeour (1989) reported that benthic communities in areas of Lake Maraetai, New Zealand, affected by

effluent were dominated by tubificid oligochaetes and chironomids. Water in affected areas was highly coloured, had visible foam rafts, high concentrations of suspended solids, and low dissolved oxygen. These are problems more typically associated with mills with only primary treatment, although the mill in question was reported to treat effluent in a system of settling and aeration ponds (Scrimgeour 1989). Several studies on riverine receiving environments have reported no association between benthic community structure and the location of mill discharges (Stone et al. 1974, Scarlett and Harris 1990, Harris et al. 1992). These studies were surveys rather than experimental investigations, and they suffer from both high variability and pseudoreplication. NCASI's (1989b) review of unpublished studies on effluent impacts on instream biota included 110 studies of riverine benthic invertebrates, representing 29 different receiving waters. At 13 of the 29 sites, at least one study reported a "negative" impact on benthic invertebrates (35 studies out of 110). These effects included decreased diversity, a shift in community composition towards more pollution-tolerant taxa, and changes (both increases and decreases) in animal abundance. However, there were some inconsistencies in what was considered an impact: some studies that were reported as showing no "negative impact" indicated that there were signs of nutrient enrichment at downstream sites, while in other cases, enrichment was considered to be a "negative impact". In general, insufficient information was presented to determine clearly what proportion of studies had demonstrated effects, which should more properly have been evaluated as differences from upstream conditions without the application of value judgments.

There have been few experimental investigations into the effects of pulp mill effluent on riverine invertebrates and periphyton. The National Council for Air and Stream Improvement (NCASI) has conducted a series of artificial stream studies (NCASI 1982, 1983, 1984, 1985, 1989a) looking at effects of biologically-treated pulp mill effluent on cold water streams; these were long term studies, ranging from 10 to 44 months in duration. They measured effects

of effluent addition on water quality, periphyton, macroinvertebrates, and trout production. While the experimental approach taken by NCASI was a substantial improvement over biological surveys of receiving environments, these studies suffered from inadequate replication, having only 2 replicates per treatment, and the results were somewhat inconsistent, making the formulation of general conclusions difficult. In general, effluent reduced periphyton growth in pools, a response attributed to a reduction in light penetration resulting from effluent colour. The response of periphyton growth in riffles was highly variable, with seasonal increases at effluent concentrations of 1.3% and 2.2%, and an inhibition of growth at 4.5 and 5.1% effluent. Macroinvertebrate density and biomass was increased by exposure to 1.3% effluent (NCASI 1982), but showed no response in a second study at a similar concentration (NCASI 1989). An addition of effluent to a 2.2% concentration initially increased, and then decreased macroinvertebrate density and biomass (NCASI 1983); exposure to 4.5% effluent resulted in a significant increase in macroinvertebrate density and biomass (NCASI 1984); and the highest effluent concentration tested (5.1%) resulted in a decrease in macroinvertebrate density and biomass (NCASI 1985). One consistent observation was higher trout survival in control streams but increased growth in effluent streams (Hall et al. 1991)

A more recent study using the same stream system compared effects of 1.5 and 5% effluent before and after a mill's conversion to chlorine dioxide substitution (60-70%). A significant increase in macroinvertebrate biomass in the 5% effluent treatment was seen post-conversion (Haley et al. 1995), otherwise no significant differences in biomass, species richness or diversity were seen in either 1.5 or 5% effluent. However, it should be noted that in the pre-conversion study only 1.5% effluent was used and there were 2 replicate streams per treatment, while in the post-conversion study both 1.5 and 5% effluent were used and only the control treatment was replicated.

More recent artificial stream studies carried out by Dubé and Culp (1996, 1997) and Lowell et al. (1995) have used smaller outdoor stream systems

containing simplified food webs, and increased replication (n=5-7). While these systems were less realistic than the large NCASI streams, these experiments were able to provide some clear conclusions about the effects of effluent. Dubé and Culp (1996, 1997) found that the addition of biologically treated bleached kraft mill effluent (BKME) increased periphyton growth at all concentrations tested (ranging from 0.1 to 10%). Chironomid growth was stimulated by all effluent concentrations, but peaked at 1%, indicating that toxic effects were starting to occur at higher concentrations. Lowell et al. (1995) found that 1 and 10% dilutions of effluent from the same mill stimulated growth and development of the mayfly *Baetis tricaudatus* . This stimulation could not be completely explained by increased periphyton growth in the effluent treatment because access to food was controlled and growth stimulation had occurred in food-unlimited conditions. Hypotheses for this stimulation included an increase in food palatability or quality, hormesis, or the action of hormone-mimicking compounds potentially present in the effluent.

The observation of stimulatory effects of pulp mill effluent is not uncommon, and the combination of stimulatory and inhibitory effects makes bioassay results difficult to interpret. These effluents contain nitrogen and phosphorus, which will stimulate algal growth in nutrient-limited situations. This can complicate interpretation of *Selenastrum* bioassays because effluent treatments may show higher growth than controls (Ahtianen et al. 1996, Priha 1996). Pulp mill effluents also contain quantities of settleable organic matter that may represent a food source to bioassay organisms and, in the field, may enrich sediments and result in increased invertebrate biomass (Södergren et al. 1988). Stimulation of *Ceriodaphnia* reproduction has been observed in chronic toxicity testing (e.g., Hatfield Consultants Ltd. 1996a, b), and causes of this are undetermined.

A1.3 Brief Overview of the Pulping Process

Effluent from a pulp mill is a highly complex mixture containing hundreds of different compounds, many of which remain unidentified (Suntio et al. 1988). To best understand the composition of pulping effluent, it is useful to consider the processes that occur within a pulp mill and, thus, the origins of effluent components. The following information has been summarized from Lavigne (1979), Sjöström (1981) and McCubbin and Folke (1993).

The objective of the pulping process is to transform wood into separate cellulose fibres, and a number of techniques for attaining this objective have been developed. There are 2 basic types of pulping processes: mechanical and chemical. In mechanical pulping, physical force is applied to wood to pull the fibres apart; chemical pulping uses various chemical solutions to dissolve the lignin that binds cellulose fibres together. Hybrid pulping is a term used to describe operations in which logs or chips are softened briefly with a chemical solution (usually sodium sulfite) before being mechanically pulped. Only chemical pulping will be discussed in any detail.

Chemical pulping may be broken into 2 main categories- sulfite and sulfate (kraft) pulping. Sulfite pulping is the oldest technique; the first sulfite mill started operations in Sweden in 1874. Kraft pulping was first patented in 1870, but did not become a feasible technique until the 1930's, with the development of a recovery furnace by Canadian G.H. Tomlinson (Sjöström 1981). The processes differ largely in the cooking chemicals used; sulfite pulping uses sulferous acid (H_2SO_3) and a solution of bisulfite salt (sodium, ammonium and magnesium salts are common, i.e., $NaHSO_3$) as the cooking liquor, while the kraft process uses a solution of sodium hydroxide (NaOH) and sodium sulphide (Na_2S). The kraft process is used on all types of wood, while the sulphite process is used mainly for pulping spruce, hemlock and fir. Kraft mills typically recover pulping chemicals in a process which results in the incineration of many of the organic compounds dissolved from the wood. Chemical recovery is not always possible in sulfite pulping, making this process less economical, and

producing an untreated effluent with considerably higher BOD and toxicity than that produced by kraft mills. Kraft pulping produces a higher quality pulp, and is the most common process worldwide; only this process will be discussed further. Of the approximately 120 mills currently in operation in Canada, 46 use the kraft process.

A kraft pulp mill's furnish arrives at the mill in the form of logs or as wood chips purchased from a chipping operation. Logs are first cut into smaller lengths and are washed with warm water to remove any dirt. This process does not add to the effluent stream because used wash water is screened and reused. Bark is then removed by either wet or dry debarking. Wet debarking produces a very toxic effluent containing high concentrations of resin acids and other naturally occurring compounds leached from the bark (McKague et al. 1977, Holmbom and Lehinten 1980, Talka and Priha 1987). Dry debarking has become more common because it removes bark by abrasion and no effluent stream is produced.

The logs are next chipped and screened, and pieces that are too small, along with removed bark, are used as fuel for the hog boilers that produce steam and electricity used in the mill. Appropriately sized chips are steamed briefly, and then move into the impregnation vessel where heated cooking liquor is added; these steps are designed to ensure that chips and cooking liquor are very close to cooking temperature when they enter the digester. The kraft digestion process is the addition of a 10% solution of NaOH and Na_2S (white cooking liquor), under temperatures of approximately 120-130°C and raised pressure. Digesters may be batch or continuously fed; continuous digesters are the newer technology, and are more common.

The digestion process dissolves the lignin that binds cellulose and hemicellulose fibres together. When cooked chips, now called pulp, are removed from the digester, the used cooking liquid (weak black liquor) is washed from the pulp. This process, called brownstock washing, removes not only cooking chemicals, but also lignin residuals and other dissolved

constituents from the pulp (Crotogino et al. 1987). Weak black liquor is routed to the chemical recovery plant where it is condensed in a series of evaporators and then undergoes a series of processes that result in the recovery of much of the cooking chemicals and the recreation of white liquor. Naturally occurring chemicals extracted from wood during the cooking process may be recovered from black liquor (as tall oil) if they have some commercial value. The remainder is burned in the recovery furnace. The digestion process and the chemical recovery plant do not add to the mill's effluent stream under proper operating conditions. However, spills and contamination of the evaporator condensate with black liquor may occur, and can result in highly toxic materials ending up in the untreated effluent as the condensate is recycled to be used in brownstock washing.

Brownstock washing is never completely effective, and lignins and other chemicals remaining in the pulp enter the bleach plant and eventually the effluent stream. Mills may employ oil-based additives to reduce foaming during brownstock washing. These additives may slightly increase the creation of 2,3.7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-dibenzofuran when the brownstock is bleached (Voss et al. 1988); this occurs when defoamers contain dibenzodioxin (the DBD content of defoamers is now regulated). Compounds remaining in brownstock include sodium salts derived from cooking liquor, naturally occurring compounds from wood (e.g., terpenes), lignins not solubilized by the cooking process, and the products of the breakdown of lignin. As lignins, lignin residuals, and other wood extractives are precursors of many of the chlorinated organic components of effluent, poor brownstock washing will lead to increased formation of these compounds (Hise and Hintz 1990). Additional measures designed to reduce the amount of lignin entering the beach plant may be implemented. This process, known as delignification, may be done in a variety of ways, including the use of gaseous oxygen under alkaline conditions, enzymes (xylenase), or ozone.

In a bleached kraft mill, the compounds created and washed from pulp in the bleach plant form the bulk of the effluent stream. The bleaching process is designed to remove as much of the remaining lignin as possible and in this way reduce colour of the pulp. Lignin removal is accomplished by making lignin soluble in alkali through the application of elemental chlorine or chlorine dioxide. The pulp is further whitened through the use of hydrogen peroxide or sodium hypochlorite. A bleach sequence typically consists of a series of applications of bleaching agents and caustic extractions. The type of bleaching agent used has important consequences for effluent composition. When elemental chlorine is used during bleaching, chlorinated organic compounds such as dioxins, furans, chlorinated resin acids and chlorinated phenolics form. Chlorinated compounds are generally more refractory and toxic than their unchlorinated forms (LeBlanc et al. 1988), and have been the focus of much research and legislation aimed at reducing effluent toxicity. The substitution of chlorine dioxide for elemental chlorine in the bleaching process will substantially reduce but not completely eliminate the formation of these compounds. Elemental chlorine often becomes substituted on aromatic compounds while chlorine dioxide breaks aromatic rings, hence the reduction in formation of chlorinated phenolics, PAHs and polychlorinated dioxins and furans. Furthermore, chlorine dioxide is a stronger oxidizing agent, requiring less chlorine per tonne of pulp bleached (Tana and Lehtinen 1996).

Pulping effluent is treated in a variety of ways prior to being discharged. The first step is usually removal of solids by screening, and then settling in a primary clarifier. Sludge from the screening process and the clarifier is usually disposed of in a landfill. The remaining effluent is pH adjusted, if required, and then passes into the secondary (biological) treatment system. Biological treatment is accomplished by a variety of methods, but most commonly by either activated sludge treatment (AST) or aerated stabilization basins (ASB). Both AST and ASB utilize bacteria and other microorganisms to break down toxic materials in the effluent. Organic compounds in the effluent provide a carbon

source for microbial growth, while oxygen, nitrogen and phosphorus must be added. AST is a very high rate process, with effluent treatment complete in a few hours to a day. Effluent is mixed with nutrients (nitrogen and phosphorus) and a sludge containing microorganisms, and is then aerated. After a period of time, effluent flows to a secondary clarifier where sludge settles. Some sludge is rerouted to the aeration tank and the rest is dewatered and landfilled or incinerated. AST's efficiency depends upon maintaining the correct ratio of sludge to nutrients to incoming effluent. This process is considerably faster and requires less land than the use of ASB, while at same time produces more sludge (requiring increased landfill capacity). Aerated stabilization basins are a slower rate process, usually requiring retention times of several days to a week. After primary treatment, effluent is pH balanced and added to the basin. Nutrients in the form of inorganic fertilizers are usually added to increase BOD removal. The basins are aerated over their initial section and then have a quiescent zone that acts to reduce discharge of settleable materials. Aerobic decomposition occurs in overlying waters of the aerated section, while anaerobic process (including dehalogenation, Amy et al. 1988) occur in sediment and in water overlying the sediment. In a well designed ASB, microbial biomass generated in system is also decomposed within the system, thus ASBs produce less sludge than AST. A properly operated ASB will require infrequent dredging to remove accumulated biomass (NCASI 1989b, McCubbin and Folke 1993). In general ASBs are slightly less efficient at removal of BOD, and are more susceptible to cold temperatures, while activated sludge plants are less efficient at removing organohalogens (e.g., chlorinated phenolics, chlorolignin, Saski et al. 1996).

Secondary treatment is effective at removing many effluent components. Volatile compounds like chloroform, turpentine, methyl mercaptans and hydrogen sulfide will be largely removed by aeration (Wilson and Hrutfiord 1975). The majority of labile organic compounds will be broken down, this is witnessed by the substantial reduction in biological (BOD) and chemical oxygen

demand (COD) that is accomplished by secondary treatment (usually >90% BOD, 45-55% COD; NCASI 1989b, Tomar and Allen 1991, Strömberg et al. 1996). Approximately 90% of resin acids, 73% of sterols, 70-85% of fatty acids and 25-70% of chlorinated phenolic compounds (13-54% in AST, Leuenberger et al. 1985) will be broken down (Bonsor et al. 1988, NCASI 1989b, Strömberg et al. 1996). Non-chlorinated phenolics will be virtually eliminated (Leuenberger et al. 1985), and removal of halogenated organics will be on the order of 15-47% for TOX, and 43-50% for the low molecular weight AOX (Tomar and Allen 1991). A substantial reduction in effluent toxicity is accomplished by secondary treatment; biologically treated effluents generally display little acute toxicity to common test biota including *Selenastrum capricornutum*, fathead minnows (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), zebrafish (*Brachydanio rerio*), and the crustaceans *Ceriodaphnia dubia* and *Daphnia magna* (Tomar and Allen 1991, McCubbin and Folke 1993, Ahtiainen et al. 1996).

A1.4 Composition of Pulp Mill Effluent

A considerable number of compounds have been identified in pulp mill effluent, and this section briefly describes some of the major effluent components. The discussion is not exhaustive as only compounds that are regularly tested for, or those that have be identified as of potential interest are discussed. Components are broken into categories based upon their origin: the first section describes components that originate from wood, the second section describes components that are added during processing. Appendix 2 presents further information regarding the concentrations and toxicity of specific effluent components.

A1.4.1 Compounds originating in wood

Most compounds in pulp mill effluent originate in the wood itself rather than in chemicals added during the pulping process, (Figure A1.1). These

compounds may be broken into two categories: compounds that are virtually unchanged by the pulping process, and residuals, which are the breakdown products of compounds occurring in wood. Residuals most often have their origin in the chemical destruction of lignins.

A1.4.1.1 Compounds unchanged by the pulping process

Compounds in effluent that are basically "washed out" during the pulping process include terpenes, sterols, fatty acids, lignins (discussed separately, see A1.4.1.2), nitrogen, phosphorus, and metals. Terpenes are volatile compounds with a carbon skeleton made up of isoprene (C_5H_8) units; their basic chemical structure is $(C_{10}H_{16})_n$ (e.g., monterpene $=C_{10}H_{16}$, sesquiterpene = $C_{15}H_{24}$, diterpene = $C_{20}H_{32}$). They are natural secondary compounds found in the essential oils and oleoresins of plants, and play a major role in plant resistance to microbes, insects and other herbivores. Defensive functions of terpenes include repellency and feeding inhibition. Insects ingesting these compounds may also experience a variety of effects including early death, reduction in food reserves, smaller size, and reduced fecundity (Painter 1958).

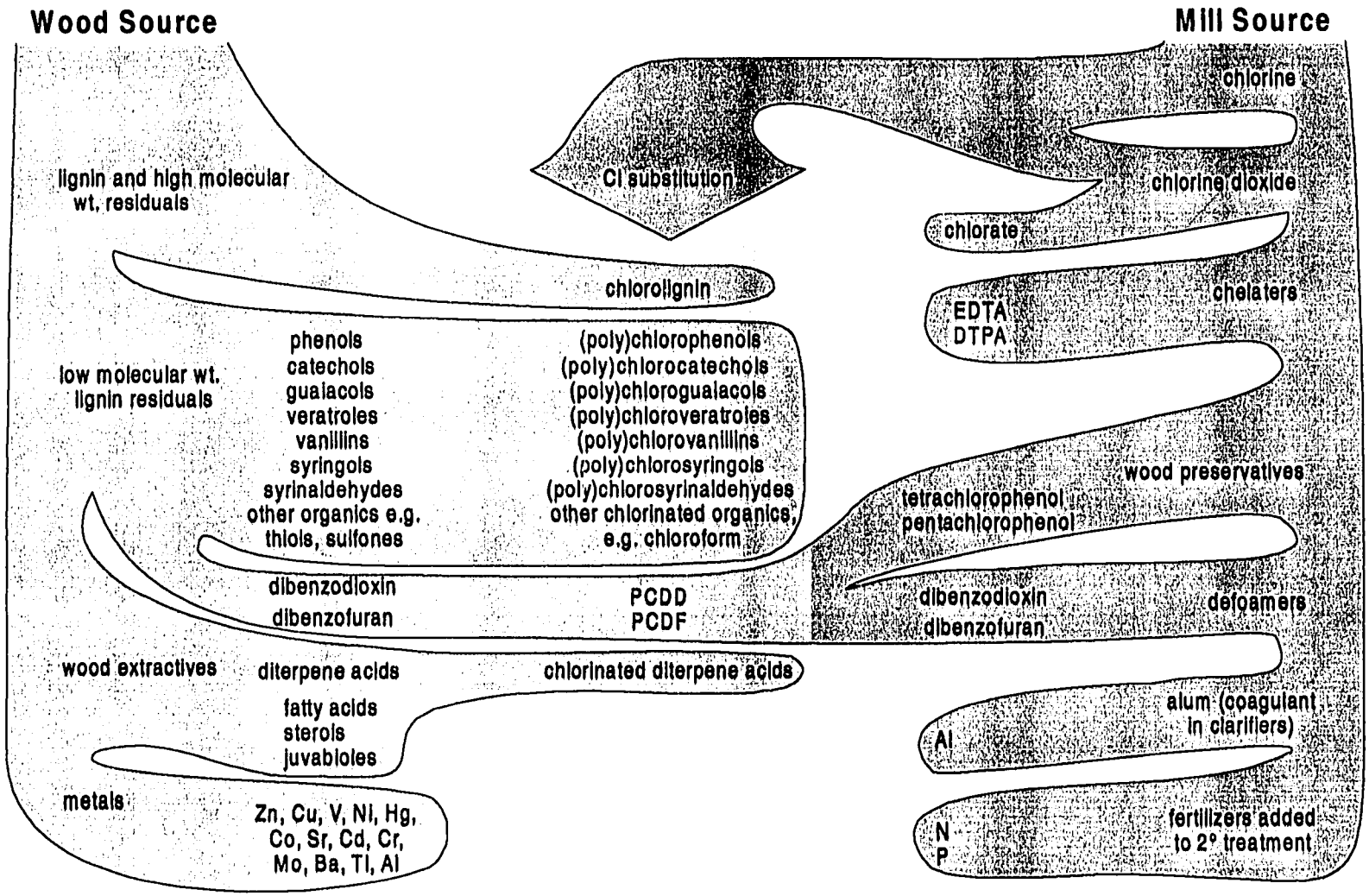


Figure A1.1 Origins of major effluent components.

Resins acids are diterpene acids found in wood resin, particularly pine and spruce, and are commonly identified as compounds responsible for a major portion of the toxicity of pulping effluents (Rogers 1973, Rogers et al. 1975; Rogers et al. 1979, D. McLeay and Associates 1987). There are seven commonly occurring resins acids, with dehydroabietic, isopimaric, abietic, and pimaric the most common. Leach and Thakore (1973) attributed over 80% of the toxicity of kraft effluent to the presence of three resin acid soaps (salts of isopimaric, abietic and dehydroabietic). Resin acids do not occur in hardwood, and this is thought to be why untreated effluents from mills using hardwood furnish are less toxic than mills using softwood. Although these compounds are very toxic, secondary treatment of effluent generally results in very effective removal of resin acids (Strömberg et al. 1996). Resin acids released in effluent are believed to sorb to sediment particles and are removed from the water column through sedimentation (Tana and Lehtinen 1996). Although fungal degradation of resin acids occurs, dehydroabietic acid has been found in elevated concentrations in sediments up to 15 km from the source (Brownlee et al. 1977).

Resin acids can be quite toxic to fish (Leach and Thakore 1973, Rogers 1973, Rogers et al. 1975), causing liver disfunction resulting in jaundice (Oikari and Nakari 1982). These compounds are also likely to affect insects because a main function of resin acids is to deter insect herbivores; the most common effect of resin acids on terrestrial insects is feeding and growth inhibition (Van Beek 1986, Harborne 1990). Resin acids are split into 2 categories: the abietic-type (1 isopropyl group at C13) and the pimaric-type acids (methyl and vinyl substituents at C13). Abietic-type acids, in particular dehydroabietic acid, are the resin acids found in the greatest concentrations in pulp mill effluent. Abietic acid and several of its derivatives have antijuvenalizing activity towards insects (Murakoski et al. 1976), meaning that exposure to these compounds may result in precocial adults or moulting to forms exhibiting both adult and larval features; in both cases sterility may result. Abietic acid also exhibits estrogenic activity,

enhancing proliferation of estrogen-dependent breast cancer cell lines *in vitro* and causing vitellogenin gene expression when fed to juvenile rainbow trout (Mellanen et al. 1996). Resin acids also exhibit toxicity toward algae; dehydroabietic acid inhibits photosynthesis of *Cryptomonas erosa* at a concentration of 500µg/L (Moore and Love 1977).

Other terpenoids include dehydrojuvabione, juvabiol and juvabione. These compounds are very toxic to fish (Leach et al. 1975) and are found in balsam fir, douglas fir, and alpine fir (Bowers et al. 1966, Leach et al. 1975) and, thus, may be found in pulping effluents from mills using these species as furnish. These compounds may also affect aquatic insects, as they are analogs of an insect growth regulating hormone, juvenile hormone (JH). The application of exogenous JH or a JH analogue can disrupt the moulting cycle and normal development of insects, and may result in death (Staal 1975). Man-made JH analogs are used as insecticides (Staal 1975). For example the products Farsenol and Methoprene, both JH analogues, have been used to control aquatic insects (Mulla et al. 1974, Mulligan and Schaefer 1990). Application of JH to eggs can reduce hatching success (Staal 1975), application to immatures can cause disorders in metamorphosis (Gelbic et al. 1994) while application to adults may result in reduced fecundity and/or egg viability and death (Hicks and Gordon 1992). Reduced reproduction in *Ceriodaphnia* in response to exposure to dehydrojuvabiol was demonstrated by O'Connor et al. (1992a) at concentrations as low as 0.5µg/l. There have been no studies of the toxicity of these compounds to aquatic insects.

Sterols are steroid-based alcohols with a hydrocarbon side chain of 8-10 carbon atoms. Sterols reported in kraft pulping effluent, in order of decreasing concentration, are: β-sitosterol, stigmastanol, stigmasterol, and campesterol (Cook et al. 1997). Although secondary treatment usually removes between 61-96% of most sterols, stigmasterol content of effluent can actually increase 2-3x after treatment in an aerated stabilization basin (Cook et al. 1997). Kraft mills release in their secondary-treated effluent between 0.2-19.2 g of sterols per ton

of pulp produced (Cook et al. 1997). Plant sterols released in effluent may be responsible for hormone disruption; female mosquitofish (*Gambusia affinis*) exposed to degradation products of β-sitosterol and stigmastanol can develop masculine secondary sexual characteristics (Denton et al. 1985, Hunsinger et al. 1988, Howell and Denton 1989). Masculinization has also occurred in wild populations of mosquitofish exposed to pulp mill effluent (Howell et al. 1980). β-sitosterol is estrogenic in rainbow trout, causing expression of the vitellogenin gene in juveniles (Mellanen et al. 1996). Compounds with estrogen activity have been reported to inhibit moulting in the cladocera *Daphnia magna* (Zou and Fingerman 1997). Because all arthropods utilize similar moulting hormones, plant sterols may therefore affect moulting in aquatic insects. Note however, that as most insects cannot synthesize a steriod nucleus, sterols are also an essential component of their diet (Fast 1964, Chapman 1982).

Fatty acids are organic acids found in natural fats, oils, and waxes and are natural constituents of most plants. Many fatty acids are essential dietary components for animals. In the pulping process, most fatty acids are recovered as a constituent of tall oil (tall oil = 50-60% fatty acids, 35-40% resin acids) and are sold; the most abundant fatty acids in pulping effluents are oleic acid and linoleic acid. Commercial uses include the manufacture of cosmetics, ointments, soaps, and flavourings. Fatty acids are not very soluble in water, degrade quickly, and are toxic only at high concentrations (Leach and Thakore 1973, Appendix 2). Both myristic and palmitic acid increase copper toxicity to *Photobacterium phosphoreum*, possibly due to the formation of lipophilic complexes (Carlson-Ekvall and Morrison, 1995).

Other constituents of pulping effluent that originate in wood are small quantities of various metals, phosphorus, and nitrogen (nitrate and ammonia) (McCubbin and Folke 1993). The majority of the nitrogen and phosphorus in effluent originates in fertilizers added in the secondary treatment system, and will therefore be discussed in a later section. Metals found in pulp effluents include zinc, copper, thallium, vanadium, chromium, cadmium, cobalt,

molybdenum, titanium, strontium, barium, tin, and lead (McCubbin and Folke 1993, Verta et al. 1996). McCubbin and Folke (1993) report that zinc is the only metal originating in wood that is found in pulp effluents in concentrations averaging higher than 50ug/l. Zinc is an essential micronutrient in the diets of many animals, including insects, due to it's role in enzyme structure. While zinc may exhibit high toxicity in laboratory testing, it associates with humic and other organic material in nature and is usually not bioavailable (McCubbin and Folke 1993).

A1.4.1.2 Lignin and lignin residuals

Lignins are a group of over 200 phenolic polymers found in all parts of plants, including tree resin (MacRae and Towers 1984). Lignins constitute 16-33% of wood (Sjöström 1981, LaFleur 1996) and provide rigidity and act as a bonding agent between cellulose fibres (Kringstad and Lindström 1984). Besides providing structural rigidity, biological activities of lignins include antifungal, antimicrobial, and antiviral action as well as inhibition of various enzyme systems including mixed function oxygenases. Activities towards insects include growth inhibition, weak juvenile hormone activity, and feeding inhibition (MacRae and Towers 1984).

The cooking process dissolves lignin, separating the cellulose fibres. In the cooking process, and in the oxygen delignification step used by many mills, lignin molecules are broken into a wide array of compounds. Figure A1.2 shows the prominent structures of softwood lignin, which is formed by the copolymerization of coumaryl, sinapyl and coniferyl alcohols (Kringstad and Lindström, 1984). The sodium hydroxide and sodium sulfide used in kraft pulping promotes cleaving of ether bonds (-O-) in this structure; it is obvious that a great variety of degradation products are possible. Both molecular chlorine and chlorine dioxide react with lignins, and with any lignin-residuals or other wood extractives that were not washed from the brownstock. Both bleaching agents oxidize aromatic compounds to form open-ring compounds, and molecular chlorine also becomes substituted on aromatic rings

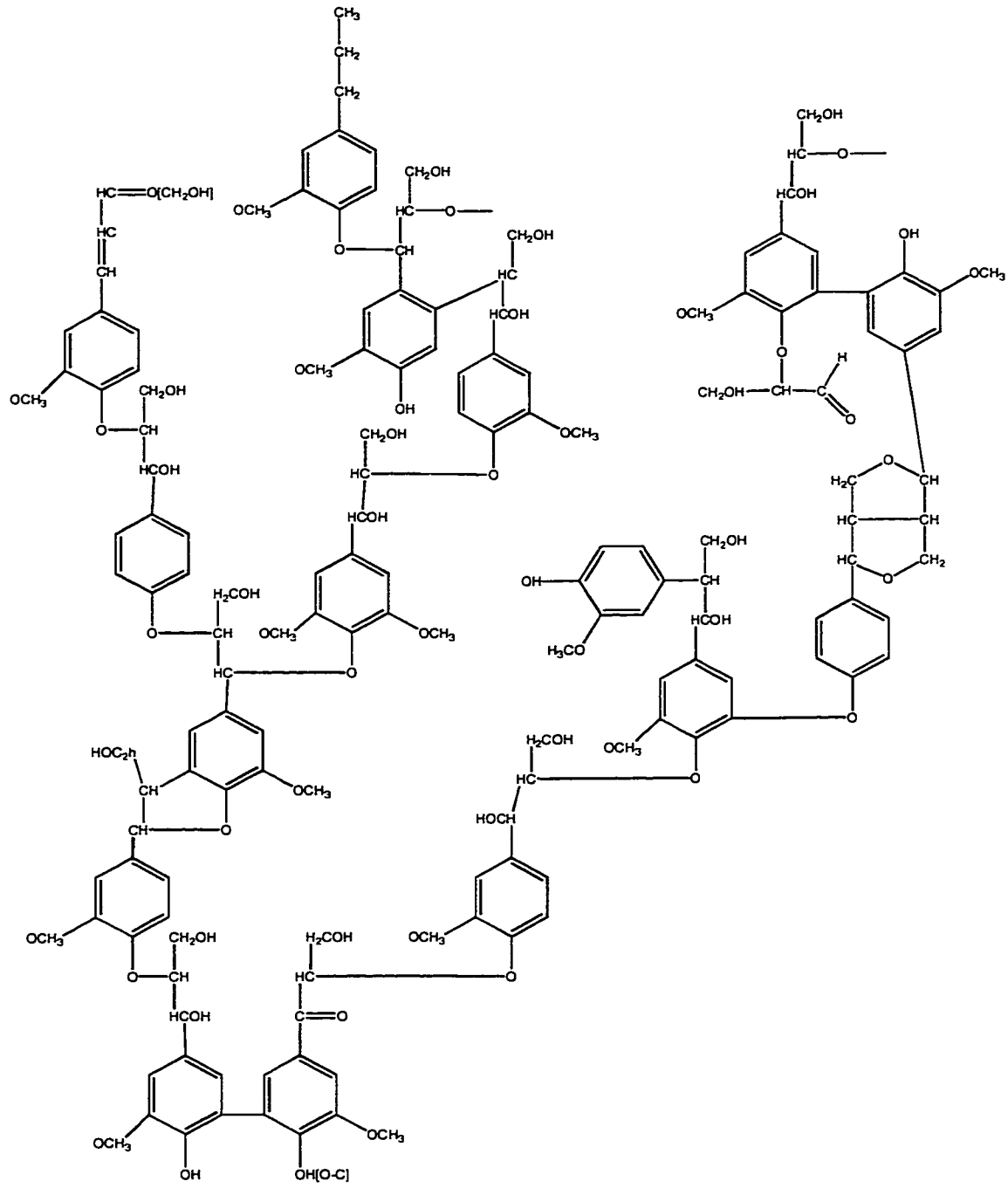


Figure A1.2 Prominent structures of softwood lignin, redrawn from Kringstad and Lindström (1984).

(LaFleur 1996). Compounds arising from breakdown of lignin include chloroform, polycyclic aromatic hydrocarbons, chlorinated dioxins and furans, and chlorinated and nonchlorinated phenolics including catechols, guaiacols,

vanillins, syringols, (Hrutfiord and Negri 1992, LaFleur 1996). Chlorine-substituted forms of lignin residuals are not only more toxic, but are also more persistent in the environment (LeBlanc et al. 1988, Kuivasniemi et al. 1986). Chlorinated organic compounds in effluent are split into two categories: a low (<1000 D) and a high(>1000 D) molecular weight group. The high molecular weight compounds are largely unidentified and the group as a whole is referred to as chlorolignins. Low molecular weight lignin residuals include phenols, catechols, guaiacols, syringols, veratroles, syringaldehydes, vanillins, and polycyclic aromatic hydrocarbons. Whether chlorinated or not, these compounds show toxicity to aquatic species (Appendix 2). The higher substituted forms are more lipophilic and, therefore, more toxic and more likely to bioaccumulate. Catechols, guaiacols, and veratroles bind to particulate matter (Remberger et al. 1986), humic materials, and chlorolignin. The mobility of compounds bound to chlorolignins is substantially increased (Grimvall et al. 1991), while those bound to particles are sedimented quickly and generally are not transported long distances in receiving waters. Under aerobic conditions, bacteria biotransform chlorophenols to anisoles, and catechols and guaiacols are methylated to veratroles (Neilson et al. 1983, Neilson et al. 1990, Remberger et al. 1986); these metabolites are more toxic and more likely to bioaccumulate than their precursors (Neilson et al. 1984). Under anaerobic conditions in sediments, chloroguaiacols and chloroveratrols are transformed to chlorocatechols (Remberger et al. 1986, Neilson et al. 1987). Catechol reduces efficiency of food assimilation in insects, resulting in decreased growth (Reese and Beck 1976). A chlorinated guaiacol caused filter-net deformities in a hydropsychid caddisfly (Petersen and Petersen 1984). Filter-net deformities have the potential to affect growth of these insects as this structure is responsible for collection of food particles.

Chloroligins represent 80-95% of the chlorinated organic material in effluent (Kringstad et al. 1984, Strömberg et al. 1996, Saski et al. 1996), and the environmental effects of these high molecular weight compounds are unclear.

The molecules are too large to pass across cell membranes (Kukkonen 1992), and produce no acute toxicity in *Daphnia magna* (Sågfors and Stark 1988). However, these compounds accumulate in sediments (Saski et al. 1996) and may breakdown to release more toxic low molecular weight compounds (Neilson et al. 1983, Millar et al. 1996). Lignins and chlorolignins are very similar in structure to natural humic substances (Kukkonen 1992), and both substantially decrease bioavailability and resultant bioconcentration of organic xenobiotics including, DDT, Benzo[a]pyrene, polychlorinated biphenyls, and various other polycyclic aromatic hydrocarbons (Landrum et al. 1985, Black and McCarthy 1988, and Servos and Muir 1989). For example, chlorinated and non-chlorinated lignins from pulp mill effluent can reduce bioconcentration of hydrophobic contaminants by 60%-80% (Kukkonen 1992, Kukkonen and Oikari 1992). It is believed that the mechanism responsible is the binding of contaminants to the lignins. This increases their solubility in water and prevents them from penetrating though cell membranes (Black and McCarthy 1988). The presence of humic material can also reduce the sorption of some hydrophobic organic contaminants to sediments (Hassett and Anderson 1982, Caron et al. 1985)

Polycyclic aromatic hydrocarbons (PAHs) are compounds containing only carbon and hydrogen atoms (usually) in the form of 2 or more fused benzene rings. Many PAHs are naturally found in petroleum products and are ubiquitous products of incomplete combustion. PAHs with 2-3 benzene rings, such as acenapthylene and acenapthene, are biodegraded in a matter of days in water (Ogawa et al. 1981), while PAHs with >3 benzene rings (e.g., fluoranthene and pyrene) are likely to be more persistent (Verschueren 1983). PAHs in general show less acute toxicity than chlorinated phenolics (Appendix 2), however many members of this class of compounds are carcinogenic. PAHs have log octanol-water partition coefficients close or above 4, indicating the potential to bioaccumulate. Cash and Culp (1996) reported that concentrations of fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, and

chrysene were elevated in periphyton tissue exposed to a 3% dilution of effluent from a bleached kraft mill. Many animals, including mammals, fish, and insects, are capable of metabolizing PAHs with microsomal cytochrome enzyme systems, however, in some cases the biotransformation results in the production of carcinogenic metabolites (e.g., bezanthracene and benz(a)anthracene, Hawkins et al. 1995). Some PAHs (e.g., anthracene) exhibit phototoxicity, in which the toxicity of the compound is substantially increased in the presence of ultraviolet light (Allred and Giesy 1985).

A1.4.2Compounds added during pulping process

Compounds found in effluents that are added by mill processes include chlorate, chelating substances, defoaming agents, surfactants, aluminum, nitrogen and phosphorus. In addition, the interaction of molecular chlorine with wood extractives and lignin residuals during pulp bleaching results in the formation of many chlorinated organics, as described in the previous section. Chlorate (ClO_3) is produced by mills using chlorine dioxide as a substitute for molecular chlorine. While chlorate is toxic to marine algae (Lehinten 1988), no adverse effects of chlorate exposure have been found in freshwater algae (Perrin and Bothwell 1992) or aquatic invertebrates (van Wuk and Hutchinson 1995). Aluminum may be added as alum to water and effluent in primary clarifiers to act as a chemical coagulant (McCubbin and Folke 1993), but is not believed to pose any environmental problems (McCubbin and Folke 1993). Chelating substances such as EDTA (ethylene diamine tetra-acetic acid) and DTPA (diethylene triamine penta-acetic acid) are used in the bleaching of pulp to stabilize hydrogen peroxide and ozone (Hinck et al. 1997). These compounds are released in the effluent and are very resistant to degradation (Hinck et al. 1997), though no harmful effects in a freshwater environment been identified (McCubbin and Folke 1993).

The most notorious compounds in pulp mill effluent are the polychlorinated dioxins and furans (PCDDs and PCDFs). There are 75 possible PCDD

congeners and 135 possible PCDFs; they are very persistent and may bioaccumulate (log K_{ow} range = 6.8-8.2). These compounds are ubiquitous in the environment in trace amounts (Jones and Stewart 1997) because they form naturally during combustion as well as during various industrial processes. Toxicity of polychlorinated dioxins and furans varies widely; tetrachlorinated forms (TCDDs and TCDFs) are the most toxic and 2,3,7,8-TCDD is considered most toxic of all (Jones and Stewart 1997). 2,3,7,8-TCDD and 2,3,7,8-TCDF are the tetrachlorinated forms found in the highest concentrations in pulp mill effluent (Amendola et al. 1989a b). TCDDs and TCDFs in pulp mill effluent originate with the condensation of phenolics during pulp bleaching; this occurs when wood chips are contaminated with polychlorinated phenols, used by sawmills as anti sap-stain agents (Luthe et al. 1993). TCDD and TCDF may also form with the chlorination of dibenzodioxin, which occurs in small quantities in the wood, but more often as a contaminant in process water or in oil-based defoamers used during brownstock washing (Berry et al. 1989, Voss et al. 1988). Strategies designed to reduce dioxin and furan release in effluents have generally been quite successful, as evidenced by a 95% reduction in TCDD/TDCF (measured as 2,3,7,8-TEQ - toxic equivalents) from 1988 to 1991 (Luthe et al. 1992).

Alkylphenol polyethoxylate surfactants are used by a variety of industries including the pulp and paper industry (Field and Reed 1996). Microbial degradation of these surfactants produces alkylphenols, including nonylphenol and octylphenol. Alkylphenols are more toxic than their precursors (Renner 1997), and appear to be estrogenic. When male rainbow trout undergoing sexual maturation were exposed for 3 weeks to a 30µg/L nominal concentration of nonylphenol, octylphenol, nonylphenol ethoxy carboxylate, or nonylphenoldiethoxylate, the fish exhibited vitellogenin production and an inhibition of testicular growth (Jobling et al. 1996). Octylphenol produced a significant increase in vitellogenin production at a concentration of 4.8µg/l, in comparison with 20.3µg/L for nonylphenol. In Field and Reeds' (1996) survey

of fifteen paper mill effluents, concentrations of nonylphenol ethoxy carboxylates ranged from below detection limits (1 mill, detection limits =0.04-0.4 µg/L) to 1269.7 µg/L (median= 22.6 µg/L). These compounds are believed to bioaccumulate, and therefore chronic field effects may occur at concentrations lower than those observed in the 3 week laboratory exposure. The use of alkyphenol ethoxylate surfactants has already been restricted in Europe (Renner 1997).

As the preceding discussion has indicated, pulp mill effluents contain a wide variety of compounds that have the potential to adversely affect the aquatic environment. However, for the most part, these compounds are released in concentrations below those which have been observed to cause effects, at least on an acute basis (see Appendix 2). Possibly the most important compound currently added by mills to the aquatic environment is phosphorus. Phosphorus and/or nitrogen are added to waste treatment systems because they are limiting nutrients for microbial growth. Insufficient quantities of nutrients in biological treatment systems will result in increased toxicity and decreased removal of biological oxygen demand and settlable solids in the effluent (McCubbin and Folke 1993). Phosphorus however, is also a limiting nutrient in most freshwater systems, and phosphorus additions *via* pulping effluents can result in greatly increased primary production in receiving waters (Bothwell 1992). Fertilization can have profound effects on an ecosystem. Probably the most comprehensive study of effects of fertilization on a riverine ecosystem is the long-term research on the Kuparak River, Alaska. Responses to increases in phosphorus content of the river included increased biomass of primary producers and some consumers (Peterson et al. 1993; Hershey et al. 1988), changes in growth rates of aquatic insects and fish (Hiltner and Hershey 1992; Peterson et al. 1993), decreases in drift in the fertilized section of the river (Hinterleitner-Anderson et al. 1992), increased body size of insects (Hershey et al. 1988), changes in community composition of benthic invertebrates (Hiltner and Hershey 1992), increased microbial activity

(Hiltner and Hershey 1992; Peterson et al. 1993) and changes in the energy base of ecosystem from heterotrophy to autotrophy (Peterson et al. 1985).

A1.4.3Other Effluent Parameters

Mills discharge quantities of both settleable and non-settleable solids in their effluents. Settleable solids include wood fibres and flocculated bacterial/algal materials. While these substances used to be of major concern in receiving waters, in mills with modern treatment systems the majority of these solids are settled prior to effluent discharge. Non-settleable solids in biologically treated effluents are largely bacterial in origin (NCASI 1978a b, Costa et al. 1979) and are generated in the secondary treatment system. A study by Zanella et al. (1978) showed that these solids could be utilized as food by a filter feeding caddisfly (*Hydropsyche*), and *Daphnia*. A tracer study (NCASI 1978b) has demonstrated that non-settleable solids labeled with ^{14}C were incorporated by periphyton, grazers (Physa, Chironomidae, Elmidae), a filter feeding Trichoptera (Cheumatopsyche), detritivores (Gammarus, Helobdella), and a macroinvertebrate-feeding fish (Notemagonus chrysoleucas). A radioisotopic study of the food web downstream of a pulp mill in the Thompson River, B.C. (Wassenar and Culp 1996) indicated that the macroinvertebrates and fish in this rivers utilize, to a substantial degree, a terrestrially-derived carbon source. Organic material in pulp mill effluent may therefore represent a route through which terrestrially-based carbon moves into these systems. Non-settleable solids also represent a phosphorus source for primary producers; as these solids decompose the majority (67-79%) of phosphorus released is in the form of orthophosphates (Lee et al. 1978).

A1.5 Conclusion

A final cautionary note about the composition of pulp mill effluent is required at this point. It is important to realize that no 2 pulp mills are identical and sweeping generalizations about effluent composition and related environmental

effects should be made with caution. Effluent composition can be highly variable, not only between mills but on a daily basis within a mill. Concentrations of wood extracts (sterols, resin acids, fatty acids) are highly dependent upon the furnish used, a significant fact since these compounds have been identified as components largely responsible for effluent effects. The wood species, age and condition of the wood when cut, storage times, and storage conditions will all have significant effects upon the concentrations of these compounds (Swan 1973 O'Connor et al. 1992b). The configuration, age, and condition of equipment will vary from mill to mill and, consequently, so will the potential for spills and suboptimal operation of equipment designed to reduce effluent toxicity. Finally, a secondary treatment system is a biological system and thus subject to greater variability than a purely chemical or mechanical system.

A1.6 Literature Cited

Ahtiainen, J., T. Nakari, and J. Silvonen. 1996. Toxicity of TCE and ECF pulp bleaching effluents assessed by biological toxicity tests. Pages 33-40 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Allred, P. M., and J. P. Giesy. 1985. Solar radiation-induced toxicity of anthracene to *Daphnia pulex*. Environmental Toxicology and Chemistry 4: 219-226.

Amendola, G., D. Barna, R. Blosser, L. LaFleur, A. McBride, F. Thomas, T. Tiernan, and R. Whittemore. 1989a. The occurrence and fate of PCDDs and PCDFs in five bleached kraft pulp and paper mills. Chemosphere 18: 1181-1188.

Amendola, G. A., R. E. Handy Jr., and D. G. Bodien. 1989b. Bench-scale study of dioxins and furan (2378-TCDD and 2378-TCDF) treatability in pulp and paper mill wastewaters. Tappi Journal 72: 189-195.

Amy, G. L., C. W. Bryant, B. C. Alleman, and W. A. Barkley. 1988. Biosorption of organic halide in a kraft mill generated lagoon. Journal of the Water Pollution Control Federation 60: 1445-1453.

Bagge, A. M., and E. T. Valtonen. 1996. Experimental study on the influence of paper and pulp mill effluent on the gill parasite communities of roach (*Rutilus rutilus*). Parasitology 112: 499-508.

Berry, R. M., B. I. Fleming, R. H. Voss, C. E. Luthe, and P. E. Wrist. 1989. Preventing the formation of dioxins during chemical pulp bleaching. Paper presented at the 75th Annual Meeting, CPPA Tech. Sect., Montreal, Jan.31-Feb3 .

Black, M. C., and J. F. McCarthy. 1988. Dissolved organic macromolecules reduce the uptake of hydrophobic organic contaminants by the gills of rainbow trout (*Salmo gairdneri*). Environmental Toxicology and Chemistry 7.

Bothwell, M. L. 1992. Eutrophication of rivers by nutrients in treated kraft pulp mill effluent. Water Pollution Research 27: 77-102.

Bowers, W. S., H. M. Fales, M. J. Thompson, and E. C. Uebel. 1966. Juvenile hormone: identification of an active compound from balsam fir. Science 154: 1020-1021.

Brownlee, B., M. E. Fox, W. M. Strachan, and S. R. Joshi. 1977. Distribution of dehydroabietic acid in sediments adjacent to a kraft pulp and paper mill. Journal of the Fisheries Research Board of Canada 34: 383-843.

Brownlee, B. G., and W. M. J. Strachan. 1977. Distribution of some organic compounds in the receiving waters of a kraft pulp and paper mill. Journal of the Fisheries Research Board of Canada 34: 830-837.

Cairns Jr., J. 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100: 47-57.

Carlson-Erkvall, C. E. A., and G. M. Morrison. 1995. Toxicity of copper in the presence of organic substances in sewage sludge. Environmental Technology 16: 243-251.

Caron, G., I. H. Suffet, and T. Belton. 1985. Effect of dissolved organic carbon on the environmental distribution of nonpolar organic compounds. Chemosphere 14: 993-1000.

Cash, K. J., and J. M. Culp. 1996. The effects of bleached kraft pulp-mill effluent on periphyton tissue. National Hydrology Research Institute, Saskatoon, Saskatchewan.

Chapman, P. M. 1990. The sediment quality triad approach to determining pollution-induced degradation. The Science of the Total Environment 97/98: 815-825.

Chapman, P. M. 1996. Presentation and interpretation of Sediment Quality Triad data. Ecotoxicology 5: 327-339.

Chapman, R. F. 1982. *The Insects. Structure and Function.* Harvard University Press, Cambridge, Massachusetts.

Cook, C. R. 1990. Organochlorine discharges from a bleached kraft pulp mill with oxygen delignification and secondary treatment. Pulp & Paper Canada 91: 71-77.

Cook, D. L., L. LaFleur, A. Parrish, J. Jones, and D. Hoy. 1997. Characterization of plant sterols from 22 US pulp and paper mills. Water Science and Technology 35: 297-303.

Costa, H. S., J. J. McKeown, and R. O. Blosser. 1979. Studies on characterization, fate, and impact of residual solids of biological treatment origin. Tappi 62: 41-46.

Crotogino, R. H., N. A. Poirier, and D. T. Trinh. 1987. The principles of pulp washing. Tappi Journal 70: 95-103.

Crow, M. E., and F. B. Taub. 1979. Designing a microcosm bioassay to detect ecosystem level effects. International Journal of Experimental Studies 13: 141-147.

D. McLeay and Associates Ltd. 1987. Aquatic toxicity of pulp and paper mill effluent: a review. Environment Canada, Fisheries and Oceans Canada.

Dale, A. R., and P. A. Chambers. 1995a. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, April and May, 1994. Northern River Basins Study, Edmonton, Alberta.

Dale, A. R., and P. A. Chambers. 1995b. Growth rate and biomass response of periphytic algae to phosphorus enrichment in experimental flumes, Athabasca River, Seasonal Variation. Northern River Basins Study, Edmonton, Alberta.

Denton, T. E., W. M. Howell, J. J. Allison, J. McCollum, and B. Marks. 1985. Masculinization of female mosquitofish by exposure to plant sterols and *Mycobacterium smegmatis*. Bulletin of Environmental Contamination and Toxicology 35: 627632.

Dubé, M. G., and J. M. Culp. 1996. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Environmental Toxicology and Chemistry 15: 2019-2027.

Dubé, M. G., and J. M. Culp. 1997. Growth responses of periphyton and chironomids exposed to biologically treated bleached-kraft pulp mill effluent. Water Science and Technology 35: 339-345.

Environment Canada Department of Fisheries and Oceans. 1992. Aquatic Environmental Effects Monitoring Requirements.

Fast, P. G. 1964. Insect lipids: a review. Memoirs of the Entomological Society of Canada 37: 3-50.

Field, J. A., and R. L. Reed. 1996. Nonylphenol polyethoxy carboxylate metabolites of nonionic surfactants in U.S. paper mill effluents, municipal

sewage treatment plant effluents, and river waters. Environmental Science and Technology 30: 3544-3550.

Gagnon, M. M., D. Bussieres, J. J. Dodson, and P. V. Hodson. 1995. White sucker (*Catastomus commersoni*) growth and sexual maturation in pulp mill-contaminated and reference rivers. Environmental Toxicology and Chemistry 14: 317-327.

Gagnon, M. M., J. J. Dodson, P. V. Hodson, G. Van Der Kraak, and J. H. Carey. 1994. Seasonal effects of bleached kraft mill effluent on reproductive parameters of white sucker (Catastomus commersoni) populations of the St. Maurice River, Quebec, Canada. Canadian Journal of Fisheries and Aquatic Sciences 51: 337-347.

Green, R. H. 1979. *Sampling Design and Experimental Methods for Environmental Biologists*. John Wiley & Sons.

Grimvall, A., H. Borén, S. Jonsson, U. Lundström, and R. Sävenhed. 1991. Organohalogens of natural and industrial origin in large recipients of bleach-plant effluents. Water Science and Technology 24: 373-383.

Haley, R. K., T. J. Hall, and T. M. Bousquet. 1995. Effects of biologically treated bleached-kraft mill effluent before and after mill conversion to increased chlorine dioxide substitution: results of an experimental streams study. Environmental Toxicology and Chemistry 14: 287-298.

Hall, T. J. 1996. A review of recent studies in North America using integrated monitoring approaches to assessing bleached kraft mill effluent effects on receiving waters. Pages 599-611 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Hall, T. J., R. K. Haley, and L. E. LaFleur. 1991. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental stream channels. Environmental Toxicology and Chemistry 10: 1051-1060.

Hall, T. J., and R. A. Miner. 1997. Integrated long-term receiving water study methodology development. 35: 315-320.

Harbone, J. B. 1990. Role of secondary metabolite in chemical defense mechanisms in plants. Pages pp126-139 *In* D. J. Chadwick and J. Marsh, eds. *Bioactive Compounds From Plants*. CIBA Foundation.

Harris, J. H., G. Scarlett, and R. J. MacIntyre. 1992. Effects of a pulp and paper mill on the ecology of the La Trobe River, Victoria, Australia. Hydrobiologia 246: 49-67.

Hassett, J. P., and M. A. Anderson. 1982. Effects of dissolved organic matter on adsorption of hydrophobic organic compounds by river- and sewage-borne particles. Water Research 16: 681-686.

Hatfield Consultants Ltd. 1996a. Fraser River Environmental Effects Monitoring (EEM) Cycle One Interpretive Report., West Vancouver.

Hatfield Consultants Ltd. 1996b. Weyerhauser Canada Ltd. Environmental Effects Monitoring (EEM) Cycle One Interpretive Report. Hatfield Consultants Ltd., West Vancouver.

Hawkins, W. E., W. W. Walker, and R. M. Overstreet. 1995. Carcinogenicity tests using aquarium fish. Pages 421-448 in G. M. Rand, ed. *Fundamentals of Aquatic Toxicology*. Taylor & Francis Ltd., Washington, D.C.

Hershey, A. E., A. L. Hiltner, M. A. J. Hullar, M. C. Miller, J. R. Vestal, M. A. Lock, S. Rundle, and B. Peterson. 1988. Nutrient influence on a stream grazer: *Orthocladius* microcommunities respond to nutrient input. Ecology 69: 1383-1392.

Hewitt, L. M., J. H. Carey, D. G. Dixon, and K. R. Munkittrick. 1996. Examination of bleached kraft mill effluent fractions for potential inducers of mixed function oxidase activity in rainbow trout. Pages 79-94 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Hicks, B. J., and R. Gordon. 1992. Effects of the juvenile hormone analog Fenoxycarb on various developmental stages of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). The Canadian Entomologist 124: 117-123.

Hiltner, A. L., and A. E. Hershey. 1992. Blackfly (Diptera: Simuliidae) response to phosphorus enrichment of an arctic tundra stream. Hydrobiologia 240: 259-.

Hilton, D. F. J. 1980. The effect of Kraft paper mill effluents on insects inhabiting the St. Francis River near East Angus, Quebec. Annales de la Societe Entomologique du Quebec 25: 179-189.

Hinck, M. L., J. Ferguson, and J. Puhaakka. 1997. Resistance of EDTA and DTPA to aerobic biodegradation. Water Science and Technology 35: 25-31.

Hinterleitner-Anderson, D., A. Hershey, and J. A. Schuldt. 1992. The effects of river fertilization on mayfly (*Baetis* sp.) drift patterns and population density in an arctic river. Hydrobiologia 240: 247-258.

Hise, R. G., and H. L. Hintz. 1990. The effect of brownstock washing on the formation of chlorinated dioxins and furans during bleaching. Tappi Journal 73: 185-190.

Holbom, B., and K.-J. Lehtinen. 1980. Acute toxicity to fish of kraft pulp mill waste waters. Paperi ja Puu-Papper och Trä 11: 673-684.

Howell, W. M., D. A. Black, and S. A. Bortone. 1980. Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia holbrooki*: evidence for environmentally-induced masculinization. Copeia 4: 676-681.

Howell, W. M., and T. E. Denton. 1989. Gonopodial morphogenesis on female mosquitofish, *Gambusia affinis affinis*, masculinized by exposure to degradation products from plant steroids. Environmental Biology of Fishes 24: 43-51.

Hrutfiord, B. F., and A. R. Negri. 1992. Chlorinated dibenzofurans and dibenzodioxins from lignin models. Tappi Journal 75: 129-134.

Hunsinger, R. N., B. R. Byran, and W. M. Howell. 1988. Unchanged gonadal morphology of mosquitofish masculinized by exposure to degraded plant sterols. Journal of Fish Biology 32: 795-796.

Hynes, H.B.N. 1971. *The Biology of Polluted Waters.* University of Toronto Press, Toronto, Canada 202p

Jobling, S., D. Sheahan, J. A. Osborne, P. Mathiessen, and J. P. Sumpter. 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology and Chemistry 15: 194-202.

Jones, K. C., and A. P. Sewart. 1997. Dioxins and furans in sewage sludges: a review of their occurrence and sources in sludge and of their environmental fate, behavior, and significance in sludge-amended agricultural systems. Critical Reviews in Environmental Science and Technology 27: 1-85.

Kennedy, C. J., R. M. Sweeting, J. A. Johansen, A. P. Farrell, and B. A. McKeown. 1996. Lethal and sublethal effects of chlorinated resin acids and chloroguaiacols in rainbow trout. Pages 391-400 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Kimball, K. D., and S. A. Levin. 1985. Limitations of laboratory bioassays: the need for ecosystem-level testing. BioScience 35: 165-171.

Kringstad, K. P., and K. Lindström. 1984. Spent liquors from pulp bleaching. Environmental Science and Technology 18: 236a-248a.

Kuivasniemi, K., V. Eloranta, and L. Halttunen-Keyrilāinen. 1986. Aging of bleached kraft mill effluent studied by degradation of chlorinated phenolic compounds and *Selenastrum* algal assays. Environmental Pollution (Series A) 41: 247-262.

Kukkonen, J. 1992. Effects of lignin and chlorolignin in pulp mill effluent on the binding and bioavailability of hydrophobic organic pollutants. Water Research 26: 1523-1532.

Kukkonen, J., and A. Oikari. 1992. Effects of kraft lignin and chlorolignin on the binding and bioavailability of Benzo(a)pyrene to *Daphnia magna* Straus. Bulletin of Environmental Contamination and Toxicology 48: 781-788.

LaFleur, L. E. 1996. Sources of pulping and bleaching derived chemicals in effluents. Pages 21-31 in M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Landrum, P. F., M. D. Reinhold, S. R. Nihart, and B. J. Eadie. 1985. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoya* in the presence of humic and fulvic materials and natural dissolved organic matter. Environmental Toxicology and Chemistry 4: 459-467.

Lapoint, T. W., M. T. Barbour, D. L. Borton, D. S. Cherry, W. H. Clements, J. M. Diamond, D. R. Grothe, M. A. Lewis, D. K. Reed-Judkins, and G. W. Saalfield. 1996. Field assessments: discussion synopsis. Pages 191-228 *In* D. R. Grothe, K. L. Dickson, and D. K. Reed-Judkins, eds. *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts*. SETAC Press.

Lavigne, J. R. 1979. *Instrumentation Applications for the Pulp and Paper Industry*. Miller Freeman Publications.

Leach, J. M., and A. N. Thakore. 1973. Identification of the constituents of kraft pulping effluent that are toxic to juvenile coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 30: 479-484.

Leach, J. M., and A. N. Thakore. 1976. Toxic constituents in mechanical pulping effluents. Tappi Journal 59: 129-132.

Leach, J. M., A. N. Thakore, and J. F. Manville. 1975. Acute toxicity to juvenile rainbow trout (*Salmo gairdneri*) of naturally occurring insect juvenile hormone analogues. Canadian Journal of Fisheries and Aquatic Sciences 32: 2556-2559.

LeBlanc, G. A., B. Hilgenberg, and B. J. Cochrane. 1988. Relationships between the structures of chlorinated phenols, their toxicity, and their ability to induce glutathionine S-transferase activity in *Daphnia magna*. Aquatic Toxicology 12: 147-156.

Lee, E., J. Mueller, and C. Walden. 1978. Non-settleable suspended solids: their impact on receiving waters. Pulp & Paper Canada 79: T290-T294.

Lehtinen, K. J., M. Notini, J. Mattsson, and L. Lander. 1988. Disappearance of Bladder-Wrack (*Fucus vesiculosus* L.) in the Baltic Sea: Relation to pulp-mill chlorate. Ambio 17: 387-393.

Leuenberger, C., W. Giger, R. Coney, J. W. Graydon, and E. Molnar-Kubica. 1985. Persistent chemicals in pulp mill effluents. Water Research 19: 885-894.

Lowell, R. B., J. M. Culp, and F. J. Wrona. 1995. Stimulation of increased short-term growth and development of mayflies by pulp mill effluent. Environmental Toxicology and Chemistry 14: 1529-1541.

Luthe, C. E., R. M. Berry, and R. H. Voss. 1993. Formation of chlorinated dioxins during production of bleached kraft pulp from sawmill chips contaminated with polychlorinated phenols. Tappi Journal 76: 63-69.

Luthe, C. E., P. E. Wrist, and R. M. Berry. 1992. An evaluation of the effectiveness of dioxins control strategies on organochlorine effluent discharges from the Canadian bleached chemical pulp industry. Pulp & Paper Canada 93: 40-49.

MacRae, W. D., and G. H. N. Towers. 1984. Biological activities of lignins. Phytochemistry 23: 1207-1220.

Martel, P. H., T. G. Kovacs, and R. H. Voss. 1996. Effluents from canadian pulp and paper mills: a recent investigation of their potential to induce mixed function oxygenase activity in fish. Pages 401-412 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

McCubbin, N., and J. Folke. 1993. A review of literature on pulp and paper mill effluent characteristics in the Peace and Athabasca River basins. Northern River Basin Study, Edmonton, Alberta.

McKague, A. B., J. M. Leach, R. N. Soniassay, and A. N. Thakore. 1977. Toxic constituents in woodroom effluent. Tappi Transactions of the Technical Section 3: TR75-TR81.

McKinney, A. D., and D. C. Wade. 1996. Comparative response of *Ceriodaphnia dubia* and juvenile *Anodonta imbecilis* to pulp mill effluents discharged to the Tennessee River and its tributaries. Environmental Toxicology and Chemistry 15: 514-517.

McMaster, M. E., K. R. Munkittrick, G. J. van der Kraak, P. A. Flett, and M. R. Servos. 1996. Detection of steroid hormone disruptions associated with pulp mill effluent using artificial exposures of goldfish. Pages 425-437 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

McMaster, M. E., C. B. Van Der Kraak, C. B. Portt, K. R. Munkittrick, P. K. Sibley, I. R. Smith, and D. G. Dixon. 1991. Changes in hepatic mixed function oxygenase (MFO) activity, plasma sex steroid levels and age at maturity of a white sucker (*Catastomus commersoni*) population exposed to bleached kraft pulp mill effluent. Aquatic Toxicology 21: 199-218.

Mellanen, P., T. Petänen, J. Lehtimäki, S. Mäkelä, G. Byland, B. Holmbom, E. Mannola, A. Oikari, and R. Santti. 1996. Wood-derived estrogens: studies

in vitro with breast cancer cell lines and *in vivo* in trout. Toxicology and Applied Pharmacology 136: 381-388.

Millar, K. R., J. H. Carey, B. K. Burnison, H. Lee, and J. T. Trevors. 1996. Degradation of the high molecular weight fraction of bleached kraft mill effluent by biological and photochemical processes. Pages 239-251 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Moore, J. E., and R. J. Love. 1977. Effect of a pulp and paper mill effluent on the productivity of periphyton and phytoplankton. Journal of the Fisheries Research Board of Canada 34: 856-862.

Mulla, M. S., T. Ikeshoji, and W. L. Kramer. 1974. Insect growth regulators for the control of aquatic midges. Journal of economic Entomology 67: 165-170.

Mulligan, F.S. and C.H. Schaefer. 1990. Efficacy of a juvenile hormone mimic, pyriproxyfen (S-31183), for mosquito control in dairy wastewater lagoons. Journal of the American Mosquito Control Association 6: 89-92.

Munkittrick, K. R., C. B. Portt, G. J. Van Der Kraak, I. R. Smith, and D. A. Rokosh. 1991. Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steriod levels of a Lake Superior White Sucker, (*Catastomus commersoni*) population. Canadian Journal of Fisheries and Aquatic Sciences 48: 1371-1380.

Munkittrick, K. R., G. J. Van der Kraak, M. E. McMaster, and C. B. Portt. 1992. Reproductive dysfunction and MFO activity in three species of fish exposed to bleached kraft mill effluent at Jackfish Bay, Lake Superior. Water Pollution Research Journal of Canada 27: 439-446.

Munkittrick, K. R., G. J. Van Der Kraak, M. E. McMaster, and C. B. Portt. 1994. Survey of receiving water environmental impacts associated with discharges from pulp mills. 2. Gonad size, liver size, hepatic EROD activity and plasma sex steriod levels in white sucker. Environmental Toxicology and Chemistry 13: 1089-1101.

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1978a. Characterization of dispersed residual solids in biologically treated pulp and paper mill effluents. NCASI Technical Bulletin 308, New York, 14p.

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1978b. A study of the fate of suspended solids from a full scale treatment plant in receiving waters. NCASI Technical Bulletin 313, New York, 27p.

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1982. Effects of biologically treated bleached kraft mill effluent on cold

water stream productivity in experimental streams-first progress report, NCASI Technical Bulletin 368, New York, 66p..

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1983. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental streams- second progress report, NCASI Technical Bulletin 413, New York, 80p..

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1984. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental streams-third progress report, NCASI Technical Bulletin 445, New York, 124p..

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1985. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity in experimental streams- fourth progress report, NCASI Technical Bulletin 474, New York, 177p.

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1989a. Effects of biologically treated bleached kraft mill effluent on cold water stream productivity as determined in experimental streams-fifth progress report, NCASI Technical Bulletin 566, New York, 158p..

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1989b. Pulping effluents in the aquatic environment-Part I: A review of the published literature, NCASI Technical Bulletin 572, New York, 245p

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1989c. Pulping effluents in the aquatic environment- Part II: A review of unpublished studies of in-stream aquatic biota in the vicinity of pulp mill discharges, NCASI Technical Bulletin 573, New York 129p.

National Council of the Paper Industry for Air and Stream Improvement, Inc. 1989d. Pulping effluents in the aquatic environment: A report of the scientific panel on pulping effluent in the aquatic environment, NCASI Special Report 89-08, New York, 40p.

Neilson, A. H., A. S. Allard, C. Lindgren, and M. Remberger. 1987. Transformations of chloroguaiacols, chloroveratroles, and chlorocatechols by stable consortia of anaerobic bacteria. Applied and Environmental Microbiology 53: 2511-2519.

Neilson, A. H., A. S. Allard, S. Reiland, M. Remberger, A. Tärnholm, T. Viktor, and L. Lander. 1984. Tri- and tetra-chloroveratrol, metabolites produced by bacterial *o*-methylation of tri- and tetra-chloroguaiacol: an assessment of their bioconcentration potential and their effects on fish reproduction. Canadian Journal of Fisheries and Aquatic Sciences 41: 1502-1512.

Neilson, A. H., A.-S. Allard, P.-A. Hynning, M. Remberger, and L. Landner. 1983. Bacterial methylation of chlorinated phenols and guaiacols:

formation of veratroles from guaiacols and high-molecular weight chlorinated lignin. Applied and Environmental Microbiology 45: 774-783.

Neilson, A. H., A.-S. Allard, P.-A. Hynning, M. Remberger, and T. Viktor. 1990. The environmental fate of chlorophenolic constituents of bleachery effluents. Tappi Journal 73: 239-247.

O'Connor, B. I., T. G. Kovacs, and R. H. Voss. 1992. The effect of wood species composition on the toxicity of simulated mechanical pulping effluents. Environmental Toxicology and Chemistry 11: 1259-1270.

O'Connor, B. I., and R. H. Voss. 1992. A new perspective (sorption/desorption) on the question of chlorolignin degradation to chlorinated phenolics. Environmental Science and Technology 26: 556-560.

Ogawa, I., G. A. Junk, and H. J. Svec. 1981. Degradation of aromatic compounds in ground-water, and methods of sample preservation. Talanta 28: 725-729.

Oikari, A. O. J., and T. Nakari. 1982. Kraft pulp mill effluent components cause liver dysfunction in trout. Bulletin of Environmental Contamination and Toxicology 28: 266-270.

Painter, R. H. 1958. Resistance of plants to insects. Annual Review of Entomology 3: 267-290.

Perrin, C. J., and M. L. Bothwell. 1992. Chlorate discharges from pulp mills: an examination of effects on river algal communities. Water Pollution Research Journal of Canada 27: 473-485.

Petersen, L. B.-M., and R. C. Petersen, Jr. 1984. Effect of kraft pulp mill effluent and 4,5,6 trichloroguaiacol on the net spinning behavior of *Hydropsyche angustipennis* (Trichoptera). Ecological Bulletins 36: 68-74.

Peterson, B. J., L. Deegan, J. Helfrich, J. E. Hobbie, M. Hullar, B. Moller, T. E. Ford, A. Hershey, A. Hiltner, G. Kipphut, M. A. Locke, D. M. Fiebig, V. McKinley, M. C. Miller, J. R. Vestal, R. Ventullo, and G. Volk. 1993. Biological responses of a tundra river to fertilization. Ecology 74: 653-672.

Peterson, B. J., J. E. Hobbie, A. E. Hershey, M. A. Locke, T. E. Ford, J. R. Vestal, V. L. McKinely, M. A. J. Hullar, M. C. Miller, R. M. Ventullo, and G. S. Volk. 1985. Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. Science 229: 1383-1385.

Priha, M.H. 1996. Ecotoxicological impacts of pulp mill effluents in Finland. Pages 637-650 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van Der Kraak, eds. *Environmental Fate and Effects of Pulp and Paper Mill Effluents*. St. Lucie press, Delray Beach, Florida.

Podemski, C. L., and J. M. Culp. 1996. Nutrient and contaminant effects of bleached kraft mill effluent on benthic algae and insects of the Athabasca river. Pages 571-580 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and

G. J. Van Der Kraak, eds. *Environmental Fate and Effects of Pulp and Paper Mill Effluents*. St. Lucie press, Delray Beach, Florida.

Reese, J. C., and S. D. Beck. 1976. Effects of allelochemicals on the black cutworm, *Agrotis ipsilon*: effects of catechol, l-dopa, dopamine, and chlorogenic acid on larval growth, development, and utilization of food. Annals of the Entomological Society of America 69: 68-72.

Remberger, M., A. Allard, and A. H. Neilson. 1986. Biotransformation of chloroguaiacols, chlorocatecols, and chloroveratrols in sediments. Applied and Environmental Microbiology 51: 552-558.

Renner, R. 1997. European bans on surfactant trigger - transatlantic debate. Environmental Science and Technology 31: 316A-320A.

Robinson, R. D., J. H. Carey, and k. R. Solomon. 1994. Survey of receiving-water environmental impacts associated with discharges from pulp mills. 1. Mill characteristics, receiving-water chemical profiles and lab toxicity tests. Environmental Toxicology 13: 1075-1088.

Rogers, I., H. Mahood, J. Servici, and R. Gordon. 1979. Identifying extractives toxic to aquatic life. Pulp & Paper Canada 80: T286-T290.

Rogers, I. H. 1973. Isolation and chemical identification of toxic components of kraft mills wastes. Pulp & Paper Canada 74: 111-116.

Rogers, I. H., J. C. Davis, G. M. Kruzynski, H. W. Mahood, J. A. Servizi, and R. W. Gordon. 1975. Fish toxicants in kraft effluents. Tappi Journal 58: 136-140.

Sågfors, P.-E., and B. Starck. 1988. High molecular mass lignin in bleached kraft pulp mill effluents. Water Science and Technology 20: 49-58.

Saski, E. K., J. K. Jokela, and M. S. Salkinoja-Salonen. 1996. Biodegradability of different size classes of bleached kraft pulp mill effluent organic halogens during wastewater treatment and in lake environments. Pages 179-193 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents*. St. Lucie Press, Delray Beach, FL.

Scarlett, G., and J. Harris. 1990. Biological monitoring of a pulp and paper mill wastewater. Environmental Monitoring and Assessment 14: 335-345.

Scrimgeour, G., and P. A. Chambers. 1996. Identification of spatial and temporal patterns in nutrient limitation with herbivory effects, Wapiti, Smoky and Athabasca rivers, 1994. Northern River Basins Study, Edmonton, Alberta.

Scrimgeour, G. J. 1989. Effects of bleached kraft mill effluent on macroinvertebrate and fish populations in weedbeds in a New Zealand hydroelectric lake. New Zealand Journal of Marine and Freshwater Research 23: 373-379.

SENTAR Consultants Ltd. 1997. A synthesis of information on ecotoxicity of pulp mill effluents in the Peace, Athabasca and Slave River Basins. Northern River Basins Study, Edmonton, Alberta.

Servos, M. R., D. C. G. Muir, and G. R. B. Webster. 1989. The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-*p*-dioxins. Aquatic Toxicology 14: 169-184.

Sjöström, E. 1981. *Wood Chemistry*. Academic Press, Inc., New York,.

Södergren, A., B. E. Bengtsson, P. Jonsson, S. Lagergren, A. Larsen, M. Olsson, and L. Renberg. 1988. Summary of results from the Swedish project 'Environment/Cellulose'. Water Science and Technology 20: 49-60.

Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. Annual Review of Entomology 20:417-460.

Stewart-Oaten, A., J. R. Bence, and C. W. Osenberg. 1992. Assessing effects of unreplicated perturbations: no simple solutions. Ecology 73: 1396-1404.

Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? Ecology 67: 929-940.

Stone, D., T. C. Griffing, and M. C. Knight. 1974. Biological monitoring of the Fraser River near Prince George, B.C. Pulp & Paper Canada : 100-106.

Strömberg, L., R. Mörck, F. de Sousa, and O. Dahlman. 1996. Effects of internal process changes and external treatment on effluent chemistry. Pages 3-19 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. Van der Kraak, eds. *Environmental Fate and Effects of Pulp and Paper Mill Effluents*. Florida, Delray Beach.

Suntio, L. R., W. Y. Shiu, and D. Mackay. 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere 17: 1249-1290.

Suter, G. 1993. Organism-level effects. Pages 175-246 in G. Suter, ed. *Ecological Risk Assessment*. Lewis Publishers, Chelsea, Michigan.

Swan, E. P. 1973. Resin acids and fatty acids of Canadian pulpwoods- a review of the literature. Department of the Environment, Canadian Forestry Service, Western Forest Products Laboratory.

Swanson, S., R. Shelast, R. Schryer, P. Kloepper-Sams, T. Marchant, K. Kroeker, J. Bernstein, and J. W. Owens. 1992. Fish populations and biomarker responses at a Canadian bleached kraft mill site. Tappi Journal 75: 139-149.

Talka, E., and M. Priha. 1987. Fractionation and identification of some biologically active compounds in bleached kraft mill effluent. Paperi Ja Puu 3: 220-228.

Tana, J., and K.-J. Lehtinen. 1996. The aquatic environmental impact of pulping and bleaching operations - an overview. Finnish Environmental Institute, Helsinki.

Tomar, P., and D. G. Allen. 1991. Removal of organochlorides from kraft pulp mill effluent by an aerated lagoon. Water Pollution Research Journal of Canada 26: 101-117.

Underwood, A. J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecological Applications 4: 3-15.

Van Beek, T.A. 1986. Terpenoid antifeedants, part I. An overview of terpenoid antifeedants of natural origin. Recueil des Travaux Chimiques des Pays-Bas 150:513-527.

van Wuk, D. J., and T. H. Hutchinson. 1995. The ecotoxicity of chlorate to aquatic organisms: a critical review. Ecotoxicology and Environmental Safety 32: 244-253.

Vander Wal, J. 1977. Relation between Nipigon Bay benthic macroinvertebrates and selected aspects of their habitat. Journal of the Fisheries Research Board of Canada 34: 824-829.

Verschueren, K. 1983. *Handbook of environmental data on organic chemicals.* Van Nostrand Reinhold Co., New York.

Verta, M., J. Ahtiainen, T. Nakari, A. Langi, and E. Talka. 1996. The effect of waste constituents on the toxicity of TCF and ECF pulp bleaching effluents. Pages 41-51 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Voss, R. H., C. E. Luthe, B. I. Fleming, R. M. Berry, and L. H. Allen. 1988. Some new insights into the origins of dioxins formed during chemical pulp bleaching. Pulp & Paper Canada 89: 151-161.

Wassenaar, L. I., and J. M. Culp. 1996. The use of stable isotopic analyses to identify pulp mill effluent signatures in riverine food webs. Pages 413-423 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

Whitney, A. N., and J. C. Spindler. 1959. Effects of Kraft paper wastes on a Montana stream. Transactions of the American Fisheries Society 88: 153.

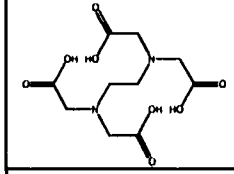
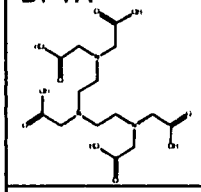
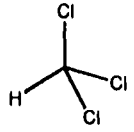
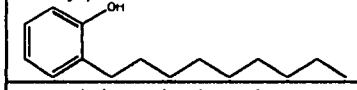
Williams, T. G., J. H. Carey, B. K. Burnison, D. G. Dixon, and H.-B. Lee. 1996. Rainbow trout (*Oncorhynchus mykiss*) mixed function oxygenase responses caused by unbleached and bleached pulp mill effluents: a laboratory-based study. Pages 379-389 *In* M. R. Servos, K. R. Munkittrick, J. H. Carey, and G. J. van der Kraak, eds. *Environmental fate and effects of pulp and paper mill effluents.* St. Lucie Press, Delray Beach, FL.

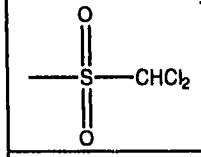
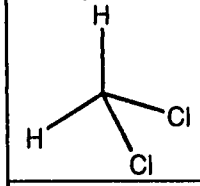
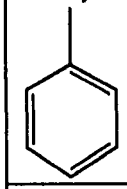
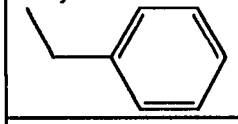
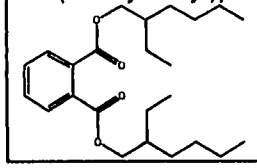
Wilson, D., and B. Hrutfiord. 1975. The fate of turpentine in aerated lagoons. Tappi Journal 76: 91-93.

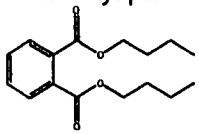
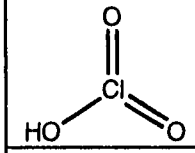
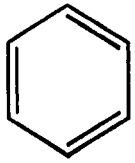
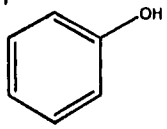
Zanella, E., J. Conkey, and M. Tesmer. 1978. Nonsettleable solids. Characteristics and use by two aquatic food chain organisms. Tappi 61: 61-65.

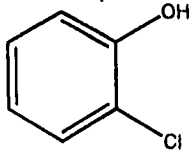
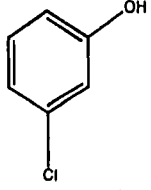
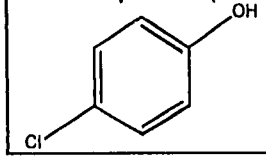
Zou, E. 1997. Effects of sublethal exposure to zinc chloride in the reproduction of the Water Flea, *Moinai irrasa* (Cladocera). Bulletin of Environmental Contamination and Toxicology 58: 437-441.

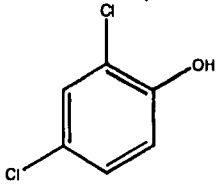
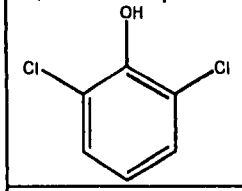
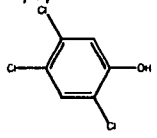
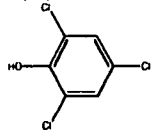
Appendix 2. Concentration, log octanol/water partition coefficient (log K_{ow}), and ecotoxicity of compounds commonly tested for in kraft pulp mill effluent (- indicates that no information was found). Please note unit difference between second and last columns.

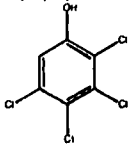
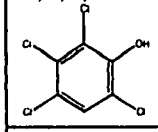
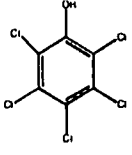
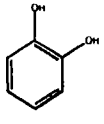
Compound	**Concentration in kraft Mill Effluents (µg/L)**	**logK_{OW}**	**Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)**
EDTA 			159 mg/L 96h LC_{50} *Lepomis macrochirus* Verscheuren 1996 11 mg/L 7d EC0 *Secendesmus quadricauda* Bringmann and Kühn 1980
DPTA 			10-100 96h LC_{50}, *Daphnia magna*, Batchelder et al. 1980 >300 96h LC_{50}, *Pimephales promelas*, Batchelder et al. 1980 111596h LC_{50}, *Lepomis macrochirus*, Batchelder et al. 1980
Chloroform 	6.5 - 108 (Voss 1983)	1.97	316 24h LC_{50} *Daphnia magna* Calleja et al. 1994 235 9d LC_{50} *Ceriodaphnia dubia* Cowgill and Milazzo 1991 3.4 9d reproduction NOEC *C. dubia* Cowgill and Milazzo 1991
nonylphenol 		4.10 - 6.36	
nonylphenol ethoxylate			1.0 96h LC_{50} *Salmo trutta*, Reiff et al. 1979 4.9 48h LC_{50} *Carrasius auratus*, Reiff et al. 1979 7.0 - 11.0 96h LC_{50} *Idus idus*, Reiff et al. 1979

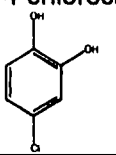
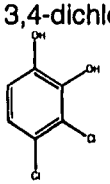
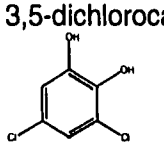
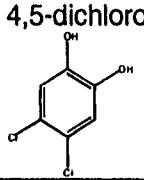
Compound	Concentration in kraft Mill Effluents (µg/L)	$\log K_{OW}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
dichlorodimethylsulfone 	6 - 429 (Voss 1983)		
trichlorodimethylsulfone	0.3 - 12.4 (Voss 1983)	7.6	
tetrachlorodimethylsulfone	0.4 - 1.1 9 (Voss 1983)		
methylene chloride 	0 - 80 (Costle et al. 1980)	1.25	
methyl benzene 		2.73	7.0 24h EC50, *Daphnia magna*, Pickering et al. 1989 5.8 96h LC50, *Salmo gairdneri*, Pickering et al. 1989 12.5 72hEC50, *Selenastrum capricornutum*, Pickering et al. 1989 28.2 95h LC50, *Poecilia reticulata*, Pickering et al. 1989
ethy lbenzene 	0 - 3 (Costle et al. 1980)	3.15	
bis(2-ethylhexyl)phthalate 	0 - 94	7.6	

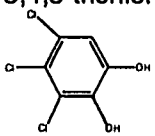
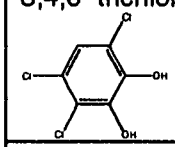
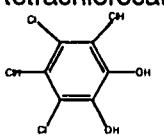
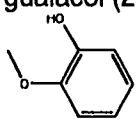
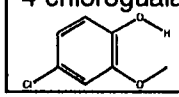
Compound	Concentration in kraft Mill Effluents (µg/L)	$logK_{OW}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
Di-n-butyl phlalate 	0-19 (Costle et al. 1980)	4.12-5.74	6.5 96h LC_{50} *Salmo gairdneri* Giam et al. 1992 1.3 96h LC_{50} *Pimephales promelas* Giam et al. 1992 0.7, 1.2 96h LC_{50} *Lepomis macrochirus* Giam et al. 1992 2.1 96h LC_{50} *Gammarus pseudolimnaeus* Giam et al. 1992 >10.0 96h LC_{50} *Orconectes mais* Giam et al. 1992
chlorate 	2 - 7500 (Dosdall et al. 1997)		3162 48h LC50, Daphnia magna Desdall et al. 1997
benzene 	0 - 3 (Costle et al. 1980)	2.13 - 2.73	203 48h LC_{50}, *Daphnia magna* Sloof et al. 1979 305 48h LC_{50}, *Daphnia pulex*, Sloof et al. 1979 1.6 8d EC_{50}, *S. capricornutum*, Herman et al.1991 >320 48h LC_{50}, *Erpobdella octoculata*, Verschueren 1996 >320 48h LC_{50}, tubificidae, Verschueren 1996 120 48h LC_{50}, *Asellus aquaticus*, Verschueren 1996 42 48h LC_{50}, *Gammarus pulex*, Verschueren 1996 130 48h LC_{50}, *Nemoura cinerea*, Verschueren 1996 34 48h LC_{50}, *Cloen dipterum*, Verschueren 1996 10 48h LC_{50}, *Ischnura elegans*, Verschueren 1996
Chlorinated Phenolics			
phenol 	0 - 29 (Costle et al. 1980)	1.46	100 24-48h LC_{50} *Daphnia magna*, Verschueren (1996) 24 96h LC_{50}, bluegill, Verschueren (1996) 32-34 96h LC_{50}, fathead minnow, Verschueren (1996) 0.23 14d LC_{50}, guppy, Verschueren (1996) 0.001 96h LC_{50}, Arctopsyche grandis, Verschueren 1996

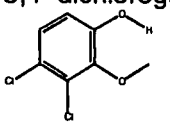
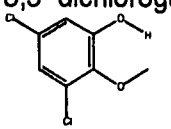
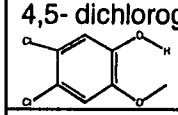
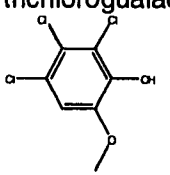
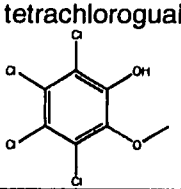
Compound	Concentration in kraft Mill Effluents (µg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
<u>chlorophenols</u> 2-chlorophenol  3-chlorophenol  4-chlorophenol	0.1 µg/L (Solomon *et al.* 1993) 6.3 Weldwood effluent (Chapter 4)	2.0 2.15-2.19 2.47-2.50 2.39-2.44	2.1 96hrLC$_{50}$,rainbow trout, Voss et al. 1980 (2-) 2.9 96h LC$_{50}$, rainbow trout, Voss et al. 1980 (2-) 3.7 7d LC$_{50}$, *Daphnia magna*, Verschueren 1996 (2-) 6.4 96hrLC$_{50}$, guppy, Konemann & Musch 1981 (2-) 7.2 96hrLC$_{50}$, bluegill, Buccafusco et al. 1981 (2-) 9.75 96hrLC$_{50}$, fathead minnow, Phipps et al. 1981 (2-) 10.9 96hrLC$_{50}$, guppy, Konemann & Musch 1981 (2-) 12.3 96h LC$_{50}$, fathead minnow, Verschueren 1996 (2-) 13.8 96hrLC$_{50}$, guppy, Verschueren 1996 (2-) 2.8 96hrLC$_{50}$, rainbow trout, Verschueren 1996 (3-) 29 96h LC$_{50}$, *Selenastrum capricornutum* (3-) 7.9 48h EC$_{50}$, *Daphnia magna*, Verschueren 1996 (3-) 0.02 96h LC$_{50}$, rainbow trout fry, NCASI 1992 (4-) 1.9 96h LC$_{50}$, rainbow trout, NCASI 1992 (4-) 2.3 7d LC$_{50}$, Daphnia magna, Verschueren 1996 (4-) 4.0 96h LC$_{50}$, bluegill, Buccafusco et al. 1981 (4-)
4-chlorophenol (continued) 			6.2 96h LC$_{50}$, fathead minnow, Verschuren 1996 (4-) 8.49 96h LC$_{50}$, guppy, Verschueren 1996 (4-)

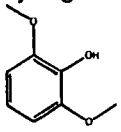
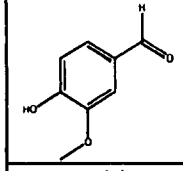
Compound	Concentration in kraft Mill Effluents (µg/L)	$logK_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
<u>dichlorophenol</u> 2,4-dichlorophenol  2,6-dichlorophenol 	9-15 (CEPA, 1991) 0.1-8.0 (Solomon *et al.* 1993) 0.8 (Costle et al. 1980) 20.7 Weldwood effluent (Chapter 4)	3.1 2.7 - 3.41 2.57 - 3.36	 9.2 96hLC_{50} Chlorella vularus Pickering et al. 1989 14 96hLC_{50} Selenastrum capricornutum Pickering et al. 1989 1.7 7dLC_{50}, guppy, Verschueren 1996 (2,4-) 2.6 96h LC_{50}, rainbow trout, NCASI 1992 (2,4-) 2.6 7d LC_{50}, *Daphnia magna*, Verschueren 1996 (2,4-) 5.5 96h LC_{50}, guppy, Verschueren 1996 (2,4-) **8.2** 96h LC_{50}, fathead minnow, Verschueren 1996 (2.4-) 3.4, 4.748h EC_{50}, *Daphnia magna*, Verschueren 1996 (2,6-) **4.1** 96h LC_{50}, rainbow fry, NCASI 1992 (2,6-) 7.8 96h LC50, guppy, Verschueren 1996 (2,6-) 29 4d EC_{50}, Selenastrum capricornutum, Verschueren 1996 (2,6-)
<u>trichlorophenols</u> 2,4,5- trichlorophenol  2,4,6- trichlorophenol 	0.1 - 6.0 (Solomon *et al.* 1993) - 1 - 51 (CEPA, 1991) 0 - 6 (Costle et al. 1980) 6.6 Weldwood effluent (Chapter 4)	3.7 3.06 - 4.10 2.8 - 4.03	0.45-2.6 CEPA 0.0012 96hr LC_{50}, guppy, Verschueren 1996 (2,4,5-) 0.9 96hr LC_{50}, rainbow trout, NCASI 1992 (2,4,5-) 0.9, 1.27 96hr LC_{50}, fathead minnow, Verschueren 1996 (2,4,5-) 3.5 7d LC_{50}, Daphnia magna, Verschueren 1996 (2,4,5-) 0.69 48h LC_{50}, Daphnia magna, Verschueren 1996 (2,4,6-) 0.730 96h LC_{50}, rainbow trout, NCASI 1992 (2,4,6-) 9.15 96h LC_{50}, fathead minnow, Verschueren 1996 (2,4,6-) 0.88 48h LC50, red killifish, Yoshioka et al. 1986 (2,4,6-)

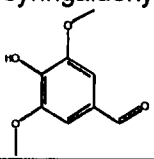
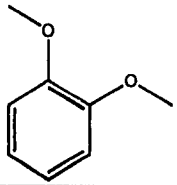
Compound	Concentration in kraft Mill Effluents (µg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
<u>tetrachlorophenol</u>	0.1-2.0 (Solomon *et al.* 1993)	4.5	
2,3,4,5- tetrachlorophenol 		4.21 - 5.3	0.44 96h LC50, fathead minnow, Hall & Kier 1984 (2,3,4,5-) 0.77 96h LC50, guppy, Konneman & Musch 1981 (2,3,4,5-) 0.5 96h LC50, rainbow trout, Voss *et al.* 1980 (2,3,4,5-)
2,3,4,6- tetrachlorophenol 		4.1 - 5.03	0.75 96h LC50, goldfish, Verschueren 1983 (2,3,4,6-) 0.21 96h LC50, rainbow trout, NCASI 1992 (2,3,4,6-) 0.48 96h LC50, rainbow trout, Voss *et al.* 1980(2,3,4,6-) 0.4 96h LC50, bluegill, Buccafusco *et al.* 1981 (2,3,4,6-) 1.4 96h LC50, guppy, Konneman & Musch 1981 (2,3,4,6-)
pentachlorophenol 	0.1-1.0 (Solomon *et al.*1993) 0 - 21 (Costle et al. 1980)	5.05 5.0 4.07-5.1	0.2 CEPA 0.16 96h LC_{50}, rainbow trout, NCASI 1992 0.53 7d LC_{50}, Daphnia magna, Verschueren 1996 0.19-0.6 96h LC_{50}, fathead minnow, Verschueren 1996 0.141, 0.21 8d LC_{50}, fathead minnow, Verscueren 1996
catechol 		0.53 - 1.28	4.0 LD_0, Daphnia , Verschueren 1996

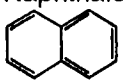
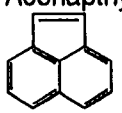
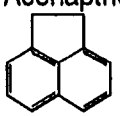
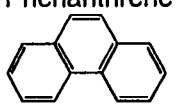
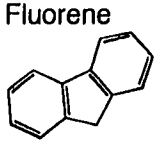
Compound	Concentration in kraft Mill Effluents (μg/L)	$logK_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
monochlorocatechol 4-chlorocatechol 	ND-0.1 (Solomon et al, 1993) 7.1 Weldwood effluent (Chapter 4)	2.0	0.7 NOEC, zebrafish egg/larvae, NCASI 1992 (4-)
dichlorocatechol 3,4-dichlorocatechol 	0.1-83 (Solomon et al. 1993) 12-90 (CEPA 1991)	3.2	1.8 EC50, Selenastrum capricornutum, NCASI 1992 (3,4-) 2.7 96h LC50, sockeye fry, Voss et al. 1980 (3,4-) 2.7 96h LC50, *O. nerca* (salmon), NCASI 1992 (3,4-)
3,5-dichlorocatechol  4,5-dichlorocatechol 	15.0 Weldwood effluent (Chapter 4)		0.5 NOEC, zebrafish egg/larvae, NCASI 1992 (3,5-) 0.1 96h LC50, rainbow trout, NCASI 1992 (4,5-) 0.5 NOEC, zebrafish egg/larvae, NCASI 1992 (4,5-)

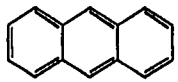
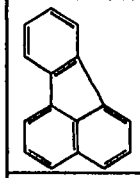
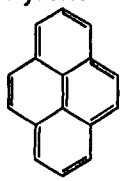
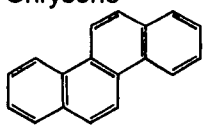
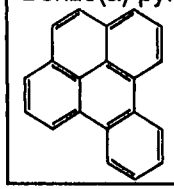
Compound	Concentration in kraft Mill Effluents (µg/L)	$logK_{OW}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
<u>trichlorocatechols</u>	0.5-1.0 (CEPA 1991) 0.1-41 (Solomon et al. 1993)		1.0 - 1.5 96hLC_{50} rainbow trout, McKague 1979
3,4,5-trichlorocatechol 	2-19 (Verschueren 1996) 3.7 Weldwood effluent (Chapter 4)	3.75	1.0 96h LC_{50}, rainbow trout NCASI 1992, (3,4,5-) 0.2 NOEC, zebrafish egg/larvae, NCASI 1992 (3,4,5-)
3,4,6- trichlorocatechol 		3.64	0.9 96h LC_{50} Brown trout, NCASI, 1992 (3,4,6-) 0.35 NOEC, zebrafish egg/larvae, NCASI 1992 (3,4,6-)
tetrachlorocatechol 	0.4-5 (Verschueren 1996) 3.2 Weldwood effluent (Chapter 4)		0.8 96h LC_{50}, rainbow trout, Voss et al. 1980
guaiacol (2-methoxyphenol) 		1.33	44 96h LC_{50}, rainbow trout, Voss et al. 1980
<u>chloroguaiacol</u> 3-chloroguaiacol 4-chloroguaiacol 	0.2- 1.0 (Solomon *et al* 1993) 280 Weldwood effluent 98.8 Weldwood effluent (Chapter 4)	2.15 2.11-2.52	0.7 NOEC zebrafish egg/larvae, NCASI 1992 (4-)

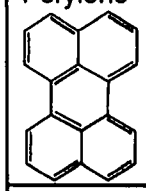
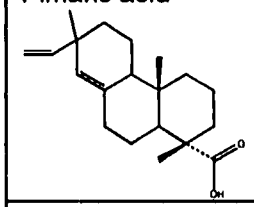
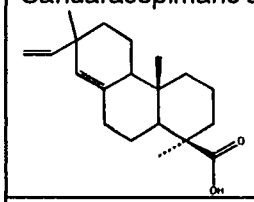
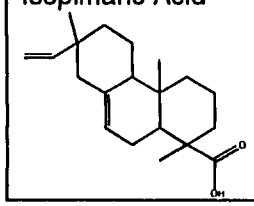
Compound	Concentration in kraft Mill Effluents (μg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
dichloroguaiacol	22-100 (CEPA 1991) 0.1-43 (Solomon *et al.* 1993)	3.2	
3,4- dichloroguaiacol 	19.0	2.55-3.23	0.27 EC_{50}, Selenastrum capricornutum, NCASI 1992 (3,4-) 2.7 96h LC50, Salmon (O. nerca), NCASI 1992 (3,4-)
3,5- dichloroguaiacol 			0.5 NOEC zebrafish egg/larvae, NCASI 1992 (3,5-)
4,5- dichloroguaiacol 	2-8 (Verschueren 1996) 33.7 Weldwood effluent (Chapter 4)	2.51 - 3.41 (4,5-)	0.5 NOEC zebrafish egg/larvae, NCASI 1992 (4,5-) 2.2 96h LC_{50}, rainbow trout, NCASI 1992 (4,5-) 3.1-6.2 96hLC_{50}, Daphnia magna, NCASI 1992 (4,5-)
trichloroguaiacol 	10-620 (CEPA 1991) 0.1-5 (Solomon *et al.* 1993) 0 (Costle et al. 1980) 2.9 Weldwood effluent (Chapter 4)	3.77 - 4.32	0.7 - 1.0 96h LC_{50}, rainbow trout, Leach 1979 1.69 96h LC_{50}, rainbow trout, *Voss et al.* 1980 0.96 48h LC_{50}, Daphnia magna, NCASI 1992 1.8 48h LC_{50}, Ceriodaphnia dubia, NCASI 1992 0.75 96hLC50 rainbow trout, Bright et al. 1997 2.2 48h LC_{50}, rainbow trout, NCASI 1992
tetrachloroguaiacol 	0.7 - 1.7 (CEPA) 0.1-5 (Solomon *et al.* 1993) 0-3 (Verschueren 1996) 0 (Costle et al. 1980) 1.7 Weldwood effluent (Chapter 4)	4.28 - 5.01	0.2-1.7 96h LC_{50} (CEPA) 0.3 96h LC_{50} rainbow trout, NCASI 1992 0.15 96h LC_{50} zebrafish egg/larvae, NACSI 1992 0.32 96h LC_{50} rainbow trout, Verschueren 1996 0.2 - 0.4 96h LC50, rainbow trout, Leach 1977

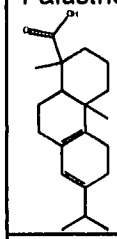
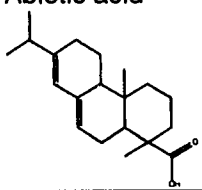
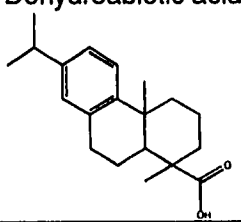
Compound	Concentration in kraft Mill Effluents (μg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
syringol 		1.15	
trichlorosyringol		4.2	0.8 96h NOEC, zebrafish egg/larvae, NCASI 1992
Vanillins 	410-2914 (Keith 1976)	1.21	121, 112 96 h LC_{50}, fathead minnow, Verschueren 1996
monochlorovanillin 5-chlorovanillin 6-chlorovanillin	4-28 (Solomon et al. 1993) 7.1 Weldwood effluent 330.0 Weldwood effluent (Chapter 4)		
dichlorovanillin	0-10 (Solomon et al. 1993) 3.9 Weldwood effluent (Chapter 4)		

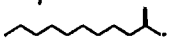
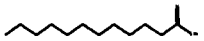
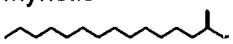
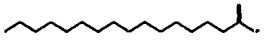
Compound	Concentration in kraft Mill Effluents (µg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
syringaldehyde 	40-55.8 (Keith 1976)		
Veratrole 		1.6	
dichloroveratrole	0.1-1.6 (4,5-) (Solomon et al. 1993)		
trichloroveratrole	0.1-1.7 (3,4,5-) (Solomon et al. 1993)		0.45 LOEC *Brachydanio rerio*, Neilson et al. 1990
tetrachloroveratrole	0.1-0.2 (Solomon et al. 1993)		0.1 LOEC *Brachydanio rerio*, Neilson et al. 1990

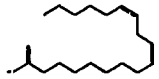
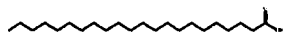
Compound	Concentration in kraft Mill Effluents (µg/L)	$logK_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
Polycyclic Aromatic Hydrocarbons			
Naphthalene 	8.5 Weldwood effluent (Chapter 4)	3.01 - 4.7	2.0-8.9 96h LC_{50}, fathead minnow, Verschueren 1996 1.0 96h LC_{50}, Daphnia pulex, Verschueren 1996 1.6 96h LC_{50}, rainbow trout, Verscueren 1996 6.08 96h LC_{50}, fathead minnow. Holcombe et al. 1984 **7.9** 96h LC_{50}, fathead minnow. Degraeve et al. 1982 >6.7 24h LC_{50}, *Aedes aegypti* Verschueren 1996 2.8 48h LC_{50}, *Chironomus tentans* Verschueren 1996
Acenapthylene 	1000 (Keith 1976)	3.55-4.08	
Acenapthene 	1.0 Weldwood effluent (Chapter 4)	3.32 - 4.49	0.06-2.1 48h LC_{50}, *Paratanytarsus*, Verschueren 1996 3.5, 41 48h EC_{50}, *Daphnia magna*, Verschueren 1996 0.67 96h LC50, rainbow trout, Holcombe et al. 1983 0.58 96h LC50, brown trout, Holcombe et al. 1983 1.60 96h LC50, fathead minnow, Holcombe et al. 1983 1.7 96h LC50, Bluegill, Bucafucso et al. 1981
Phenanthrene 	2.0 Weldwood effluent (Chapter 4)	4.57	0.10 96h LC_{50}, *Daphnia pulex*, Verschueren 1996 >sat 96 h LC_{50}, fathead minnow, Verschueren 1996 0.5 48h LC_{50}, *Chironomus tentans*, Verschueren 1996 0.5 24h LC_{50}, *Aedes aegypti*, Verschueren 1996
Fluorene 	0.0371-0.0676 (Neilson et al. 1992) 0.69 Weldwood effluent (Chapter 4)	3.70 - 4.38	0.43 48h EC50, *Daphnia magna*,

Compound	Concentration in kraft Mill Effluents (µg/L)	logK_{ow}	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
Anthracene 		4.45	0.027 48h LC_{50} Aedes aegypti, Vershueren 1996 0.035, 3.03 48h LC_{50}, *Daphni magna*, Verschueren 1996 0.001, >0.03 24hEC_{50}, *Daphnia pulex*, Verschueren 1996
Fluoranthene 	0 (Costle et al. 1980) 1.9 Weldwood effluent (Chapter 4)	4.7 - 6.5	54 96h EC_{50} *Selenastrum capricornutum*, Vershueren 1996 4.0 96h LC_{50}, bluegill, Verschueren 1996 320 48h LC_{50}, *Daphnia magna*, Vershueren 1996 0.1, 0.2 24h LC_{50}, fathead minnow, Verschueren 1996 320 48h LC_{50}, *Daphnia magna*, LeBlanc 1980 >0.099 24h LC50 *Artemia*, Abernathy et al. 1986
Pyrene 	1.4 Weldwood effluent (Chapter 4)	4.45 - 6.7	
Chrysene 		5.61	0.228 48h NOEC, *Daphnia magna*, Eastmond et al. 1984 1.9 2h LC_{50}, *Daphnia magna*, Verschueren 1996 1.7 24h LC_{50}, *Aedes aegypti*, Verschueren 1996
Benzo(a) pyrene 		6.04	0.008 12h LC_{50}, *Aedes aegypti*, Verschueren 1996 0.005 96h LC_{50}, *Daphnia pulex*, Verschueren 1996 >4.0 72h EC_{50}, *Chlamydomonas reinhardtii*, Pickering et al. 1989 0.015 72h EC_{50}, *Selenastrum capricornutum*, Pickering et al. 1989.

Compound	Concentration in kraft Mill Effluents (µg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
Perylene 		6.12, 6.5	0.00075 24h EC_{50}, *Daphnia magna*, Verschueren 1996 0.00015 48h EC_{50}, *Brachydanio rerio*, Verschueren 1996
Resin Acids			
Pimaric acid 	0-790 (Costle et al. 1980) 196.3, 800 (Keith 1976) 150 Weldwood effluent (Chapter 4)		0.8 96h LC_{50}, rainbow trout, Leach and Thakore (1976)
Sandaracopimaric acid 	45 (Keith 1976)		
Isopimaric Acid 	160-590 (Costle et al. 1980) 184.3 (Keith 1976)		0.7 96h LC_{50}, *Oncorhynchus nerka*, Servizi et al. (1986) 1.3 96h LC_{50}, *Daphnia magna*, Servizi et al. (1986 0.4 96h LC_{50}, rainbow trout, Kutney et al. (1981) 0.4 96h LC_{50}, rainbow trout, Leach and Thakore. (1976)

Compound	Concentration in kraft Mill Effluents (µg/L)	$\log K_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (Isomer)
Palustric Acid 			0.5 96h LC_{50}, rainbow trout, Leach and Thakore. (1976) 0.32 96h LC_{50}, rainbow trout juveniles, Leach and Thakore. (1978)
Abietic acid 	0-2500 (Costle et al. 1980)		1.2 96h LC_{50}, *Oncorhynchus nerka* , Servizi et al. 1986 0.7 96h LC_{50}, rainbow trout, Leach and Thakore. (1976)
Dehydroabietic acid 	0-1000 (Costle et al. 1980) 110 Weldwood effluent (Chapter 4)		2.1 96h LC_{50}, *Oncorhynchus nerka*, Servizi et al. 1986 5.5 96h LC_{50}, *Daphnia magna*, Servizi et al. 1986 1.1 96h LC_{50}, rainbow trout, Leach and Thakore. (1976) 1.03-1.85 96h LC_{50}, rainbow trout, Davis and Hoos. (1975)
chlorodehydroabeitic	0 - 700 (Costle et al. 1980) 6 (Claeys et al. 1983) (12-) 17 (Claeys et al. 1983) (14-)		1.03 96h LC_{50}, *Oncorhynchus mykiss*, Kennedy et al. 1995 0.6 - 0.9 96 LC_{50}, rainbow trout, Leach 1980

Compound	Concentration in kraft Mill Effluents (μg/L)	$logK_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
dichlorodehydroabietic	0 - 65 (Costle et al. 1980) 39 (Claeys et al. 1983)		0.19 96h LC_{50}, *Oncorhynchus mykiss*, Kennedy et al. 1995 0.6 - 1.02 LC_{50}, *Oncorhynchus mykiss*, Leach 1980
Fatty Acids			
Capric 	280 Weldwood effluent (Chapter 4)	1.88	20 - 31 96h LC_{50}, red killifish, Onitsuka et al. 1989 41 96h LC_{50}, *Gammarus*, Onitsuka et al. 1989
Lauric 		4.2	Saturation 96h LC_0, red killifish, Onitsuka et al. 1989 Saturation 96h LC_0, Gammarus, Onitsuka et al. 1989
Myristic 	20 - 23.2 (Keith 1976) 1800 Weldwood effluent (Chapter 4)	4.15	2.74 30 min EC_{50}, *Photobacterium phosphoreum*, Carlson-Ekvall and Morrison 1995 150 96h LC_{50}, red killifish, Onitsuka et al. 1989
Palmitic 	388.5- 430 (Keith 1976) 6900 Weldwood effluent (Chapter 4)		4.10 30 min EC_{50}, *Photobacterium phosphoreum*, Carlson-Ekvall and Morrison 1995

Compound	Concentration in kraft Mill Effluents (µg/L)	$logK_{ow}$	Effect Concentration (mg/L) concentration & test type, test species, reference (isomer)
Linoleic 	0-510 (Costle et al. 1980) 700 Weldwood effluent (Chapter 4)		
Linolenic 	0 - 170 (Costle et al. 1980) 550 Weldwood effluent (Chapter 4)		
Oleic 	0-810 (Costle et al. 1980) 1400 Weldwood effluent (Chapter 4)	7.73	35.0 30 min EC_{50}, *Photobacterium phosphoreum*, Carlson-Ekvall and Morrison 1995 205 96h LC_{50}, *Pimephales promelas*, Verschueren 1996 217 96h LC_{50}, Red Killifish, Verschueren 1996 (Na salt)
Behenic 	180 -Weldwood effluent (Chapter 4)		

Literature Cited

Alexander, H. C., W. M. McCarty, E. A. Bartlett. 1978. Toxicity of perchlorethylene, trichloroethylene, 1,1,1-trichloroethane, and methylene chloride to fathead minnows. Bulletin of Environmental Contamination and Toxicology 20:344-352.

Batchelder, T.L., A. H.C., and W.M. McCarty. 1980. Acute fish toxicity of the versene family of chelating agents. Bulletin of Environmental Contamination and Toxicology 24: 543-549.

Bright, D., P. Hodson, K.-J. Lehtinen, B. McKague, J. Rodgers, and K. Solomon. 1997. Evaluation of ecological risks associated with the use of chlorine dioxide for the bleaching of pulp: scientific progress since 1993. *3rd International Conference on Environmental Fate and Effects of Pulp and Paper Mill Effluents*, Rotorua, New Zealand.

Bringmann, G., and K. R. 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Research 14: 231-241.

Buccafusco, R. J., S. J. Ellis, and G. A. LeBlanc. 1981. Acute toxicity of priority pollutants to bluegill (*Lepomis macrochirus*). Bulletin of Environmental Contamination and Toxicology 26: 446-452.

Calleja, M.C., G. Persoone, and P. Geladi. 1994. Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to non-vertebrates. Archives of Environmental Contamination and Toxicology 26: 69-78.

Canadian Environmental Protection Act. 1991 Priority Substances List Assessment Report No. 2, Effluents for Pulp Mills Using Bleaching. 60p.

Carlson-Erkvall, C. E. A., and G. M. Morrison. 1995. Toxicity of copper in the presence of organic substances in sewage sludge. Environmental Technology 16: 243-251.

Claeys, R.R., L.E. LaFleur, and D.L. Borton. 1980. Chlorinated organics in bleach plant effluents of pulp and paper mills. Pages 335-345 in R. L. Jolley, W. A. Brungs, and R. B. Cumming, eds. *Water chlorination: environmental impact and health effects.* Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Costle, D.M., J.D. Denit, and R.W. Dellinger. 1980. Development document for effluent limitations guidelines and standards for the pulp, paper and paperboard and the builders' paper and board mills - point source categories. United States Environmental Protection Agency, Washington, D.C.

Cowgill, U.M., and D.P. Milazzo. 1991. The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals utilizing the three-brood test. Archives of Environmental Contamination and Toxicology 20: 211-217.

Davis, J. C., and R. A. W. Hoos. 1975. Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays. Journal of the Fisheries Research Board of Canada 32: 411-416.

DeGraeve, G. M., R. G. Elder, D. C. Woods, and H. L. Bergman. 1982. Effects of naphthalene and benzene on fathead minnows and rainbow trout. Archives of Environmental Contamination and Toxicology 11: 487-490.

Dellinger, R. W. 1980. Development Document for Effluent Limitation Guidelines and Standards for the Pulp, Paper and Paperboard and the Builder's Paper and Board Mills. U.S. Environmental Protection Agency, Washington, D.C.

Hall, L.H., and L.B. Kier. 1984. Molecular connectivity of phenols and their toxicity to fish. Bulletin of Environmental Contamination and Toxicology 32: 354-362.

Hattula, M.L., V.-M. Wasenius, H. Reunanen, and A.U. Arstila. 1981. Acute toxicity of some chlorinated phenols, catechols and cresols to trout. Bulletin of Environmental Contamination and Toxicology 26: 295-298.

Hermand, D. C. 1991. The relationship between toxicity and bioconcentration of volatile aromatic hydrocarbons by the alga *Selenastrum capricornutum*. Chemosphere 22:665-676.

Holcombe, G. W., G. L. Phipps, and J. T. Fiandt. 1983. Toxicity of selected priority pollutants to various aquatic organisms. Ecotoxicology and Environmental Safety 7: 400-409.

Keith, L. H. 1976. Identification of organic compounds in unbleached treated kraft paper mill wastewaters. Environmental Science and Technology 140: 555-564.

Kennedy, C. J., R. M. Sweeting, J. A. Johansen, A. P. Farrell, and B. A. McKeown. 1995. Acute effects of chlorinated resin acid exposure on juvenile rainbow trout, *Oncorhynchus mykiss*. Environmental Toxicology and Chemistry 14: 977-982.

Konemann, H., and A. Musch. 1981. Quantitative structure-activity relationships in fish toxicity studies part 2: the influence of pH on the QSAR of chlorophenols. Toxicology 19: 23-228.

Kuhn, R., M. Pattard, K.-D. Pernak, and W. Winter. 1989. Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. Water Research 23: 501-510.

Kutney, J. P., M. Singh, E. Dimitriadis, G. M. Hewitt, P. J. Salisbury, B. R. Worth, J. A. Servizi, D. W. Martens, and R. W. Gordon. 1981. Studies

related to biological detoxification of kraft pulp mill effluent. II. The biotransformation of isopimaric acid with *Mortierella isabellina*. Canadian Journal of Chemistry 59: 3350-3355.

Leach, J. M., and A. N. Thakore. 1976. Toxic constituents in mechanical pulping effluents. Tappi Journal 59: 129-132.

Leach, J.M. 1980. Loadings and effects of chlorinated organics from bleached pulp mills. Pages 325-334 in R. L. Jolley, W. A. Brungs, and R. B. Cumming, eds. *Water chlorination: environmental impact and health effects*. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Leach, J.M., and A.N. Thakore. 1973. Identification of the constituents of kraft pulping effluent that are toxic to juvenile coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 30: 479-484.

Leach, J.M., and A.N. Thakore. 1976. Toxic constituents in mechanical pulping effluents. Tappi Journal 59: 129-132.

LeBlanc, G.A. 1980. Acute toxicity of priority pollutants to water flea (*Daphnia magna*). Bulletin of Environmental Contamination and Toxicology 24: 684-691.

National Council of the Paper Industry for Air and Stream Improvement. 1992. A compilation of data on chemical and biological properties of 28 chlorinated phenolic compounds, Research Triangle Park, NC.

Neilson, A. H., A.-S. Allard, P.-A. Hynning, M. Remberger, and T. Viktor. 1990. The environmental fate of chlorophenolic constituents of bleachery effluents. Tappi Journal 73: 239-247.

Phipps, G. L., G. W. Holcombe, and J. T. Fiandt. 1981. Acute toxicity of phenol and substituted phenols to the fathead minnow. Bulletin of Environmental Contamination and Toxicology 26: 585-593.

Pickering, Q., D.O. Carle, A. Pilli, T. Willingham, and J.M. Lazorchak. 1989. Effects of pollution on freshwater organisms. Journal of the Water Pollution Control Federation 61: 998-1042.

Reiff, B., R. Lloyd, M.J. How, D. Brown, and A. J.S. 1979. The acute toxicity of eleven detergents to fish: results of an interlaboratory exercise. Water Research 13: 207-210.

Saarikoski, J., and M. Viluksela. 1982. Relation between physicochemical properties of phenols and their toxicity and accumulation in fish. Ecotoxicology and Environmental Safety 6: 501-512.

Servizi, J. A., D. W. Martens, R. W. Gordon, J. P. Kutney, M. Singh, and E. Dimitriadis. 1986. Microbiological detoxification of resin acids. Water Pollution Research Journal of Canada 21: 119-129.

Sloof W. 1979. Comparison of the susceptibility of 22 freshwater species to 15 chemical compounds. I. Sub(acute) toxicity tests. Aquatic Toxicology 4:113-128.

Solomon, K., H. Bergman, R. Hugget, D. Mackay, and B. McKague. 1993. A review and assessment of the ecological risks associated with the use of chlorine dioxide for the bleaching of pulp.

Verschueren, K. 1983. *Handbook of environmental data on organic chemicals.* Van Nostrand Reinhold Co., New York.

Voss, R. H. 1983. Chlorinated neutral organics in biologically treated kraft mill effluents. Environmental Science and Technology 17: 530-537.

Voss, R. H., J. T. Wearing, R. D. Mortimer, T. Kovacs, and A. Wong. 1980. Chlorinated organics in kraft bleaching effluents. Paperi ja Puu. 62: 809-814.

Voss, R.H. 1983. Chlorinated neutral organics in biologically treated kraft mill effluents. Environmental Science and Technology 17: 530-537.

Yoshioka, Y., T. Mizuno, Y. Ose, and T. Sato. 1986. The estimation for toxicity of chemicals on fish by physio-chemical properties. Chemosphere 15: 195-203.

Appendix 3

Appendix 3 contains data on effluent composition and toxicity for the period of January 1993 to December 1995. The data has been summarized from monthly reports by Weldwood of Canada Ltd., Hinton Division, to Alberta Environmental Protection, in compliance with the mill's water license. There are five tables: A3.1 contains general quality parameters, A3.2 contains the results of dioxin and furan analyses, A3.3 contains results of analysis for heavy metal composition, A3.4 contains data from analyses of resin and fatty acids, and finally, A3.5 gives the results of chronic toxicity tests that have been conducted as a part of the mill's environmental effects monitoring program. Empty cells indicate that analysis for that parameter was not required during that sampling period. Data are available courtesy of Weldwood of Canada, Hinton Division, and Alberta Environmental Protection.

Table A3.1 Summary of general effluent quality parameters for January 1993-December 1995. Shaded cells indicated that data was calculated with one or more censored values; one half of the detection limit was used in cases where the value was reported as below the detection limit.

	BOD1	BOD5	NO3	NO2	NH3	TKN	TP	DP	AOX	Total Phenol	Chlor Phenol	Chlorate	Chlorite	Bioassay		Town COD
Date	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppb	ppm	ppm	trout	Daphnia	ppm
Jan-93	11.5	25.4	0.60	0.08	0.48	5.70	0.71	0.41	11.1	0.053	62			100	100	349
Feb-93	11.0	21.1	0.46	0.09	0.55	6.15	0.64	0.35	12.1	0.100	60.9	<.5	<10	100	100	136
Mar-93	8.3	17.2	0.29	0.25	1.08	3.10	0.51	0.18	8.6	0.079	52.4	ND	5.8	100	100	518
Apr-93		18.1	0.09	0.58	1.63	4.30	0.94	0.25	7.7	0.870	80.9	ND	1.83	100	100	185
May-93		16.7	0.08	0.18	0.95	3.98	0.51	0.16	5.7	0.098	30.14	12.68	4.45	100	***	194
Jun-93		17.3	0.19	0.15	1.33	3.96	0.60	0.22	8.8	0.055	24.4	<1	<1	100	100	213
Jul-93		17.0	0.08	0.16	1.61	2.80	0.59	0.19	2.6	0.011	12.9	0	0	100	100	133
Aug-93		12.5	0.10	0.37	2.18	3.16	0.61	0.42	1.6	0.098	ND	<1	<1	100	100	136
Sep-93		17.6	0.15	0.30	1.60	5.00	0.91	0.30	2.3	0.027	1.8	<1	<1	100	100	124
Oct-93		16.4	0.10	0.18	2.63	5.20	1.46	0.76	2.7	0.015	ND	<1	1.2	100	100	100
Nov-93	10.7***	20.4	0.09	0.11	0.91	3.54	0.66	0.10	1.7	0.068	ND	6.7	<1.0	100	100	634
Dec-93	10.3	20.4	0.31	0.28	1.83	5.10	0.60	0.12	1.6	0.119	ND	<1	3.7	100	100	1336
Jan-94	15.6	30.1	0.06	0.05	1.06	4.90	0.62	0.04	2.3	0.084	ND	14	2.6	100	100	432
Feb-94	9.2	20.7	0.05	0.08	2.11	5.55	0.62	0.18	2.8	0.011	0.49	<1	2.5	100	100	2449
Mar-94	8.7	17.9	0.08	0.19	1.95	4.32	0.77	0.34	2.5	0.014	0.16	2.1	3.3	100	100	1688
Apr-94		14.6	0.05	0.17	1.26	3.40	0.64	0.14	1.7	0.021	0.12	<1	3	100	100	418
May-94		18.9	0.63	0.18	1.37	3.92	0.50	0.18	1.9	0.005	ND	<1	<1	100	100	189
Jun-94		13.0	0.44	0.21	1.52	2.80	0.86	0.43	1.8	0.020	ND	<1	<1	100	100	517
Jul-94	5.5	13.9	0.10	0.20	1.96	3.18	0.85	0.47	2.1	0.009	ND	<1	<1	100	100	541
Aug-94		13.5	0.07	0.17	2.09	3.38	0.87	0.51	2.0	0.018	ND	<1	<1	100	100	348
Sep-94	4.3	10.5	0.11	0.52	1.89	3.45	0.85	0.55	2.06	0.005	0.23	<1	<1	100	100	407
Oct-94	5.9	12.0	0.29	0.52	2.04	3.95	0.87	0.50	1.8	0.004	0.28	<1	<1	100	100	564
Nov-94	8.6	17.7	0.15	0.19	0.79	3.68	0.65	0.18	2.4	0.007	<.10	4.7	<1	100	100	1232
Dec-94	8.7	17.0	0.36	0.25	1.45	3.38	0.80	0.35	2.9	0.017	ND	1.0	2.4	100	100	726
Jan-95	8.2	15.8	0.17	0.22	1.10	3.08	0.88	0.44	2.9	0.003		34	3.1	100	100	1072
Feb-95	6.3	12.7	0.10	0.41	1.29	3.14	0.98	0.57	2.5	0.006	0.10	<1	<1	100	100	1823
Mar-95	8.1	17.7	0.21	0.34	1.19	3.38	0.54	0.19	2.8	0.006	ND	1.4	<1	100	100	1131
Apr-95		12.0	0.10	0.32	1.01	2.68	0.33	0.16	2.4	0.005				100	100	1802
May-95		13.7	0.09	0.31	1.44	3.10	0.43	0.23	2.2	0.030				100	100	205
Jun-95		10.5	0.05	0.33	1.62	3.24	0.57	0.40	1.8	0.051				100	100	210
Jul-95		11.2	0.07	0.29	1.59	3.92	0.69	0.46	1.7	0.024				100	100	148
Aug-95		12.4	0.05	0.28	1.99	5.02	0.54	0.30	1.9	0.018				100	100	389
Sep-95		17.7	0.05	0.12	0.70	3.43	0.54	0.29	2.0	0.005				100	100	
Oct-95		15.2	0.05	0.09	0.78	3.46	0.58	0.33	1.8	0.007				100	100	139
Nov-95		18.8	0.05	0.05	0.91	4.40	0.45	0.07	2.0	0.006				100	100	147
Dec-95	11.8	27.0	0.05	0.05	0.81	5.80	0.71	0.08	2.7	0.009				100	100	155

Table A3.1 continued.

Date	Temp C	BOD Removal %	%CL02 substitution	RA ppm	Chloroform ppb	TSS ppm	Cond min µmhos	Cond max µmhos	pH min	pH max	colour c.u.
Jan-93	21	89.4		0.026		60					976
Feb-93	23	91.8		0.090		51	1965	2189	7.8	8.2	1063
Mar-93	24	92.1	50	ND		36	2041	2213	7.8	8.2	934
Apr-93	24	89.8	45	ND	8.1	34	1886	2491	7.7	8.1	905
May-93	29	91.2	63	ND		31	1675	1934	7.6	7.9	755
Jun-93	31	91.1	52	ND		29	1390	1733	7.5	7.8	1050
Jul-93	33	92.0	100	0.013	2.1	29	1414	1776	7.7	8.0	508
Aug-93	33	93.2	100	ND		19	1290	1572	7.6	7.9	308
Sep-93	30	92.1	100	0.138		28	1348	1675	7.6	8.0	417
Oct-93	26	92.6	100	0.012	ND	33	1505	1721	7.9	8.1	341
Nov-93	24	91.2	100	ND		34	1442	1654	8.1	8.3	338
Dec-93	23	91.7	100	0.046		28	1429	1620	7.9	8.1	355
Jan-94	23	88.6	100	0.790	ND	36	1425	1591	8.2	8.4	345
Feb-94	24	91.1	100	0.081		29	1608	1771	8.0	8.3	376
Mar-94	25	91.9	100	0.120		19	1599	1803	7.8	8.1	348
Apr-94	26	91.8	100	ND	ND	21	1366	1544	8.0	8.2	256
May-94	30	89.1	100	ND		25	1230	1566	8.0	8.2	314
Jun-94	32	92.0	100	ND		17	1432	1687	7.7	7.8	283
Jul-94	33	92.4	100	ND	ND	16	1409	1740	7.6	7.7	292
Aug-94	33	91.9	100	ND		40	1257	1686	7.9	8.1	302
Sep-94	31	93.7	100			19	1023	1685	7.4	7.9	303
Oct-94	23	93.2	100	0.084	ND	23	1321	1945	7.9	8.2	285
Nov-94	22	91.7	100	0.076		35	1366	1920	7.8	7.9	372
Dec-94	22	91.5	100	0.027		29	1625	2317	7.8	8.1	320
Jan-95	23		100	0.203	ND	28	1373	1935	7.7	8.0	259
Feb-95	24		100	ND		27	1554	2335	7.6	7.8	223
Mar-95	25		100	0.020		30	1474	2449	7.6	7.9	278
Apr-95	26		100	0.008		22	1161	2044	7.5	7.9	239
May-95	31		100	0.020		23	1145	1731	7.8	8.0	291
Jun-95	33		100	ND		10	1045	1805	7.7	7.8	304
Jul-95	34		100	0.040	ND	21	896	1845	7.7	7.9	334
Aug-95	33		100	0.034		17	1081	1779	7.9	8.2	398
Sep-95	30		100	0.013		24	1033	1668	7.6	8.1	336
Oct-95	23		100	0.010		29	1111	1706	7.7	8.1	308
Nov-95	23		100	0.010		29	1061	1961	7.8	7.9	249
Dec-95	23		100	0.070		33	1027	1870	7.5	7.7	333

Table A3.2 Dioxin and furan content of effluent for January 1993-December 1995.

	TCDF		PCDF			HCDF					SCDF			OCDF
Date	total pg/L	2378 pg/L	total pg/L	12378 pg/L	23478 pg/L	total pg/L	12347 pg/L	12367 pg/L	2346 pg/L	123789 pg/L	total pg/L	12346 pg/L	1234789 pg/L	pg/L
Jan-93	ND	26.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Feb-93	19.0	11.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mar-93	26.0	15.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Apr-93	13.0	15.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
May-93	13.0	ND	2.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Jun-93	14.0	6.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Jul-93	12.0	5.8	3.4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aug-93														
Sep-93														
Oct-93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nov-93														
Dec-93														
Jan-94	ND	6.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Feb-94														
Mar-94														
Apr-94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
May-94														
Jun-94														
Jul-94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aug-94														
Sep-94														
Oct-94														
Nov-94														
Dec-94														
Jan-95														
Feb-95														
Mar-95														
Apr-95														
May-95														
Jun-95														
Jul-95	6.3	6.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aug-95														
Sep-95														
Oct-95														
Nov-95														
Dec-95														

Table A3.2 continued.

	TCDD		PCDD		HCDD				SCDD		OCD
Date	total pg/L	2378 pg/L	total pg/L	12378 pg/L	total pg/L	123478 pg/L	123678 pg/L	123789 pg/L	total pg/L	1234678 pg/L	pg/L
Jan-93	ND	7.7	ND	ND	ND	ND	ND	ND	ND	ND	ND
Feb-93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	89.0
Mar-93	4.7	ND	ND	ND	ND	ND	ND	ND	ND	9.7	ND
Apr-93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	40.0
May-93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Jun-93	3.9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Jul-93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aug-93											
Sep-93											
Oct-93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nov-93											
Dec-93											
Jan-94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Feb-94											
Mar-94											
Apr-94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
May-94											
Jun-94											
Jul-94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aug-94											
Sep-94											
Oct-94											
Nov-94											
Dec-94											
Jan-95											
Feb-95											
Mar-95											
Apr-95											
May-95											
Jun-95											
Jul-95	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	36.0
Aug-95											
Sep-95											
Oct-95											
Nov-95											
Dec-95											

Table A3.3 Effluent heavy metal concentrations (µg/L)for January 1993-December 1995.

Date	Al	Be	Cd	Cr	Co	Cu	Pb	Mn	Hg	Mo	Ni	Ag	Thallium	V	Zn
Jan-93															
Feb-93															
Mar-93															
Apr-93	0.664	<.0002	<.003	0.009	<.011	0.003	0.002	0.415	<0.0005	<.015	<.006	<.002	<.016	<.007	0.023
May-93															
Jun-93															
Jul-93															
Aug-93															
Sep-93															
Oct-93	0.217	<0.0001	<0.001	<0.001	<0.001	0.004	<0.001		<0.0005	0.001	0.002	<0.001	<0.002	0.001	0.029
Nov-93															
Dec-93															
Jan-94															
Feb-94															
Mar-94															
Apr-94	0.324	<0.001	<0.001	0.019	<0.005	<0.002	<0.001	0.322	<0.0005	<0.004	<0.01	<0.003	<0.05	<0.004	0.044
May-94															
Jun-94															
Jul-94															
Aug-94															
Sep-94															
Oct-94	0.330	<0.001	<0.001	0.010	<0.005	0.014	<0.001	0.314	<0.0005	<0.004	<0.006	<0.003	<0.050	0.004	0.033
Nov-94															
Dec-94															
Jan-95															
Feb-95															
Mar-95															
Apr-95	0.413	<0.001	<0.001	<0.004	<0.005	0.016	<0.001	0.124	<0.0005	<0.004	<0.006	<0.003	<0.050	<0.004	0.024
May-95															
Jun-95															
Jul-95															
Aug-95															
Sep-95															
Oct-95															
Nov-95															
Dec-95															

Table A3.4 Resin and fatty acid content of effluent (mg/L) for January 1993-December 1995. Shaded cells indicated that data was calculated with one or more censored values; one half of the detection limit (DL) was used in cases where the value was reported as <DL. Detection limits were 0.01 mg/L, with the exception of April 1995 when the DL was 0.001 mg/L, and August and September 1995, when the DL was 0.005 mg/L.

Date	Abietic	14-chloro-dehydro-abietic	12-chloro-dehydro-abietic	dehydro-abietic	12,14,di-chloro-dehydro-abietic	Isopimaric	Levo-pimaric	Neoabietic	Pimaric	Sandarac opimaric	Palustric	Myristic	Palmitic	Linoleic	Oleic	Stearic	Linolenic	Arachidic	9.10-dichloro-stearic
Jan-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Feb-93	N.D.	N.D.	N.D.	0.03	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	N.D.	N.D.	0.01	N.D.	0.03	N.D.	N.D.	N.D.	N.D.
Mar-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Apr-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
May-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jun-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jul-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aug-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sep-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.03	N.D.	0.03	0.01	N.D.	N.D.	N.D.
Oct-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Nov-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dec-93	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jan-94	0.01	N.D.	N.D.	0.19	N.D.	0.16	0.01	0.01	0.26	N.D.	0.05	N.D.	0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Feb-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02	0.05	N.D.	N.D.	0.02	N.D.	N.D.	N.D.
Mar-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	0.05	N.D.	0.04	0.02	N.D.	N.D.	N.D.
Apr-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
May-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jun-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jul-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aug-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sep-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Oct-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.04	N.D.	0.02	0.02	N.D.	N.D.	N.D.
Nov-94	N.D.	N.D.	N.D.	0.01	N.D.	0.013ndr	N.D.	N.D.	0.02	N.D.	N.D.	N.D.	0..014	N.D.	N.D.	0.01	N.D.	N.D.	N.D.
Dec-94	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	N.D.	0.01	N.D.	N.D.	N.D.

Table A3.4 Continued.

Date	Abietic	14-chloro-dehydro-abietic	12-chloro-dehydro-abietic	dehydro-abietic	12,14,di-chloro-dehydro-abietic	Isopimaric	Levo-pimaric	Neoabietic	Pimaric	Sandarac opimaric	Palustric	Myristic	Palmitic	Linoleic	Oleic	Stearic	Linolenic	Arachidic	9.10-dichloro-stearic
Jan-95	0.01	N.D.	N.D.	0.04	N.D.	0.03	0.03	N.D.	0.08	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.
Feb-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Mar-95	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	0.01ndr	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Apr-95	N.D.	N.D.	N.D.	0.00	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	0.002n	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
May-95	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jun-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Jul-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.04	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Aug-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Sep-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.006dn	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Oct-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Nov-95	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Dec-95	N.D.	N.D.	N.D.	0.01	N.D.	0.03	N.D.	N.D.	0.01	N.D.	N.D.	N.D.	0.01	N.D.	N.D.	0.01	N.D.	N.D.	N.D.

Table A3.5 Results of chronic toxicity tests using *Ceriodaphnia dubia*, *Selenastrum capricornutum*, and *Pimephales promelas*

	Ceriodaphnia dubia - 7 day												Fathead Minnow - 7 day				Algal growth			
	Mortality				Reproduction				Mortality				Growth				Growth			
Date	NOEC	LOEC	LC25	LC50	NOEC	LOEC	LC25	LC50	NOEC	LOEC	LC25	LC50	NOEC	LOEC	LC25	LC50	NOEC	LOEC	LC25	LC50
Jan-93																				
Feb-93																				
Mar-93																				
Apr-93																				
May-93																				
Jun-93																				
Jul-93																				
Aug-93																				
Sep-93																				
Oct-93																				
Nov-93																				
Dec-93																				
Jan-94																				
Feb-94																				
Mar-94																				
Apr-94																				
May-94																				
Jun-94																				
Jul-94																				
Aug-94																				
Sep-94																				
Oct-94	80	100	82(68-	>100	60	80	67(54-	75(70-	100	N/A	>100	>100	80	100	98(92-	N/A	>100	N/A	>100	>100
Nov-94																				
Dec-94																				
Jan-95	100	N/A	>100	>100	50	100	55(43-	75(69-	100	N/A	>100	>100	100	N/A	>100	>100	12.5	25	22(18-	>100
Feb-95																				
Mar-95																				
Apr-95	100	N/A	>100	>100	50	100	59(52-	79(74-	100	N/A	>100	>100	100	N/A	>100	>100	12.5	25	18(10-	>100
May-95																				
Jun-95																				
Jul-95																				
Aug-95	100	>100	>100	>100	25	50	43(36-	59(51-	100	>100	>100	>100	100	>100	>100	>100	50	100	82(46-	>100
Sep-95																				
Oct-95																				
Nov-95	>100	>100	97	>100	60	80	76(65-	>100	100	>100	>100	>100	80	100	>100	>100	100	>100	>100	>100
Dec-95																				

Appendix 4

Appendix 4 contains a detailed description of the artificial stream system that was designed for my field experiment (Chapter 3). This appendix was originally published as: Culp, J. M., C. L. Podemski and C. Casey 1996. Design and application of a transportable experimental stream system for assessing effluent impacts on riverine biota. Project Report No. 128, Northern River Basins Study, Edmonton, Alberta.

ACKNOWLEDGEMENTS

We thank J. Banner, B. Christie, N. Glozier, E. Marles and J. Mollison who provided valuable assistance during the design and construction of the artificial streams. This research was supported by the Northern Rivers Basins Study and the National Hydrology Research Institute (Environment Canada). CLP was supported by a Postgraduate Scholarship from the National Sciences and Engineering Research Council of Canada (NSERC).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

1.0 INTRODUCTION

Artificial streams (i.e., mesocosms) have been used as tools for investigating ecological interactions in running waters since the 1960s (see Lamberti and Steinman 1994 for a review). This approach has been used to investigate a variety of ecological phenomena including: effects of environmental factors such as irradiance, temperature, and nutrients on algae (McIntire 1966a; McIntire 1966b; Bothwell 1988; Bothwell 1989); and to examine specific trophic relationships among algae, insects, and fish (Lamberti et al. 1987; Schlosser 1988; Lamberti et al. 1989; Culp et al. 1991; Scrimgeour et al. 1991). In the Northern Rivers Basin Study, mesocosms will be used to investigate effects of treated pulp mill effluents and nutrients on complex food webs, including primary and secondary producers.

The high degree of spatial heterogeneity and challenge of obtaining true replicates in natural environments like the Athabasca River often makes it difficult to predict or verify, quantitatively, the impacts of complex effluents on riverine biota. Therefore, an important advantage of mesocosm research lies in our ability to investigate complex, benthic food webs in model systems that simulate specific riverine conditions. It is important to recognize that the goal of mesocosm research is to simulate, rather than reproduce exactly, key aspects of the riverine environment. By locating the experimental system beside the study river, the stream mesocosm can be supplied with natural river water under ambient water temperature and light regimes. Multiple trophic level communities can be examined by seeding the mesocosms with natural substratum and biota (i.e., biofilm, invertebrates, and fish) from the river.

The objective of this project was to design and construct a transportable mesocosm for testing impacts of nutrients and contaminants from pulp mill effluents on abundance and taxonomic composition of aquatic invertebrate communities in the Athabasca River. The experimental apparatus will be used in a variety of experimental settings, and the results will be used to address impacts of nutrients, contaminants, and their interaction on benthic invertebrate and biofilm productivity. This information is critical for assessing nutrient and contaminant impacts on biota (Questions 1A, 4A & 5) and for preserving aquatic life and habitat (Question 6). The report describes mesocosm design, specific hydraulic characteristics, important details and procedures of installation, and results obtained during initial tests of the system at the Hinton, Alberta experimental site.

2.0 DESIGN AND CONSTRUCTION

This section provides a brief description of the experimental stream facility (Plate 1) and basic requirements for setting up such a system as it was constructed in the summer of 1993. The facility was designed to withstand air and water temperatures near 0°C since it would be used for experiments conducted during autumn and early spring. Throughout the description, metric units are used except for materials which are normally sold in Imperial units.

2.1 SITE REQUIREMENTS

The site for the facility requires a cleared 9 x 5 m area. The site must be accessible by road since some materials require transport by truck trailer. Availability of a water intake system and electrical power is required, otherwise generators and pumping systems must be added to the facility design. Ground at the site should be made as level as possible because a flat working surface will save a great deal of time and effort during setup of platforms and installation of plumbing. A layer of gravel or crushed rock spread over the site will drain excess water away from the working area.

2.2 STREAM FACILITY DESIGN

The experimental stream facility consists of 16 circular tanks placed in pairs on platforms. Water from the study river is pumped into a head tank reservoir and delivered through a system of pipes to the tanks. Water flow to individual tanks is controlled by a gate valve, and water movement in each stream is created by a belt-driven propeller system. Water depth in the tanks is maintained by an overflow drain and wastewater is returned to the study river.

2.2.1 Water Delivery

In Autumn 1993, water from the study river was pumped via a water intake and pumping system operated by the mill into a head tank, then gravity-fed to the streams. Water demand by the system depends upon the flow rate chosen by the experimenter. In the first run of this system, water flow was set at 2 L/min to each stream; therefore, water in excess of 32 L/min was required. The head tank was a 378 L polyethylene tank placed on a 1.22 m (4 ft) high platform. Schematic diagrams of the head tank and head tank platform are shown in Figures 1 and 2, and both are pictured in Plate 2. Water input was controlled by gate valves at each stream; therefore, the flow rate into each stream could be calibrated. The head tank and all water delivery lines were wrapped with heat tape (Plate 3) and insulated to prevent freezing (Plate 4). Figures 3 and 4 show diagrams of the system. A materials list for the water delivery system is

found in Appendix A.

2.2.2 Streams and Platforms

The streams were circular tanks (107 cm diameter; 42 inch) made out of polyester fibreglass. These were constructed by cutting 38 cm (15 inch) sections of 107 cm pipe and bonding a flat sheet of fibreglass to one end. A 25 cm (10 inch) diameter section of pipe was then centred in the larger pipe and the bottom cut out to form a standpipe. A completed stream is shown in Plate 5.

Streams were placed on eight, 74 cm (29 inch) high platforms, two to a platform. Each platform was 1.22 m x 2.44m (4 x 8 feet) long, made of 1.9 cm (3/4 inch) plywood and 7.6 x 7.6 x 0.6 cm (3 x 3 x 1/4 inch) angle aluminium. Plans for the platforms are given in Figure 5, and materials are listed in Appendix A. When assembling this system, care must be taken to ensure that the platforms are completely level and that pairs of tables sharing 2.54 cm (1 inch) feed lines are level with each other.

2.2.3 Wastewater System

A 3.5 cm (1 3/8 inch) drain hole was cut in the standpipe approximately 27 cm above the bottom of the tank. Height of this drain determined water depth in the streams. A 2.54 cm (1 inch) MTxS male adapter served as the drain on the inside wall of the tank. The adapter was screened with fibreglass door screen (Plate 6 a) and was screwed into a 1 inch elbow on the other side of the tank wall (Plate 6 b). Rubber gaskets placed on either side of the tank wall prevented leakage. Water flowing into the drain passed through a 1 inch pipe running vertically down through the platform (Figure 6). The 2.54 cm (1 inch) drain lines emptied into 5.1 cm (2 inch) drain lines which ran under the platforms (Plate 7 a & b) and were connected to a wooden trough (Plate 8). The 5.1 cm (2 inch) drain lines were heat taped and insulated. Wastewater from the troughs was returned to the study river through a length of 10.2 cm (4 inch) Big-O hose (Plate 9). A materials list for the wastewater system is provided in Appendix A.

2.2.4 Motor and Propeller System

Current velocity in the stream was created by a belt-driven propeller system. The motor assembly was comprised of a geared-head motor (250 rpm, 1/40 amp) driving a 22.6 mm pulley. A flat drive belt transmitted power 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 clamped to the top, outside edge of the circular tank (Plate 10). A 16 mm x 230

mm long, copper strut extending downward from the aluminium 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 was a 22.8 cm (9 inch) aluminium fan blade which rotated at a no-load speed of 115 rpm. Materials for the motor and propeller system are listed in Appendix A.

2.2.5 Contaminant Delivery System

Contaminants were delivered continuously to individual streams by peristaltic pumps and a series of insulated tubes for solution delivery. In the first run of this system, 2 Masterflex L/S Nema, type 13, wash down controllers and cartridge pump heads were used to deliver solutions to ten of the 16 streams. Pumps were kept in insulated boxes to keep them within approved operating temperatures (Plate 11). Solutions were stored in insulated containers (Plate 12) and immersion heaters were used to heat the solutions to approximately 27 /C. Tubes carrying contaminant solutions were run through 1.9 cm (3/4 inch), foam, pipe insulation to the streams. The tubes were then fed into small holes drilled into the water delivery spouts. Heating of solutions and insulation of all supply lines is recommended to prevent the thin supply lines (< 2 mm) from freezing.

2.2.6 Electrical System

If power is supplied by an existing electrical source, a step-down transformer may be required to deliver correct voltage and amperage to the experimental facility. In the autumn 1993 experiments, the 600 volt a.c. electrical power supplied by the mill was stepped down by a 7.5 KVA distribution transformer which provided four 120 volt, 15 amp circuits. Each of the circuits was used as follows: two circuits, consisting of four duplex outlets each, were needed to power the circulation motors; one circuit of two duplex outlets supplied power to the metering pumps; and one circuit of two duplex outlets was used for other needs as they arose.

3.0 OPERATION

This section provides a brief description of the operation of the artificial stream system as it was used in the initial run of the system during autumn, 1993.

3.1 SUBSTRATUM

A variety of substrata types including natural and artificial materials can be used in the mesocosms. In our initial operation of this system, the bottom of each stream was covered with a thin layer of thoroughly washed, crushed rock. One hundred and sixty stones (mean surface area = 535 cm^2) were collected from the study river, and ten were placed in each stream on top of the crushed rock, as illustrated in Plate 13. Using substratum from the study river provided a natural community of biofilm and invertebrates for experimental tests that incorporate multiple trophic levels. Porcelain tiles (23.5 cm^2) were used to provide a standardized substratum to compare biofilm development and accumulation. Two sets of tiles were used; one set was placed on top of the crushed rock, the other was suspended above the bottom on aluminum bars (Plate 14).

3.2 WATER DEPTH

Water depth in the streams was controlled by adjusting the height of the drain tube. In the initial experiment, mean water depth over gravel in the streams was 27 cm (x = 26.9 cm, SE = 0.09) and 22 cm (x = 22.5, SE = 0.14) over rocks. Note that water depths in this experiment were measured at the highest point of each rock in the stream. Water depth over suspended tiles was 10 cm (10.0 cm, SE = 0.6).

3.3 CURRENT VELOCITY AND CONTAMINANT MIXING

A test stream was set up in the laboratory in order to determine the range of water velocities that could be produced in the streams. In this test, the stream substratum was similar to that used in the autumn 1993 experiments, and velocity was measured with a Nixon Instruments velocity meter. Specifically, the substratum consisted of a thin layer of gravel upon which larger rocks were placed. The test stream did not contain tiles suspended over the stream bottom. Measurements were taken at 21 locations around the stream at three depths: immediately below the surface, at the mid-point of the water column, and just above the highest point of each rock. For the tests, water depth in the stream was approximately 31 cm. Mean water velocity, measured approximately 4 cm below the water surface, was 0.196 m/sec (n=21, SE = 0.029), and ranged from 0.006 to 0.479 m/sec. Average mid-water velocity was

0.20 m/sec (n=21, SE = 0.0223) with velocities ranging from 0.021 to 0.344 m/sec. Velocities just above rock surfaces ranged from 0.006 to 0.378 m/sec with a mean velocity of 0.225 m/sec (n=25, SE = 0.0194). During the autumn experiment, we suspended tiles on aluminium bars above the stream bottom and found that these created enough drag to reduce overall current velocity in streams relative to our laboratory tests. The mean velocity in streams at the mill site was 0.26 m/sec (n= 150, SE = 0.014, mean depth = 25 cm). Observations of dye traces indicate that contaminants mix within the first quarter of stream length (Plate 15).

3.4 HYDRAULIC RESIDENCE TIME AND WATER TEMPERATURES

Hydraulic residence time in streams was adjusted by changing the inflow rate. In the initial run of this system, inflow was set to 2 L/min. The tanks have a volume of 227 L resulting in a residence time of just under 2 h.

Water temperature was monitored by placing a Ryan thermograph in one of the streams and another in the head tank (Plate 16). Temperatures in the head tank reflect temperatures in the incoming river water. In contrast, relatively long residence times in streams resulted in some heating or cooling of water depending upon ambient air temperatures. Figure 7 shows heating and cooling of water in experimental streams relative to incoming water over a 72 h period.

3.5 MAINTENANCE

The stream system required a moderate amount of regular maintenance as motors had to be inspected daily for loose or misaligned belts. Loose belts were commonplace, particularly in the first three weeks of operation but were relatively easy to fix. In addition, motors were lubricated with non-toxic grease every three days to prevent seizing of propeller shafts. Leaves falling into the streams during autumn had to be removed daily as they caught on the propellers and caused them to become unbalanced and rotate unevenly. Drain screens were cleaned daily. The 4 inch water delivery lines were flushed on a weekly basis to remove any build-up of sand that settled in the lines (Plate 17). Finally, contaminant delivery lines had to be inspected for blockages and tubing changed as required, this was only a problem in lines carrying pulp mill effluent.

4.0 REFERENCES

Bothwell, M.L. 1988. Growth rate responses of lotic diatoms to experimental phosphorus enrichment: the influence of temperature and light. Canadian Journal of Fisheries and Aquatic Sciences 45: 261-270.

Bothwell, M.L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Canadian Journal of Fisheries and Aquatic Sciences 46: 1293-1301.

Culp, J.M., N.E. Glozier, and G.J. Scrimgeour. 1991. Reduction of predation risk under the cover of darkness: Avoidance responses of mayfly larvae to a benthic fish. Oecologia 86: 163-169.

Lamberti, G. A. and A.D. Steinman. 1994. Research in artificial streams: applications, uses and abuses. Journal of the North American Benthological Society 12: 313-384.

Lamberti, G.A., L.R. Ashkenas, S.V. Gregory, and A.D. Steinman. 1987. Effects of three herbivores on periphyton communities in laboratory streams. Journal of the North American Benthological Society 6: 92-104.

Lamberti, G.A., S.V. Gregory, L.R. Ashkenas, A.D., A.D. Steinman, and C.D. McIntire. 1989. Productive capacity of the periphyton as a determinant of plant-herbivore interactions in streams. Ecology 70: 1840-1856.

McIntire, C.D. 1966a. Some factors affecting respiration of periphyton communities in lotic environments. Ecology 47: 918-930.

McIntire, C.D. 1966b. Some effects of current velocity on periphyton communities in laboratory streams. Hydrobiologia 27: 559-570.

Schlosser, I.J. 1988. Predation risk and habitat selection by two size classes of a stream cyprinid: experimental test of a hypothesis. Oikos 52: 36-40.

Scrimgeour, G.J., J.M. Culp, M.L. Bothwell, F.J. Wrona, and M.H. McKee. 1991. Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly-diatom systems. Oecologia 85: 343-348.

5.0 FIGURES

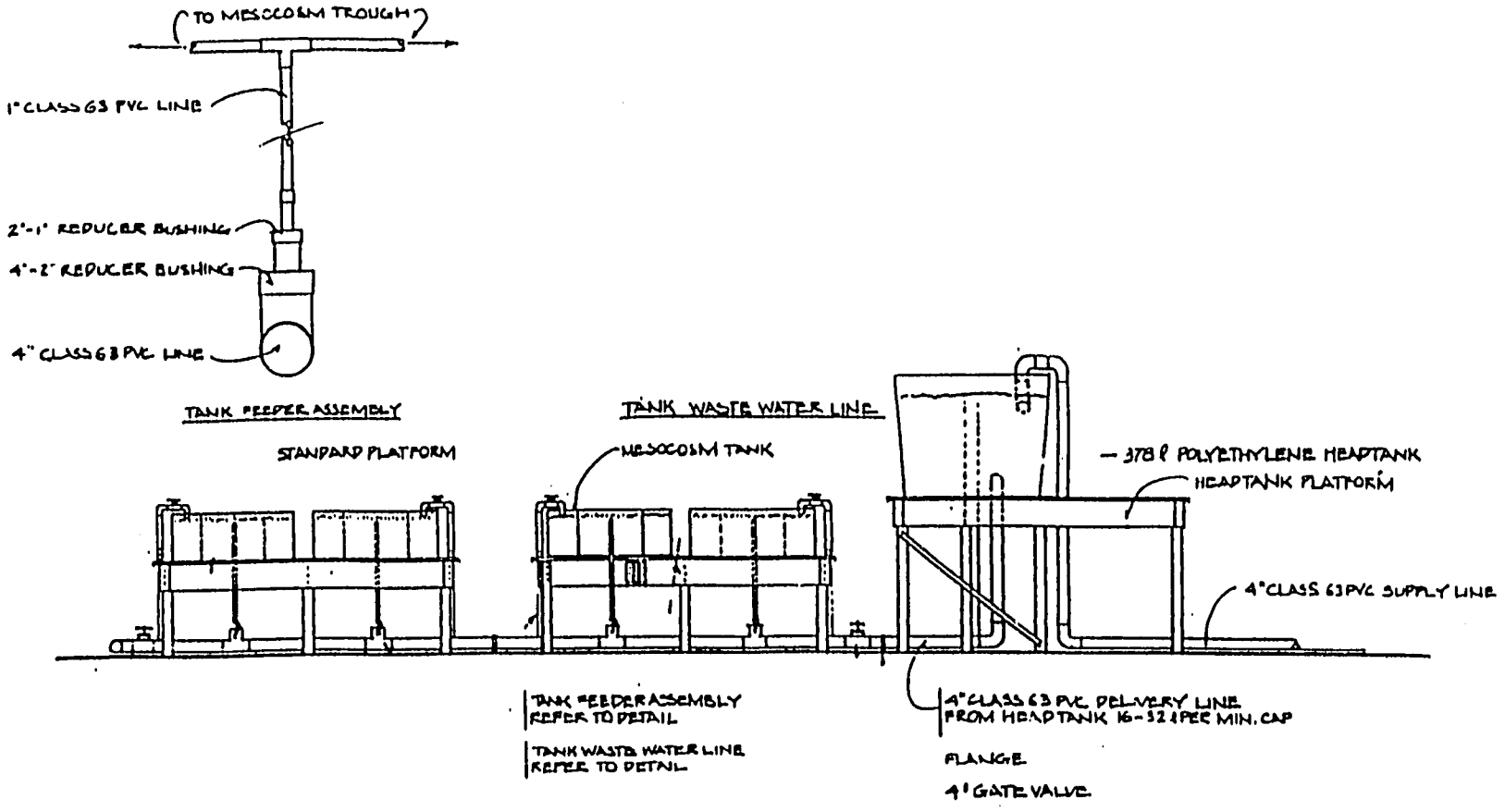


Figure 1. Side view of experimental stream facility showing: head tank and head tank platform, stream tanks and stream platforms, and associated plumbing for the water delivery and wastewater systems. Note inset of tank feeder assembly.

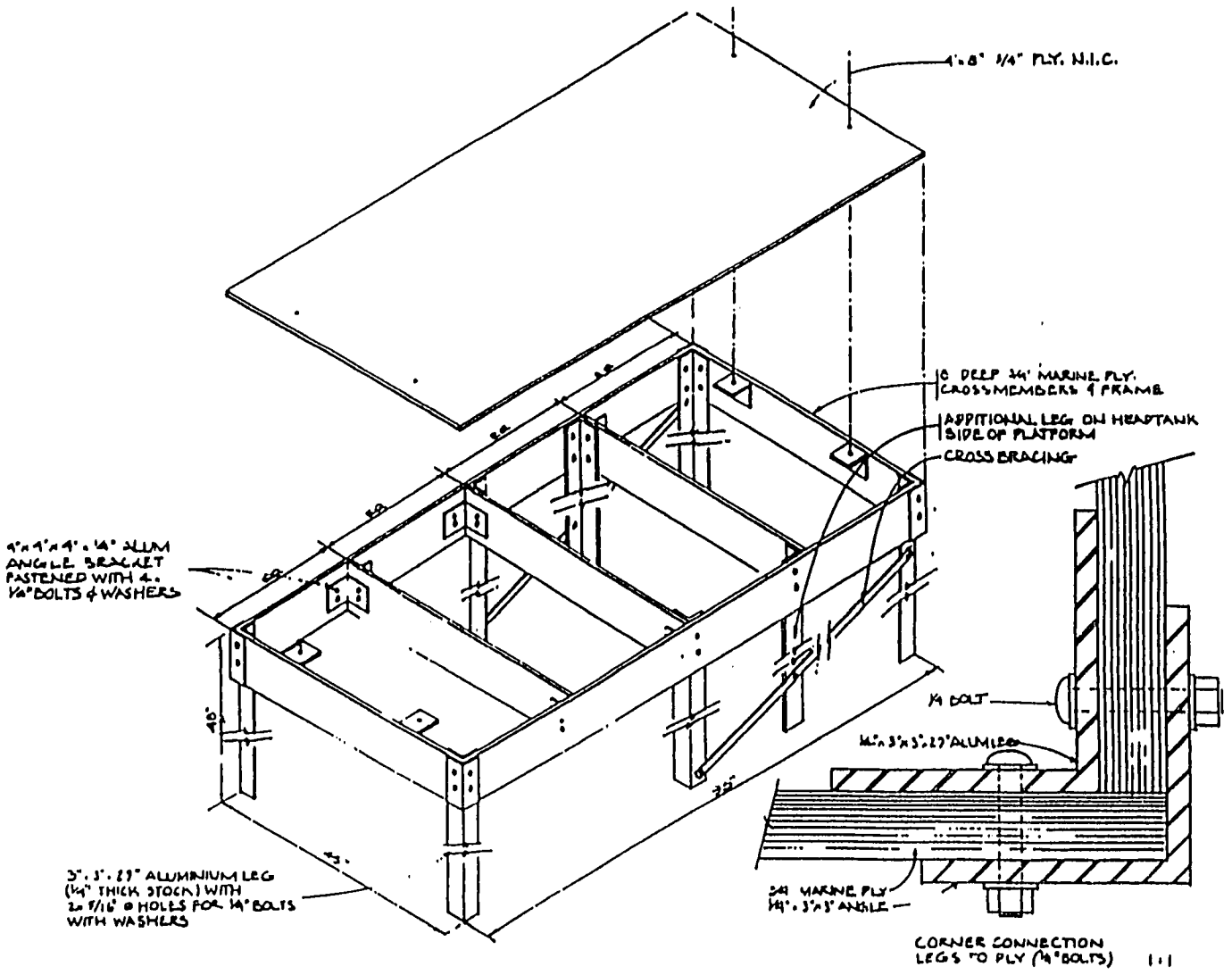


Figure 2. **Schematic diagram of the head tank platform used to support the head tank for the experimental stream facility.**

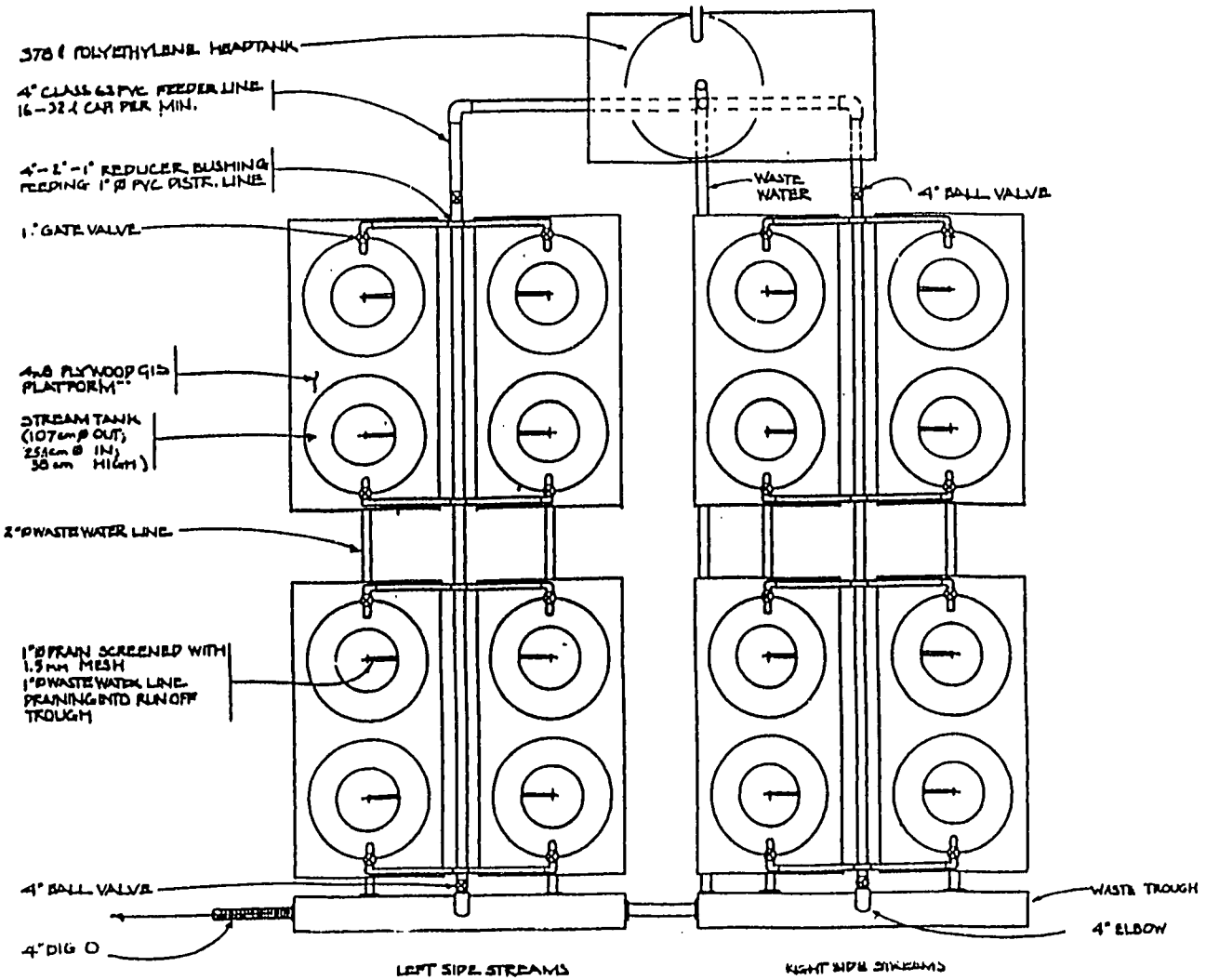


Figure 3. Overhead view of experimental stream facility showing plumbing used for water delivery and wastewater systems.

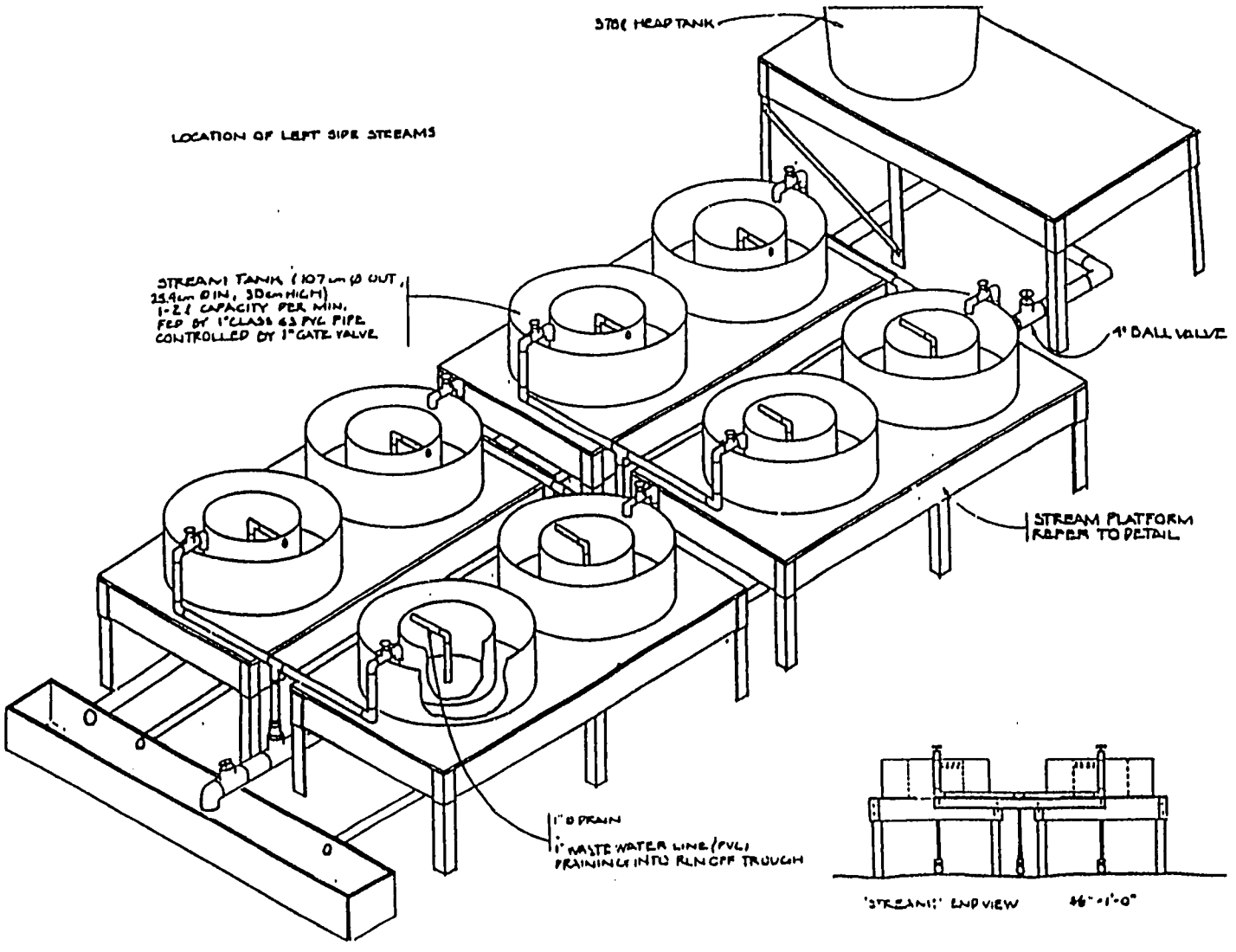


Figure 4. Oblique view of stream facility showing water delivery and wastewater systems. Note inset of stream end view.

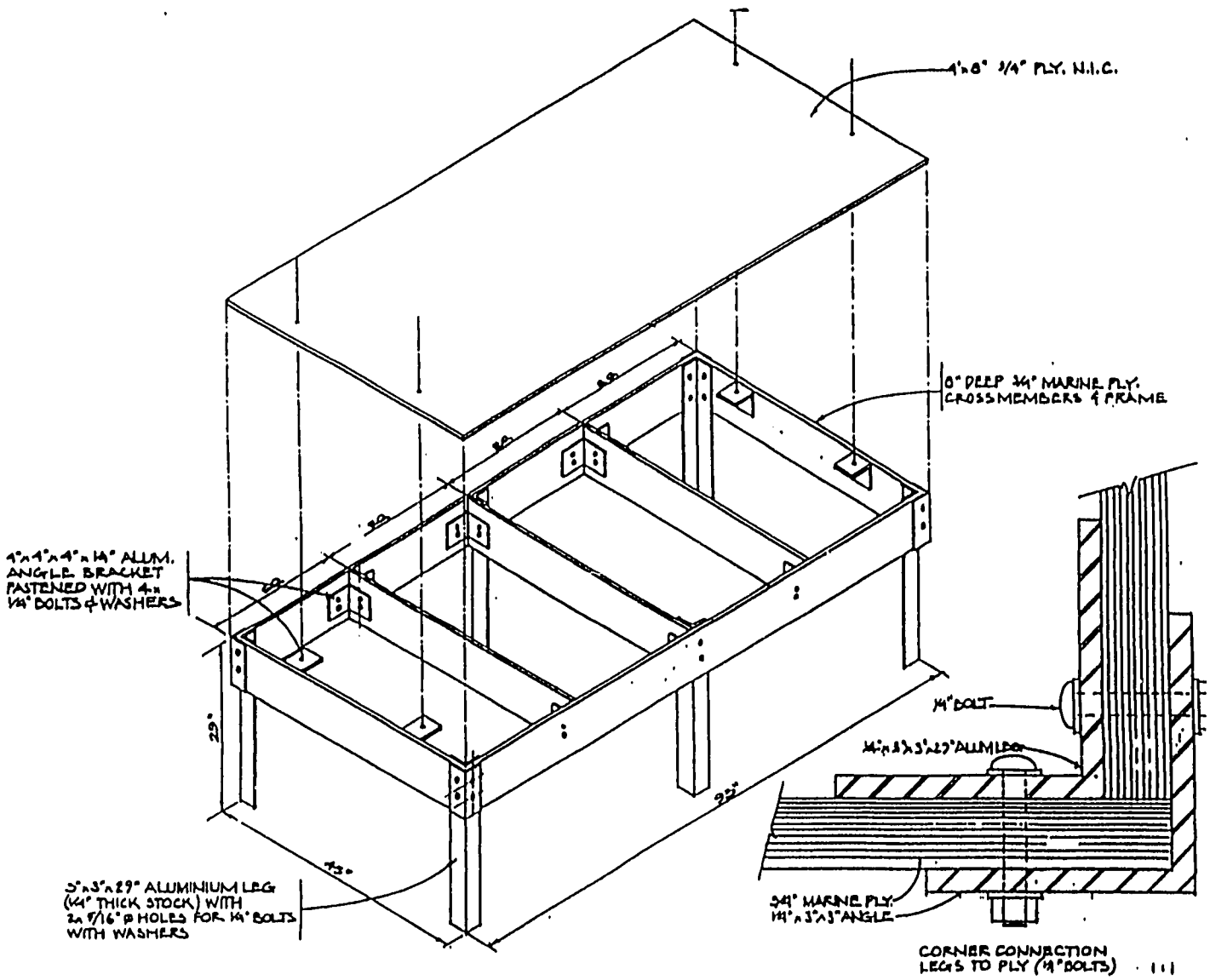


Figure 5. Schematic diagram of a stream platform used to support two stream tanks.

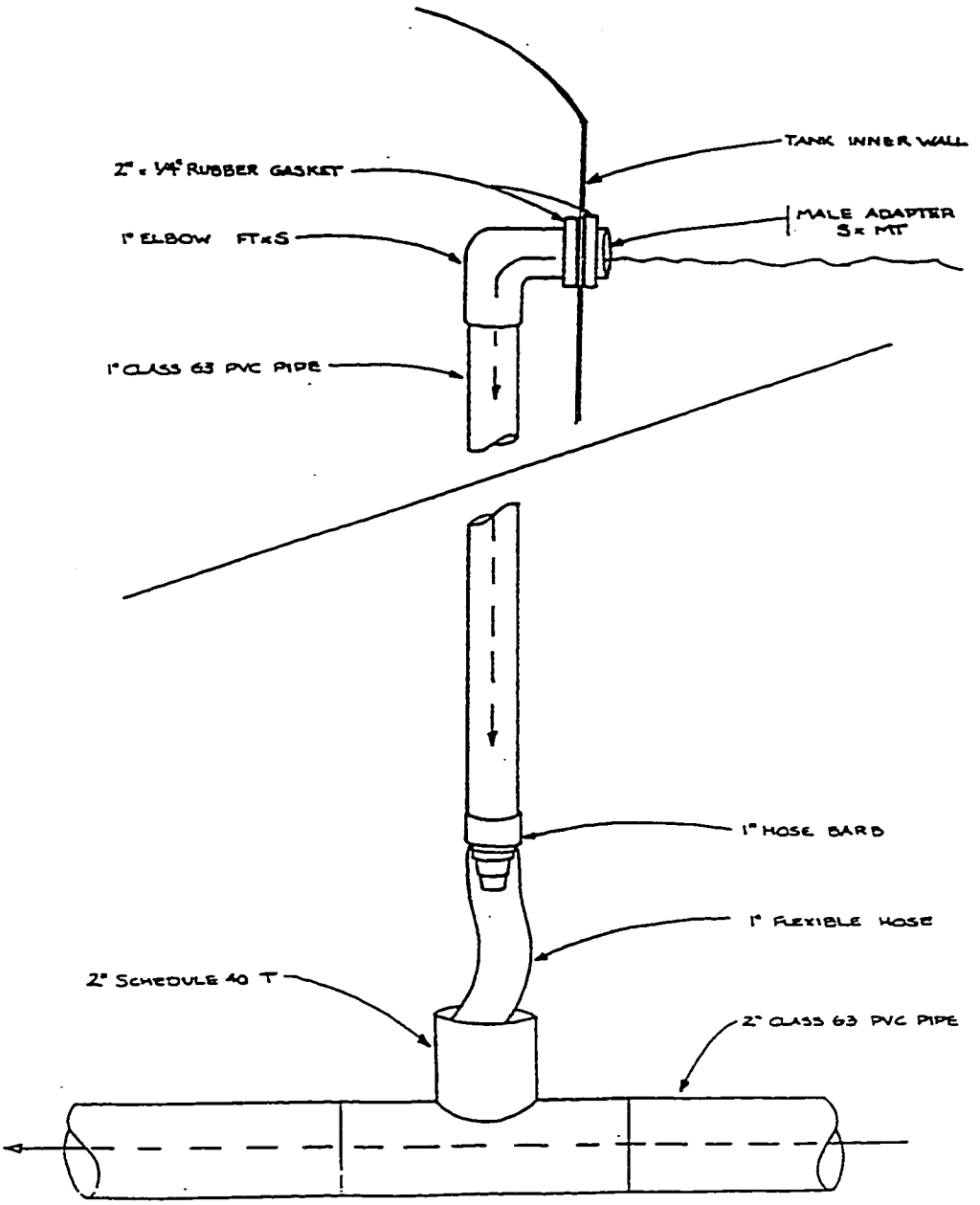


Figure 6. **Close-up, schematic view of the plumbing used for the drain line from each stream tank and connection to the shared 2 inch drain line running under the platforms.**

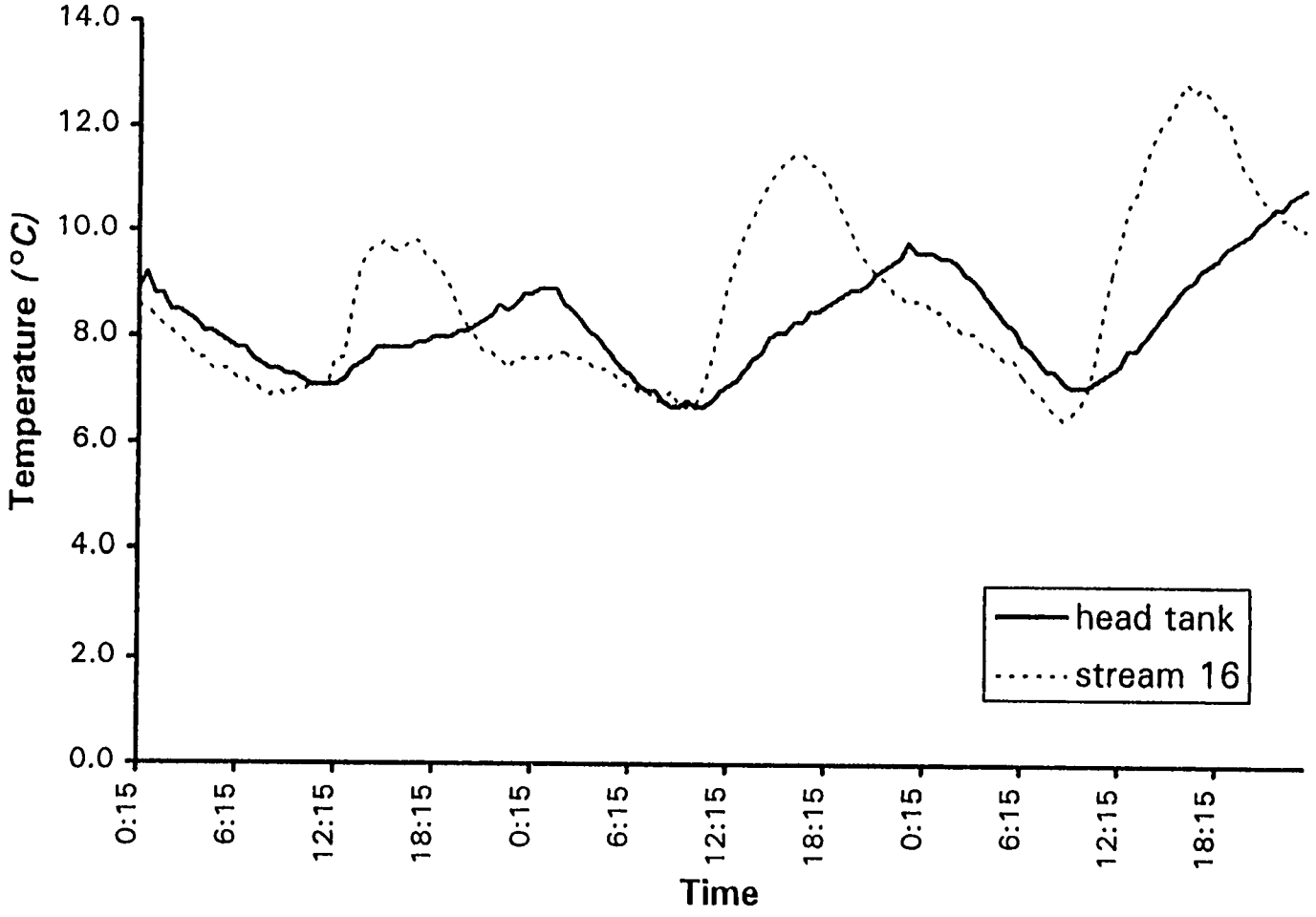


Figure 7. **Variation in water temperature in experimental streams relative to ambient river water temperature in the head tank over a 72 hour period.**

6.0 PLATES

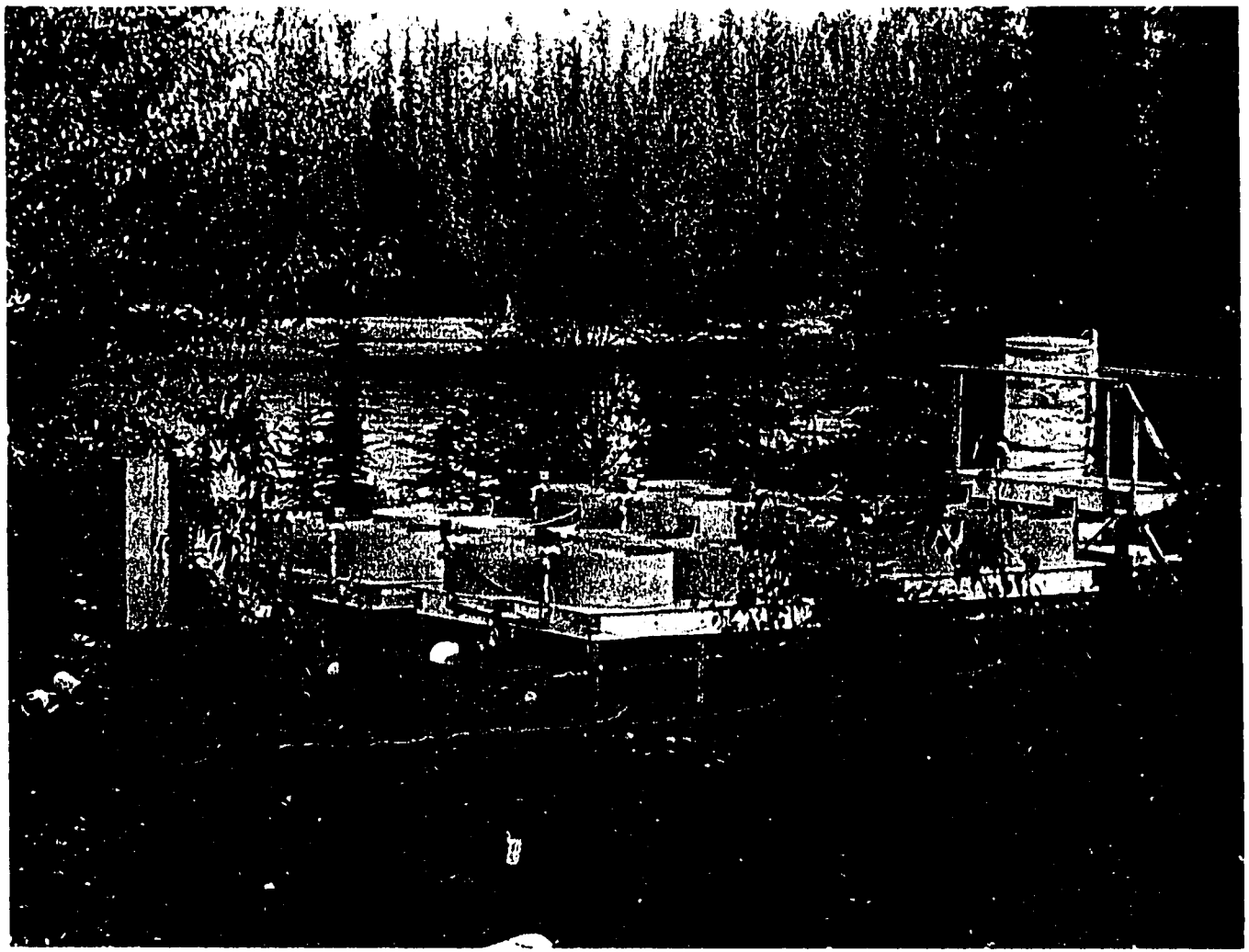


Plate 1. View of experimental stream facility assembled at Hinton, Alberta (autumn 1993).

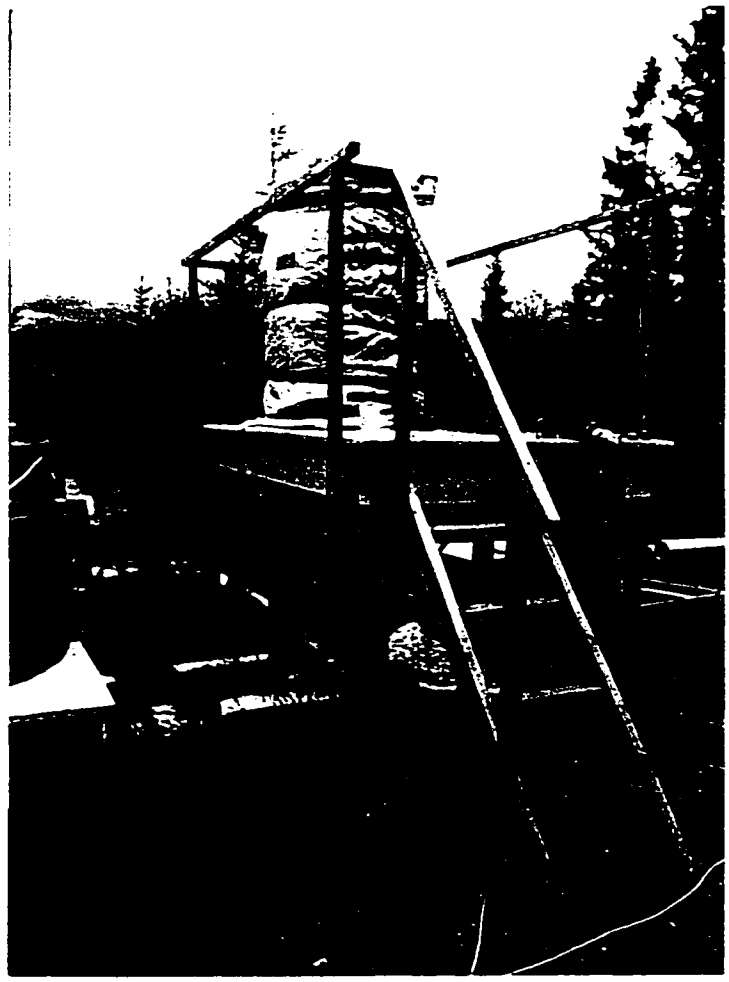


Plate 2. Head tank and head tank platform used for experimental stream facility.

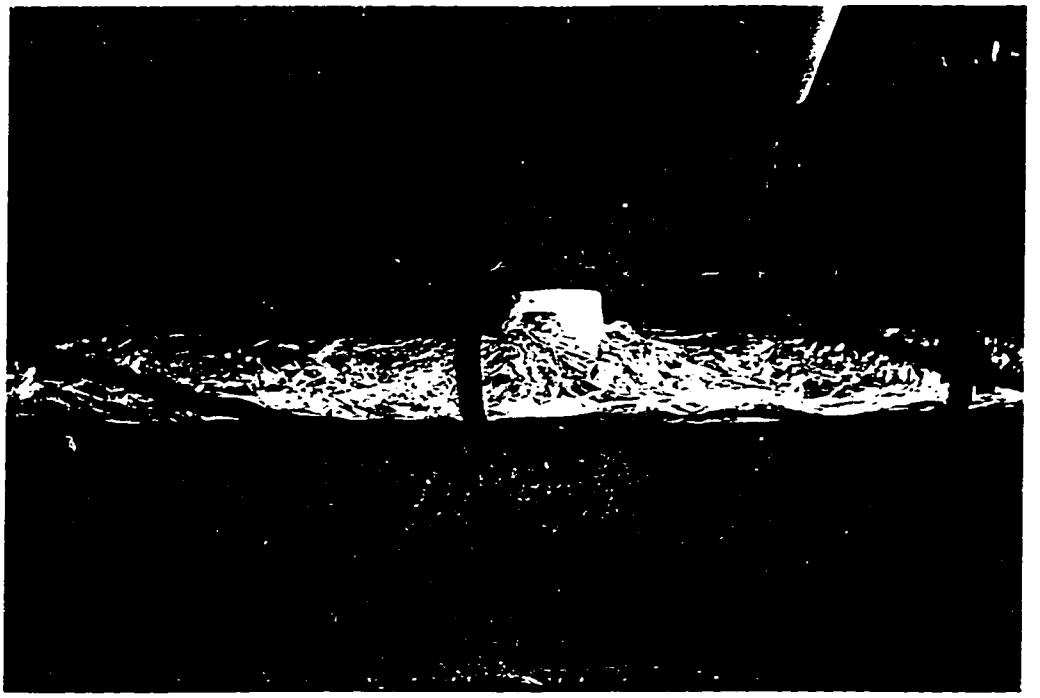


Plate 3. Water delivery line wrapped with heat tape to prevent freezing.

Plate 4. Insulating head tank to prevent freezing.

Plate 5. Completed stream tank showing: water intake, centre standpipe with drain, and motor assembly (attached to side of tank).

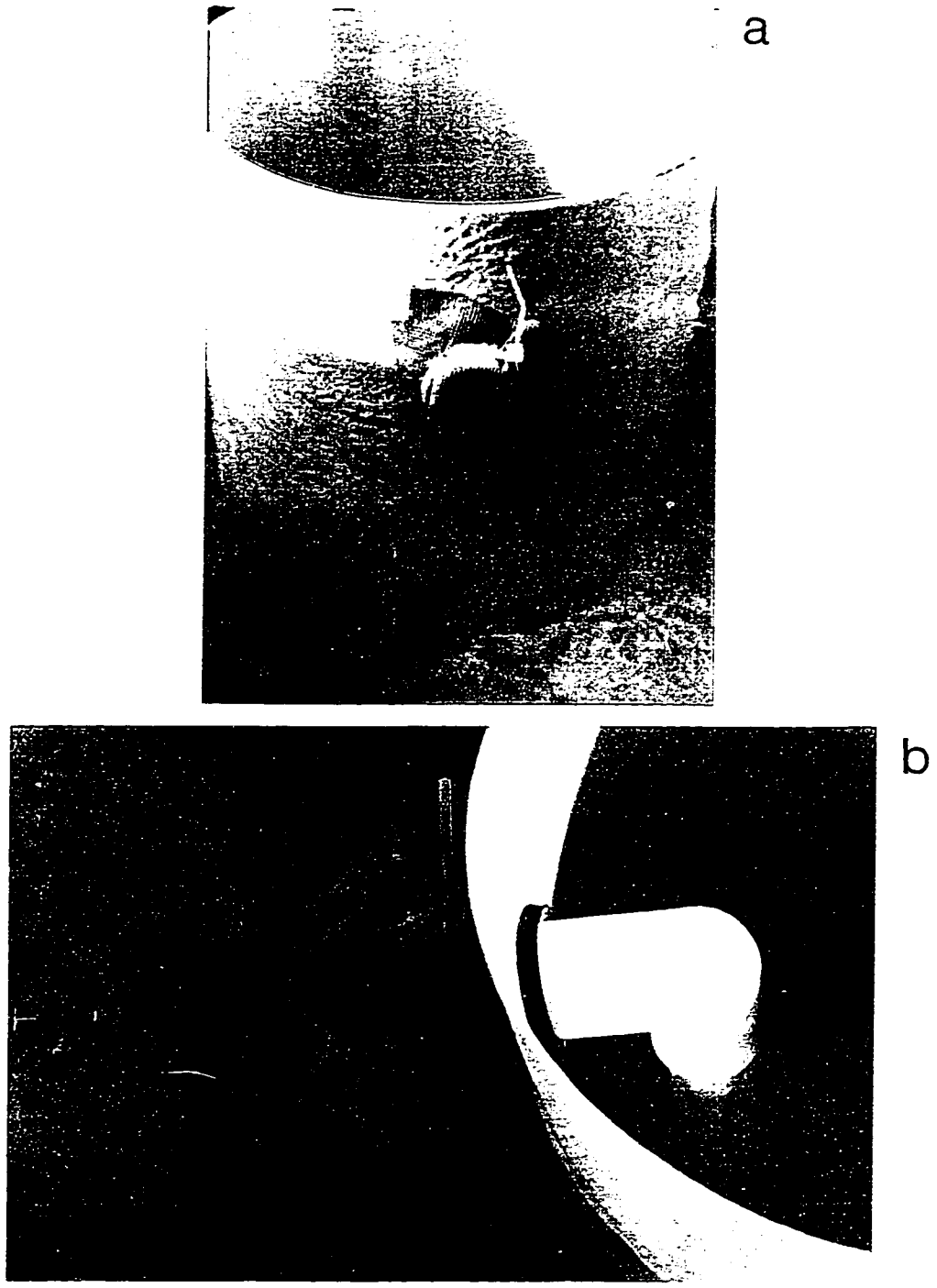


Plate 6. Drain assembly showing (a) fibreglass screen and (b) elbow connection.

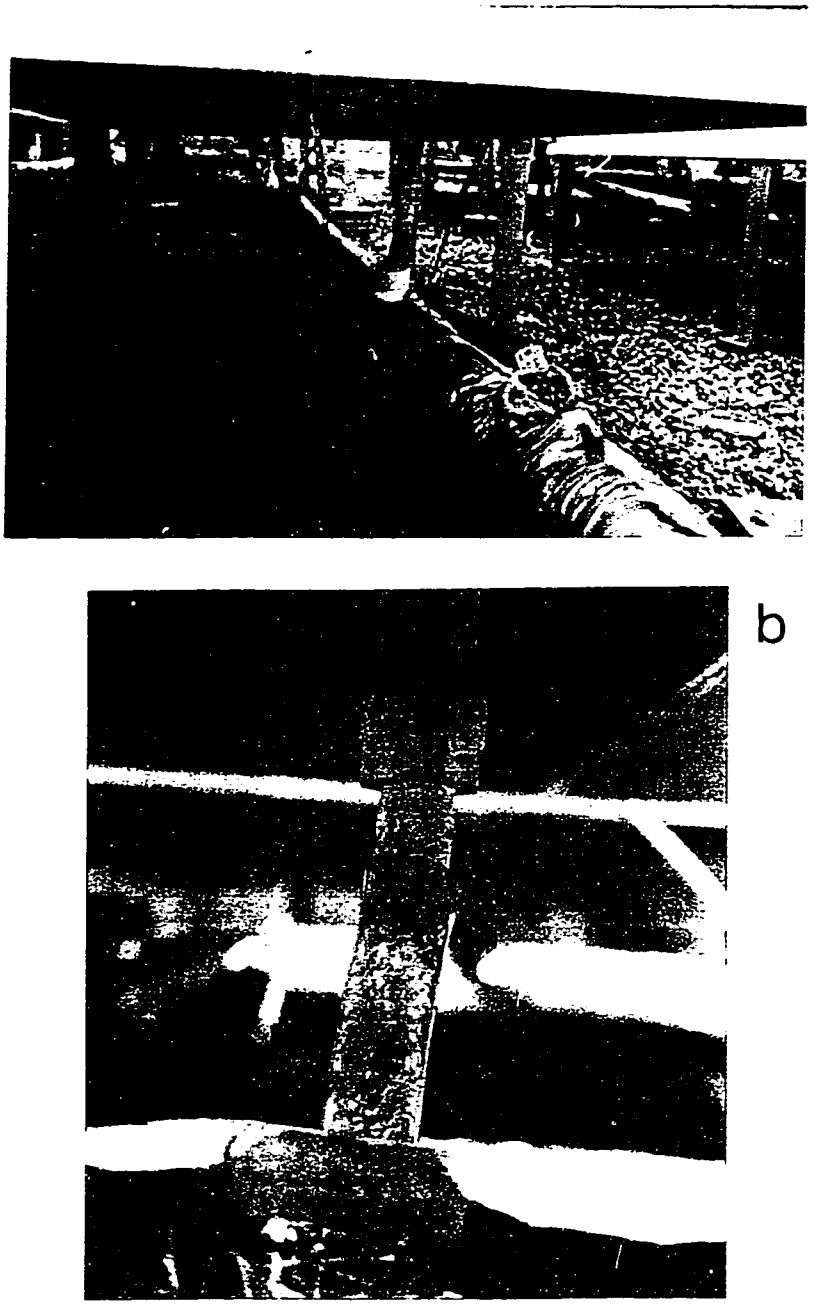


Plate 7. Waste lines showing (a) 1 inch drain lines from each tank and their (b) connection to the 2 inch drain lines running under the platforms. Note insulation of 2 inch drain lines.

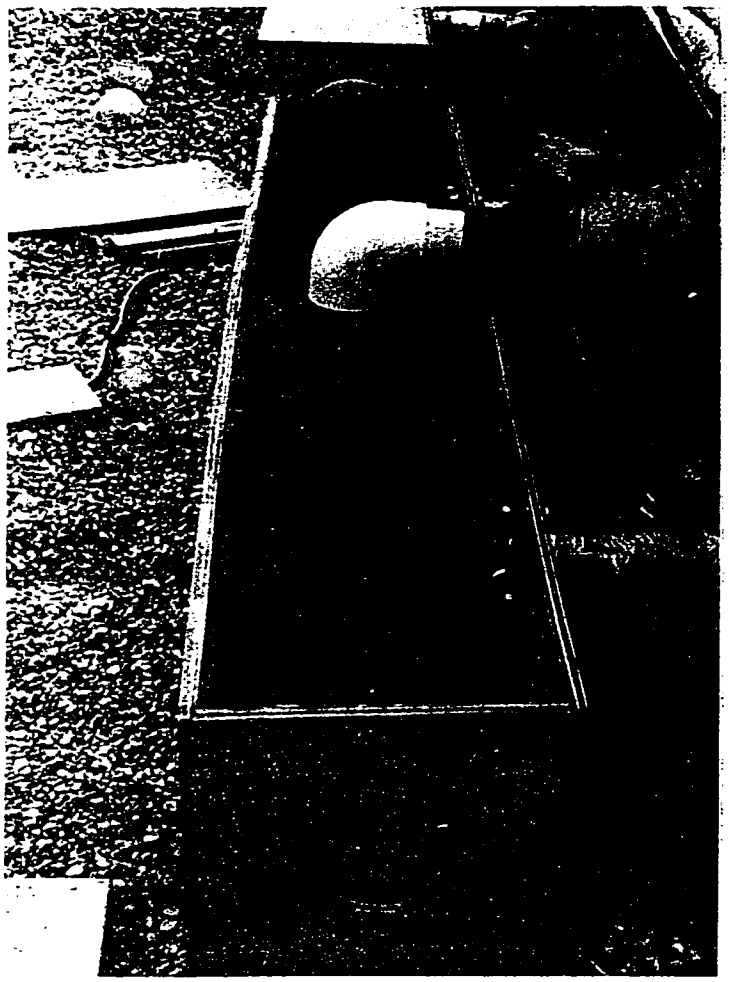


Plate 8. Two inch drain line and wooden collection trough located at downstream end of the stream facility.

Plate 9. View of completed stream facility showing: head tank and head tank platform, streams and stream platforms, motor assemblies, drain lines and collection troughs, and 4 inch Big-O hose used to return wastewater to the study river.

Plate 10. Motor assembly and propeller system showing: attachment to tank wall, copper strut and grease nipple, belt and pulley system, and propeller shaft and propeller.

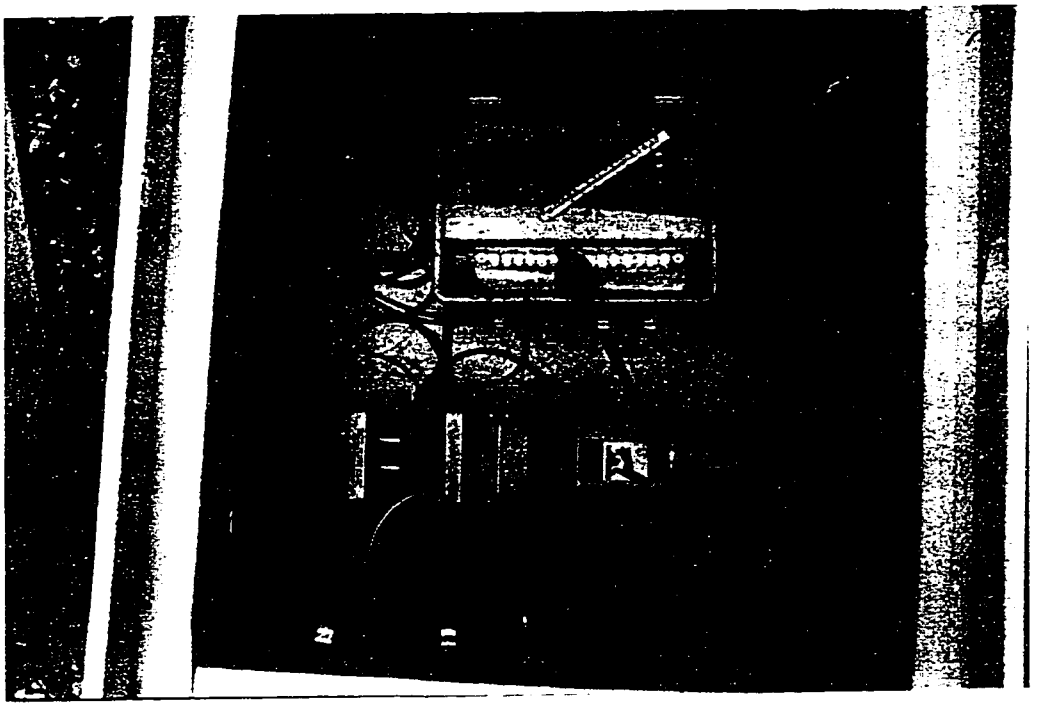


Plate 11. Contaminant solution pump and insulated holding box used to maintain the pump within approved operating temperatures.

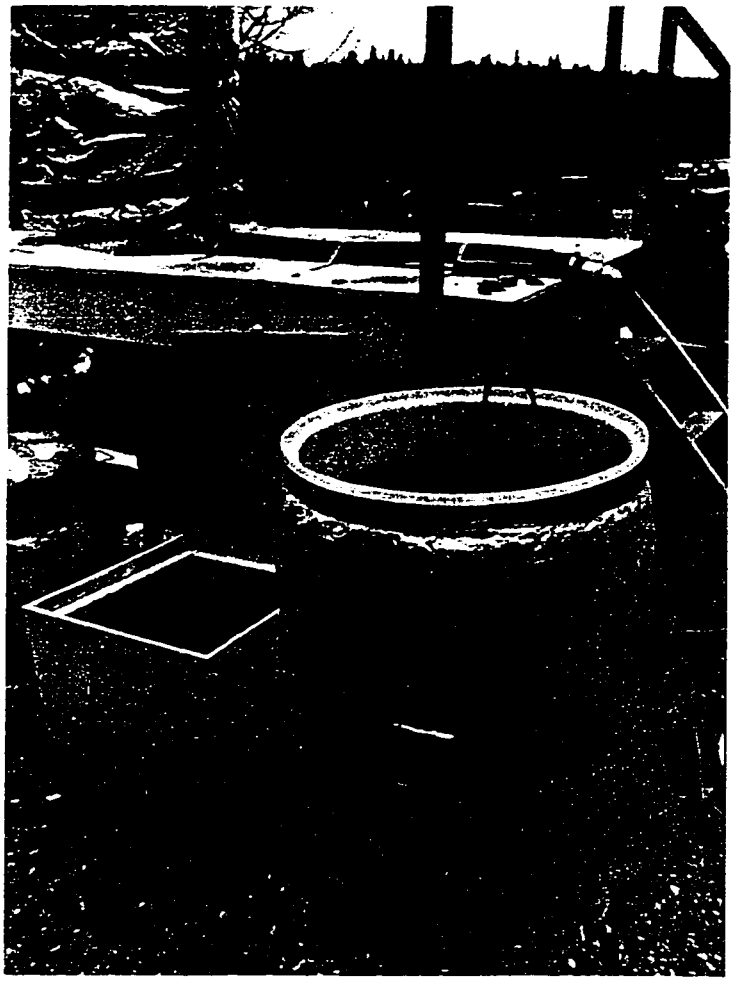


Plate 12. Insulated storage container used for contaminant solutions.

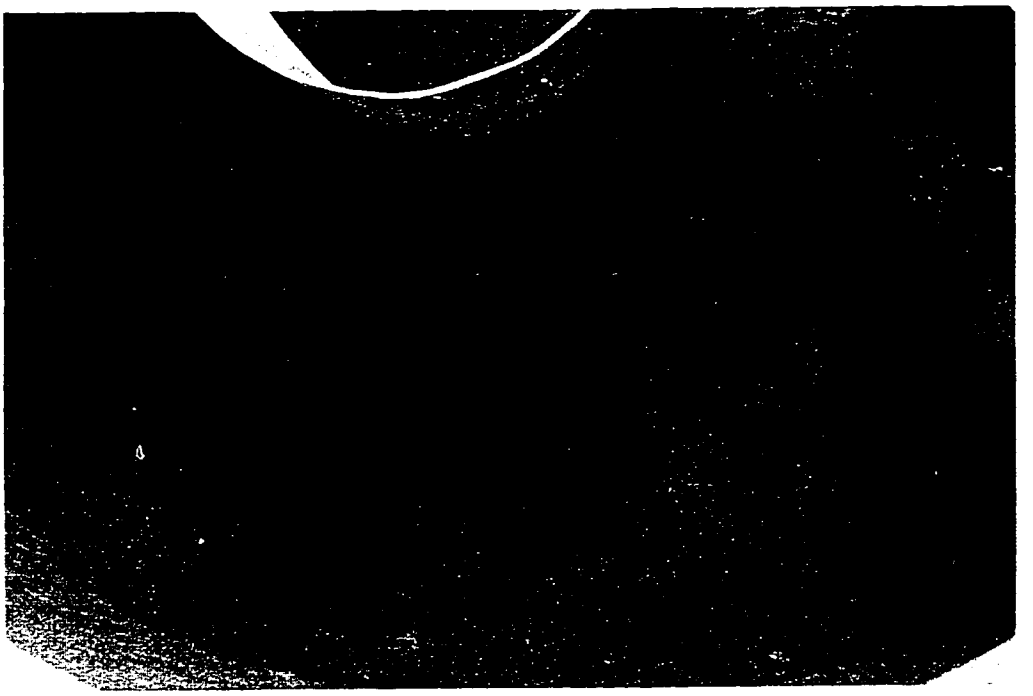


Plate 13. Placement of stones in the artificial streams. Stones were collected from the Athabasca River upstream of the mill effluent outfall.

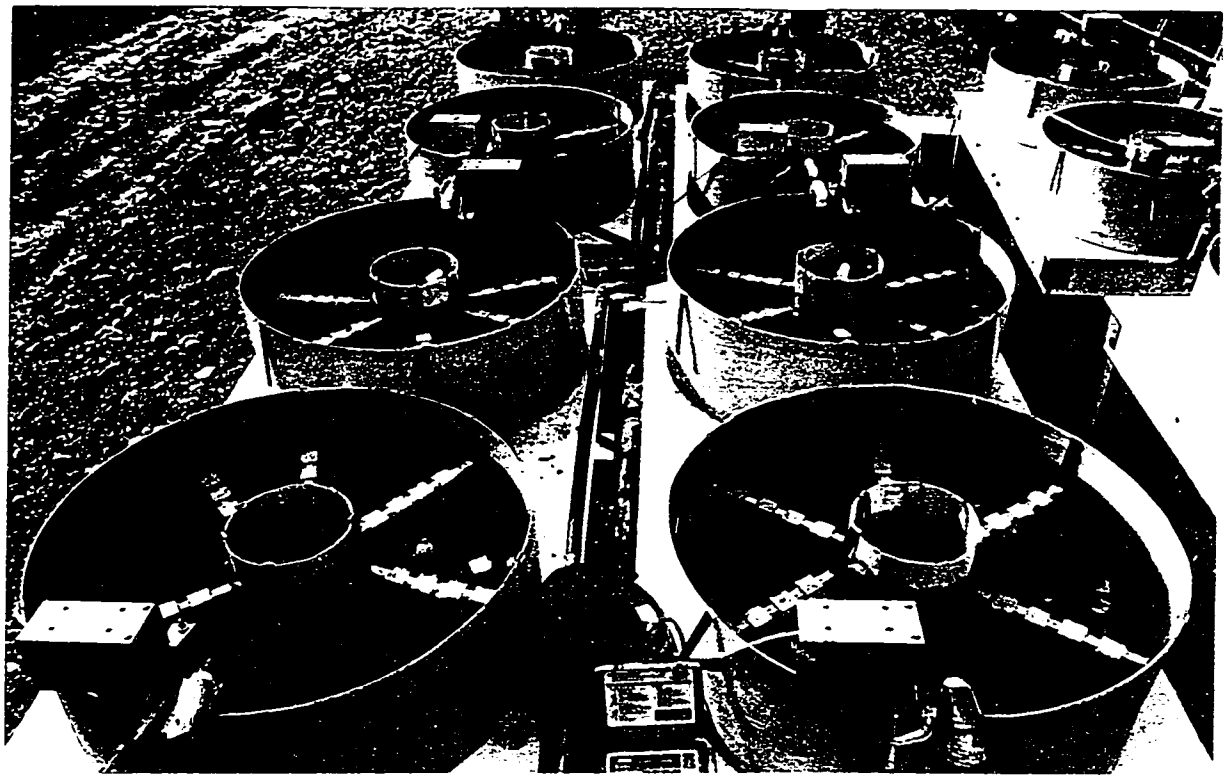


Plate 14. Experimental streams showing placement of porcelain tiles on the gravel and suspended from aluminium bars.

Plate 15. Dye trace showing mixing within first quarter of stream length.

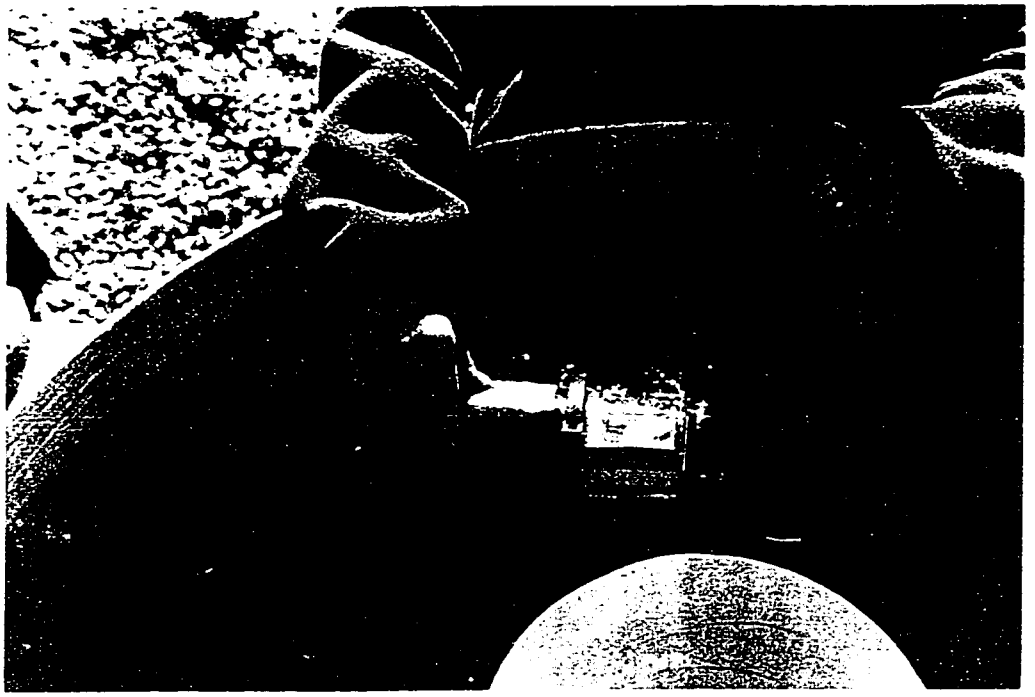


Plate 16. Ryan thermograph used to monitor stream temperature.

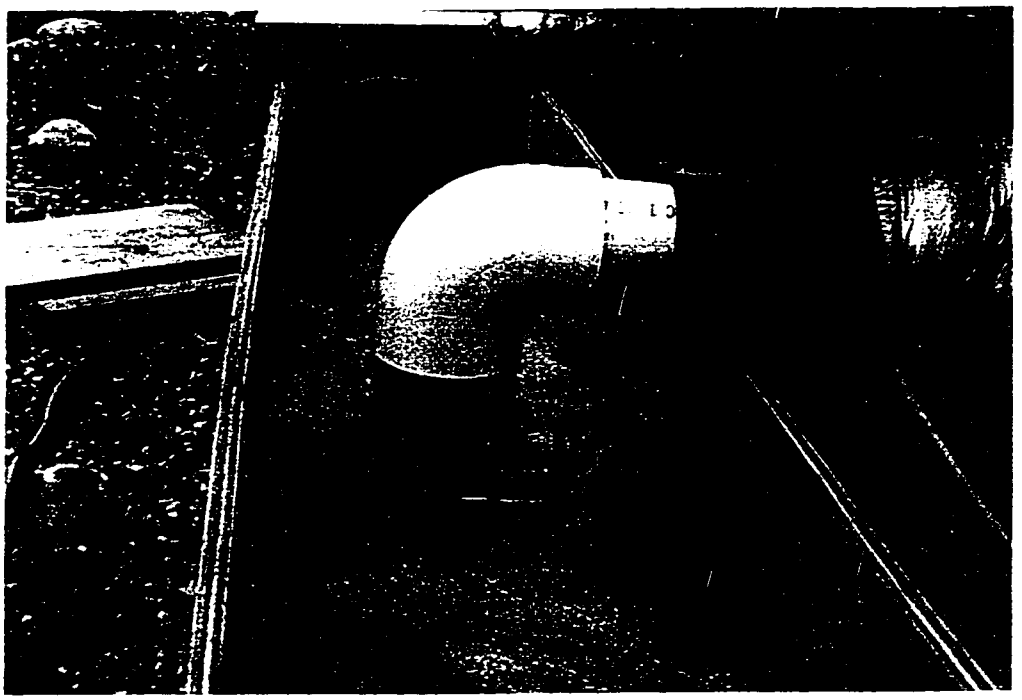


Plate 17. Four inch water delivery line being flushed into wooden trough at downstream end of facility.

Appendix A. Components of Artificial Stream.

(1) Materials list for water delivery system.

Description	Dimension	Quantity
Class 63 PVC pipe	4 "	110'
Sch 40 PVC tee	4"	9
Sch 40 PVC 90 deg. elbow	4"	6
PVC ball valve	4"	4
PVC reducer bushing	4" to 2"	8
PVC reducer bushing	2" to 1"	8
Class 63 PVC pipe	1"	60'
Gate valve	1"	16
Sch 40 PVC 90 deg. elbow	1"	48
Sch 40 PVC tee	1"	8
PVC pipe flange with gasket	4"	1
Vanstone flange with gasket	4"	2
Polyethylene tank with lid	378 litre	1
Bolts	3 1/2"	18

(2) Materials list for platforms (includes materials for head tank platform).

Description	Dimension	Quantity
3/4" plywood	4' x 8'	9
3/4" plywood	4' x 8"	18
3/4" plywood	8' x 8"	18
Aluminum angle brackets	4"x 4"x 4"x 1/4"	162
Aluminum angle stock	3" x 3"x 1/4"	120'
Aluminum stock	1" x 1/4"	24'
Bolts with washers	1/4"	56
Latex paint		

(3) Materials list for wastewater system.

Description	Dimension	Quantity
Class 63 PVC pipe	2"	70'
Sch 40 PVC pipe caps	2"	4
Sch 40 PVC tee	2"	16
PVC socket flanges with gasket	2"	4
Flexible tubing (silicone recommended)	1"	16'
Class 64 PVC pipe	1"	32'
Sch 40 PVC 90 deg. elbow FTxS	1"	16
Sch 40 PVC male adapter SxMT	1"	16
Circular rubber gasket	2"x1/4" with 1" hole	32
3/4' plywood	4' x 8'	8
Vanstone flanges with gaskets	6"	2
Vanstone flanges with gaskets	4"	1
Class 63 PVC pipe	6"	2'
Flexible pipe strapping		
Big O hose	4"	*

*quantity dependent upon distance to receiving water

(4) Materials list for motors

Description	Dimension	Quantity
250 RPM, 1/10 H.P., Geared-head 110 V motor		1
1591 DBU Hammond box		1
Pulley	22.6 mm diameter	1
Pulley	49.3 mm diameter	1
Copper tube	16 mm x 230 mm	1
Copper tube	8 mm x 230 mm	1
Brass bar	3" x 3/4"	1
Stainless steel shaft	4" x 1/4"	1
Aluminum fan blade	9"	1
Grease nipple		1
C/R oil seal		1
Flat belt	14.5" x 0.5"	1
Set screws	10-32	2
NC bolt	1/2" x 1"	1
Nuts	1/2"	2
Lock washer	1/2"	1
SJTW wire	8' 16-3 gauge	1
U ground plug		1
Sheet aluminum	8" x 15" x 0.032"	1
Angle aluminum	8" x 1.5" x 2"	1
Aluminum box tube	3" piece of 1"x1"x1/8"	1
Fuse holder		1
Fuse	1/2 amp	1

Appendix 5

Procedures for Periphyton Enumeration

The periphyton samples will be agitated and aliquots drawn off for enumeration in either Sedgewick-Rafter Cells or Palmer Cells, depending on density of sample. Filamentous eukaryotic algae will be counted by the cell. Colonial algae will be counted by the cell as well. For filamentous cyanobacteria number of cells will be estimated by dividing filament length (in the field) by average cell length. Diatoms will be counted but not identified beyond centric/pennate level in wet mounts.

Permanent quantitative diatom slides will be prepared for each site. These slides will be made at several dilutions to ensure that one of the dilutions will be an appropriate thickness for counting. At least 500 valves will be counted for each sample.

All counts will be reported as cells per cm of substrate, provided that the surface area of the sample is provided with the sample. One set of slides will be prepared for deposit in the collection of your choosing. There is an excellent curated diatom collection in Toronto, or you may wish to have them placed in the collection at the Academy of Natural Sciences in Philadelphia.

I currently have a graduate student, Anessa Dodge, that will be counting these samples. She is currently enrolled in our phycology class, and will be working on a diatom project for her thesis starting this summer. I have over twenty years experience in diatom and algal taxonomy, having done my first funded work as a student in this area in 1974. In order to assure accuracy we will take the following measures.

1. The standard procedure for diatom work in my laboratory is to have students keep an exhaustive photo record of their taxa. Thus, they examine a diatom slide for some time, taking photographs of all taxa. Coordinates of all photos are recorded so that we can return to individual specimens if necessary. We have a diamond scriber for circling unusual or new taxa. I

then sit down with them and help them identify the taxa to the lowest taxonomic level possible. They then put the photos on cards and begin to make a reference set with which to identify diatoms during counts. I also examine the slide to see if I can find taxa the have missed.

2. Once the reference set of photos with measurements and notes is assembled, the student begins the count. Any new taxa observed are photographed, given a designation such as *"Navicula* species 3" to keep track of them during counts, and are later identified when sufficient photos have been taken to make a determination. Especially difficult taxa (ones I cannot find after a short search), carry the genus and species number designation for most of the study. I then sit down with the student at some point and we go through the more scattered literature to find the names of these taxa. If we still cannot find the taxa, we must consider the possibility that the taxa are new species, varieties, etc. At the conclusion of the study, if we have a number of such potentially new taxa, we will make a trip to the Academy of Natural Sciences in Philadelphia and examine their new species file. If a search in this file does not result in a taxonomic identification, the taxa will be described as new.
3. Non-diatom algae will be identified using the same protocol, only we will document these species by capturing the images on video in order to save photo expenses. Specimens of non-diatom algae are often not identifiable to species even when fresh and in excellent shape. For example, *Oedogonium, Spirogyra, Hougeotia, Zygnema,* and *Vaucheria* species cannot be identified without reproductive stages, which are very rarely in field samples. Flagellates often lose their flagellar structure in Lugol's (or any preservative for that matter). Discoloration by the Lugol's can also make determination of genus difficult when color cues are needed for recognition of the division in which the algae belongs. However, I have many years experience in identification of field samples, and feel confident I will be able to be as accurate as anyone with similar problematic samples.

Sizes of algae will be recorded, so that with problematic filamentous algae we will at least be able to determine if more than one species within such genera are present in the sample.

4. All samples will be worked blind (with number codes known only to me). To check the quality and replicability of the student's work, I will pick some of these samples and check them to see if she is getting all of the taxa. I will check all of the slides at first during the primary training period. Then I am confident that she is doing well, the frequency of such checks will decrease.
5. For further QA/QC assurance, we will examine 10% of the samples twice (again blind). I will have Anessa examine one of these replicates every 10 samples she does (without letting her know that it is a replicated sample). I am not sure at this point what level of similarity is acceptable in such situations, but I will calculate both a quantitative similarity index (Ruzicka's) and qualitative similarity index (Jaccard's) to help in assessing her skill. If replicates are grossly dissimilar, I will examine them myself to see what level of dissimilarity I produce. All replicate data will be reported. Cost of the 10% replicated samples is included in the cost per sample (i.e. charges assessed for 10 samples pay for 10 samples plus 1 replicate).

PERSONNEL

Anessa Dodge: Anessa Dodge is a master's level graduate student interested in water quality analysis using diatoms as indicator species. She is conducting her research on the river mouth areas of several rivers emptying into Lake Erie. She is studying the periphytic diatom flora as collected on artificial substrates. This work will bolster her skills as a diatomist, as will as help support her during the summer, when the work will primarily take place. We anticipate being able to finish most of the samples by the end of August (particularly if the number of samples is near 50). If upwards of 100 samples are needing analysis, then it will likely take longer. Data will be provided as it is completed, so that you will have a good idea of our progress. I suspect that the first samples will take a long time to complete, but that as summer progresses,

Anessa's speed will improve. She strikes me as a very bright student, and I am confident in her abilities.

Jeff Johansen: I have done algal and diatom floristics for over 20 years. I started doing phycological research as an undergraduate student, and now have over 40 referreed publications in the area of phycology. A full curriculum vitae is attached.

I have worked on algae in lakes, rivers, saline springs, oceans, subaerial habitats, and soils. I am broadly interested in the taxonomy and ecology of freshwater algae. I have regularly attended the North American Diatom Symposia and the meetings of the Phycological Society of America. E am familiar with a variety of multivariate methods, including clustering, PCA, CANOCO, and weighted averaging. I would be interested in providing analysis of the data for free if Anessa and I could be included as an authors on some publications. Indeed, this arrangement would be preferable to me.

Facilities

We have the finest light microscopes currently available. We have a Zeiss Axioscope photomicroscope with camera and video capabilities (3 chip camera, high resolution monitor). This microscope has the high end Nomarski DIC objectives (63X, 100K, both with 1.4 NA, plus a condenser with 1.4 NA). We also have an Olympus B-Max photomicroscope with high end Nomarski DIC objectives (60X, 100K, both with 1.4 NA, and 1.4 NA condenser). These microscopes will be suitable for recognizing diatoms to the lowest taxonomic levels to which they are described. We have an additional Olympus BH-2 photomicroscope with Nomarski DIC Optics. This is also an excellent microscope, but does not have the resolution of the newer microscopes. The paper on the algae of Seneca Cavern shows the resolution of this microscope, which is still well above average.

We also have an excellent collection of algal and taxonomic references, including the appropriate volumes in the Pascher's *Suessvasser Flora* (the

Krammer and Lange-Bertalot series), most diatom floristic texts in the *Nova Hedwigia/Bibliotheca Diatomologica* series, a number of the *Bihliotheca*

Phycologica volumes, the *Binnengevasser* volumes, a complete set of the journal Diatom Research, the multi volume algal flora of Poland edited by Starmach, the series on algal genera put out by Bourelly, Prescott's flora of the Western Great Lakes Region, the Patrick and Reimer volumes on The Diatoms oS the United States, Simonsen's Atlas and Catalogue on the Diatom Types of Friedrich Hustedt, all of the proceedings volumes of the International Diatom Symposium, Several major floras by Hustedt, and a host of reprints on diatom and algal taxonomy. We follow the taxonomic system of Krammer and Lange-Bertalot for the most part, but can recognize morphotypes (such as those of *Achnanthes minutissima*) if desired.