FINAL REPORT

EFFECTS OF WATER TEMPERATURE AND TREATED PULP MILL EFFLUENT ON SURVIVAL AND GROWTH OF *DAPHNIA MAGNA* (CLADOCERA: DAPHNIDAE) AND *TAENIONEMA* (PLECOPTERA: TAENIOPTERYGIDAE)

Effects of water temperature and treated pulp mill effluent on survival and growth of *Daphnia magna* (Cladocera: Daphnidae) and *Taenionema* (Plecoptera: Taeniopterygidae)

Prepared for:

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EXECUTIVE SUMMARY

The objective of this report is to determine whether the ecological effects of treated bleached kraft pulp mill effluent on aquatic invertebrates vary with water temperature. To address this objective, we initially summarized existing studies that evaluated the interactive effects of water temperature on the toxicity of a variety of compounds to aquatic invertebrates. This review indicated that there is a moderately large amount of information indicating that toxic effects of contaminants or other physicochemical stressors can vary with water temperature. These interactive effects between contaminant concentrations and water temperature raise the issue of whether toxic effects of pulp mill effluents that are typically evaluated at 15°C to 20°C, are representative of those at lower temperatures. This is relevant to rivers receiving pulp mill effluent in Alberta, which have lowest dilution capacity in winter, when water temperatures are near 0°C for five months or more. Our review of the literature also showed a general paucity of information evaluating the effects of water temperature and pulp mill effluent on aquatic invertebrates.

Based on these information gaps, we completed three sets of experiments to evaluate the effect of water temperature on toxicity of sodium chloride (a reference toxicant) and treated bleached kraft pulp mill effluent on two aquatic invertebrates. The first set of experiments evaluated the effects of water temperature (i.e., 7ºC, 13ºC, 20ºC) and varying concentrations of sodium chloride (NaCl) (0, 5000, 5400 and 5800 mg NaCl/L) on survival and growth of *Daphnia magna* over a 6-day period. Results from this experiment showed that survival of *Daphnia magna* was significantly affected by water temperature with greater number of individuals surviving at 20°C compared with 13°C and 7°C, respectively. Similarly, significantly greater number of individuals survived at 0 and 5000 mg NaCl/L compared with 5400 and 5800 mg NaCl/L. However, the low number of organisms that survived the experimental period precluded analysis of the effects of NaCl on growth.

The second set of experiments evaluated the effects of water temperature (i.e., 7ºC, 13ºC, 20ºC) and varying concentrations of treated bleached kraft pulp mill effluent (i.e., of 0%, 5%, 10%, 50%) on survival and growth of *Daphnia magna.* While survival of *Daphnia magna* was not significantly affected by either water temperature or pulp mill effluent concentration, body length (i.e., growth) and the production of neonates increased significantly with increasing water temperature and pulp mill effluent concentration over an 8-day period.

The third set of experiments evaluated the effects of water temperature (i.e., 3ºC, 9ºC, 15ºC) and varying concentrations of treated bleached kraft pulp mill effluent (i.e., of 0%, 15%, 30%) on survival and growth of small and large larval *Taenionema* over a 14-day period. Survival of *Taenionema* was not significantly affected by water temperature, or pulp mill effluent or the interaction of these factors, and all individuals survived the 14-day trials. In contrast, growth of both small and large larvae was significantly affected by water temperature and pulp mill effluent concentration. Larval growth was highest when larvae were maintained under high water temperatures and high concentrations of pulp mill effluent.

Taken together, our data indicate that both *Daphnia magna* and *Taenionema* can grow and develop in the even moderately high concentrations of the treated bleached kraft pulp mill effluent used in our experiments. While our experiments were not designed to identify causal mechanisms, increased growth of *Daphnia magna* and *Taenionema* with increasing concentration of treated bleached kraft pulp mill effluent likely arises from increased quantity and quality of algal and bacterial food resources. Similarly, our finding of increased growth of both species with increasing water temperature likely arises from the positive relationship between metabolic rate and temperature. The effects of different water temperatures and treated bleached pulp mill effluent concentrations that resemble conditions during spring, summer and fall are not well understood.

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SCOPE

This final report presents the findings arising from research completed under the Sustainable Forest Management Research Program 1998-1999 Assignment Agreement #8 titled: "Biomonitoring of pulp mill effluent in Alberta rivers: effects of seasonal temperature changes on the sensitivity of aquatic invertebrates to toxins and pulp mill effluents".

INTRODUCTION

Rationale and Objective

Predicting the toxicological effects of pulp mill effluents on aquatic biota is difficult because pulp mill effluents are chemically complex and their toxicity can result from interactive effects of numerous chemicals (Cairns et al. 1975, Walden 1976, Leung and Sell 1982, Davis et al. 1988, McCubbin and Folke 1993). Toxic effects of industrial effluents also can be modified by the physical and chemical characteristics, including water temperature, of the receiving waters (Lohner and Fisher 1990, Centeno et al. 1993). Studies evaluating the toxic effects of pulp mill effluents on aquatic biota are typically completed at standardized water temperatures of 15-20^{o}C. This experimental approach assumes that toxicity of pulp mill effluents within this temperature range is representative of toxicity at other temperatures.

The main objective of this report is to determine whether toxic effects of pulp mill effluents vary with water temperature, especially low temperatures. This is relevant to rivers receiving pulp mill effluent in Alberta that have lowest dilution capacity in winter and water temperatures close to 0^{o}C for five or more months. To achieve this objective, a literature review was initially conducted to identify evidence for temperature-specific toxic effects on aquatic biota. The review included a summary of earlier biomonitoring studies on the effects of various effluents on benthic macroinvertebrates in Alberta during winter. The literature review, combined with bioassay experiments, conducted as part of this study, will help define the season when aquatic invertebrates are likely to be most sensitive to treated kraft pulp mill effluent. Three different experiments were designed to evaluate the interactive effects of: i) water temperature and sodium chloride (NaCl) concentrations on survival and growth of *Daphnia magna* (Experiment 1), ii) water temperature and pulp mill effluent concentrations on survival and growth of *Daphnia magna* (Experiment 2), and iii) water temperature and pulp mill effluent concentrations on survival and growth of the stonefly *Taenionema* spp (Experiment 3).

Departures From Initially Proposed Work

Revisions to the research proposal were made in March 1998 after it was submitted to Alberta Environment in December 1997 (Casey 1997, 1998). These revisions were based on discussions with experts on the rearing and testing of Daphnidae (Environment Canada, Ontario Ministry of Environment and a private consultant), and comments received from representatives of Weyerhaeuser (Grande Prairie, Alberta, and Seattle, USA) and Alberta Forest Products Association (AFPA Environmental Committee). The revisions are discussed in detail in the Methods and Materials and Discussion sections. The major change to the study design was the use of *Daphnia magna* instead of *Ceriodaphnia dubia* as the experimental organism. This change in protocol was implemented because: i) *Daphnia magna* are more easily reared in the laboratory than are *Ceriodaphnia dubia*, ii) ARC has extensive experience in rearing *Daphnia magna* compared with *Ceriodaphnia dubia* cultures, and iii) *Daphnia magna* are larger than *Ceriodaphnia dubia*.

LITERATURE REVIEW

Materials and Methods

Two extensive literature searches were conducted using keywords and combinations of words in the title and descriptor fields of 20 different on-line computer databases (Table 1). These searches provided access to many scientific disciplines, especially aquatic and toxicological sciences, and a range of academic, technical and pulp and paper industry databases (Table 1). The first literature search focused on the effects of water temperature and toxicity on various groups of invertebrates including zooplankton, molluscs and aquatic insects. Keywords including effluent, wastewater, pulp mill, winter and river, were used to reduce the results of the search to 238 unique publications. For the second search, the same computer databases were queried using the same keywords, but temperature and toxicity were excluded so that this search would focus on other possible effects of stressors (e.g., nutrients) under winter conditions. This search produced 152 publications. The titles of all 390 publications were examined for potential relevance to this study and relevant abstracts were reviewed. Full articles were reviewed only if they were thought to be of value to the study. Other related literature, especially recent studies such as Northern River Basins Study (NRBS) reports and summaries, were also examined for useful information. Analysis of relevant temperature-toxicity data taken from earlier studies was also used in the review.

Table 1. Databases searched to evaluate the effects of water temperature on toxicity of various compounds on aquatic biota.

Database	Years Scheduled
Toxline®	1965-1998
CAB Abstracts	1972-1998
EMBASE	1974-1998
BIOSIS PREVIEWS®	1969-1998
Pascal	1973-1998
IAC BUSINESS A.R.T.S.	1976-1998
Scisearch® Cited Ref. Sci.	1974-1998
Life Sciences Collection	1982-1997
Water Resour. Abs.	1967-1998
Energy Sci. Tec.	1974-1998
CAB HEALTH	1983-1997
Aquatic Sci & Fish Abs.	1978-1998
Zoological Record Online®	1978-1997
NTIS	1964-1998
Pollution Abs.	1970-1998
JICST-Eplus	1985-1998
CRIS/USDA	1997-1998
Biol. & Agric. Index	1983-1998
Env. Bib.	1974-1998
PAPERCHEM	1967-1998

Results

Response of Macroinvertebrates to Pulp Mill and Municipal Effluents in Alberta

Studies on the effects of treated pulp mill and municipal (i.e., sewage treatment plant) effluents on benthic macroinvertebrate communities in the open-water seasons (spring and fall) have shown increases in densities of pollution-tolerant (Chironomidae and Oligochaeta) and pollution-intolerant (Ephemeroptera and Plecoptera) taxa immediately downstream of effluents (e.g., Anderson 1989, Noton et al. 1989). Changes in composition and diversity of zoobenthos communities downstream of effluents have also been documented in these studies. Effects on these communities might be caused by stress induced by the effluent or alternatively, by differences in the habitat, especially substratum, among monitoring sites. Enrichment of zoobenthos communities was likely caused by nutrient loads in the effluents that increase food sources (e.g., epilithic algae) resulting in increased population densities. Experimental studies have shown that nutrients in bleached kraft pulp mill effluents can increase the productivity of stream food webs (Hall et al. 1991, Bothwell 1992, Culp and Podemski 1996, Dubè and Culp 1996). However, sub-lethal effects on food web productivity caused by bleached kraft pulp mill effluents are also known to occur (Owens 1991). For example, Dubè and Culp (1996) found the growth of Chironomidae larvae was inhibited at highest concentrations (5% and 10%) relative to the lowest concentration (1%) of effluent tested. Higher concentrations of effluent might also reduce the production of benthic algae as a result of increased colour caused by the effluent, that in turn reduces the amount of light reaching the substratum (Hall et al. 1991). Sub-lethal effects of toxins are typically determined using standard bioassay tests in the laboratory at room temperature, such as the 7-day reproduction and survival test using *Ceriodaphnia dubia* (Environment Canada 1992). Longer-term non-routine experimental tests may be necessary to identify sub-lethal effects for biota with longer life cycles, such as aquatic insects.

In Alberta, at least 4 surveys of benthic macroinvertebrate communities have been conducted in the Wapiti-Smoky and Athabasca rivers during winter months. These studies focussed on zoobenthos communities of erosional habitats with the exception of one study that also included some depositional sites. To date, detailed results of the winter studies have not been summarised together to determine if there are similar patterns among different pulp mill effluents, river basins and different seasons (spring, fall and winter).

Pilot studies were initially conducted to determine the feasibility of collecting biomonitoring data in ice-covered rivers and to quantify potential effects under winter conditions. The first two studies showed an enrichment of zoobenthos communities downstream of separate bleached kraft pulp mill and municipal effluents in the Wapiti-Smoky River system in 1991 (February; 10 sites) and 1992 (January; 11 sites) (Noton et al. 1992, Terrestrial & Aquatic Environmental Managers 1992). These results showed no clear evidence of toxic effects on major zoobenthos groups. However, toxic effects on lower taxonomic groups may have been masked by the overall enrichment of major groups. For example mayfly larvae of *Rhithrogena* (Ephemeroptera) showed reductions in densities downstream of the separate municipal and pulp mill effluents relative to upstream sites in both years. Densities of *Rhithrogena*, however, may have been affected by differences in the substratum texture and/or food base; epilithic algae (chlorophyll a) was at much greater mass at sites immediately downstream of the effluents. For example, Clifford et al. (1992) found that in contrast to most zoobenthos taxa, Heptageniidae larvae that include *Rhithrogena* colonised smooth tiles more readily than rough tiles with greater mass of algae in two separate stream studies. Stonefly larvae of *Taenionema* (Plecoptera; Taeniopterygidae) showed different results at the same sites in separate years (Noton et al. 1992). In 1991, *Taenionema* densities increased downstream of the municipal effluent and decreased downstream of the pulp mill effluent; but the opposite pattern was found in 1992.

Another biomonitoring survey was conducted in winter (February and March) 1993 as part of the Northern River Basins Study (Saunders and Dratnal 1994). Samples were collected at nine sites from the upper Athabasca River to downstream of Fort McMurray. The following interpretation of the data was based on an examination of the raw data

presented in Saunders and Dratnal (1994). No attempt was made to examine the responses of individual taxa or to conduct statistical analysis. Densities of pollution-intolerant and pollution-tolerant zoobenthos increased downstream of the municipal effluent at Athabasca town and the combined municipal-bleached kraft pulp mill effluent at Hinton, compared to sites a short distance upstream of the effluents. Data from other sites in this study were not taken at sites immediately upstream and downstream of other effluents. Thus it was not possible to make inferences on the effects of these effluents on zoobenthos communities in the rest of the Athabasca River sampled.

In 1994, Weyerhaeuser Canada Ltd. conducted a winter survey (February-March) of zoobenthos at 14 erosional sites and 6 depositional sites in the Wapiti-Smoky river system as part of the federal Environmental Effects Monitoring (EEM) program (Terrestrial & Aquatic Environmental Managers Ltd. 1995, Golder 1996). In both the erosional and depositional habitats, there were increased densities of major zoobenthos groups downstream of the pulp mill effluent. Similar to both of the earlier studies in this river system (Noton et al. 1992, Terrestrial & Aquatic Environmental Managers 1992), densities of *Rhitrogena* larvae decreased in erosional habitats downstream of each of the same municipal and pulp mill effluents. However, as noted above, this effect may have been caused by differences in substratum texture and food (i.e., corresponding increases in algal mass were also found in this study; Terrestrial & Aquatic Environmental Managers 1995) rather than toxic effects associated with the effluents. Compared to 1991 (Noton et al. 1992), densities of *Taenionema* decreased sharply downstream of both effluents indicating the possibility of sub-lethal effects.

In general results of all 4 winter biomonitoring surveys (Noton et al. 1992, TAEM 1992, Saunders and Dratnal 1994, Terrestrial & Aquatic Environmental Managers Ltd. 1995, Golder 1996) showed similar patterns for major taxonomic groups of zoobenthos compared to open-water seasons (e.g., Anderson 1989, Noton et al. 1989). Enrichment of zoobenthos communities generally occurred downstream of treated municipal and bleached kraft pulp mill effluents, but sub-lethal effects on individual taxa may have been masked by this major effect. In conclusion, individual biomonitoring surveys are not designed to evaluate the sensitivity of zoobenthos taxa at different seasonal temperatures. But detailed analysis of individual populations in winter compared to open-water surveys would provide insight on the sensitivity of various taxa with known or uncertain sensitivities to pulp mill and other effluents in Alberta.

Water Temperature and Effects of Toxins on Aquatic Invertebrates

Water temperature is an important environmental factor that can potentially affect the response of an organism to toxic substances. Lower metabolic rates of invertebrates caused by low water temperatures can reduce their activity and growth rates. These changes in metabolism and activity of invertebrates might affect their sensitivity to a specific toxin or environmental condition (e.g., levels of dissolved oxygen in the water column).

In the following review of toxicity studies, sensitivity of different invertebrate taxa to a toxin is based on comparisons of toxicity levels (LC50 and EC50) from experiments or standard bioassays used to determine the effect of toxins at different temperatures. For example, an invertebrate species with low LC50 values at the lowest temperature compared to higher temperatures would indicate that the species is more susceptible or sensitive to the toxin at the low temperature. Different toxicity levels at different temperatures indicate temperature-specific tolerances.

Pulp and Paper Mill Effluents

Recent reviews of the effects of pulp and paper effluents on aquatic biota indicate the need to conduct experiments on the effects of seasonal temperatures on toxicity. In the extensive review of pulp and paper mill effluents and toxicity on aquatic biota, McLeay et al. (1987) concluded that the effects of environmental temperatures received little attention and there were no toxicity data under low water temperatures. In a review of hazard assessment of pulp and paper effluents, Owens (1991) suggested that biomonitoring should be conducted to determine the season of greatest stress

in ecosystems. Colodey and Wells (1992) reviewed the effects of pulp and paper effluents on estuarine and marine ecosystems in Canada. They suggested that realistic environmental conditions, including temperature, should be used when conducting non-standard bioassays in conjunction with field biomonitoring. Ecotoxicological studies using aquatic invertebrates were conducted as part of the Northern River Basins Study. These studies, however, were based on the laboratory tests conducted at 23°C (Day and Reynoldson 1995, Dobson et al. 1996).

In conclusion, there is a need for experimental studies designed to evaluate the toxicity of pulp and paper effluents at temperatures representative of seasonal levels in northern rivers of Alberta and Canada.

Other Contaminants

In a review of the effects of temperature on toxicity of various chemicals to aquatic biota, Cairns et al. (1975) found most of the literature focussed on fish and few studies evaluated toxicity to invertebrates. Cairns et al. (1975) concluded that it was difficult to make scientifically justifiable generalisations from the literature because of inadequate data. Mayer and Ellersieck (1986) compiled a database of 4,901 acute toxicity tests developed over a 20-year period by the Columbia National Fisheries Research Laboratory, United States Fish and Wildlife Service. Sixty-six species of aquatic fauna were tested with 410 chemicals. Most of the data arose from experiments using fish as the test organism and pesticides as the toxicant. Overall, insects followed by crustaceans, fishes and amphibians showed decreasing levels of sensitivity to toxins. Mayer and Ellersieck (1986) found that toxicity of most chemicals generally increased with temperature although the opposite pattern was found for some chemicals. An increase in toxicity may be related to increased metabolism leading to increased sensitivity to toxins at higher temperatures. The conclusions of Mayer and Ellersieck (1986), however, were based on fish species at temperatures ranging from about 7->20°C. Only two of 26 tests were conducted at temperatures <7°C. Mayer and Ellersieck (1986) recommended more research on temperature and toxicity of chemicals to invertebrates. They also suggested that because of inherent variation and frequent exceptions in acute toxicity data, it was most appropriate to test the chemical and species of concern under the environmental conditions of interest.

Toxicity and Water Temperature

All pertinent publications were reviewed for data that could be used to examine the relationship between water temperature and toxicity. In the following analysis, the data included the responses of various aquatic invertebrates to toxins, contaminants and environmental conditions (e.g., dissolved oxygen). The data sets for each invertebrate species were included in the analysis when the experimental design was rigorous having standardised and consistent methodologies for various temperatures of each test, defined toxicological endpoints [e.g., LC50]) and experiments with two or more water temperatures (Tables 2-6). With one exception (a marine mollusc), all studies were for freshwater invertebrates.

Data from 11 studies that specifically describe effects of water temperatures and a toxicant group were compiled into major taxonomic groups (Tables 2-6). Data for these taxa made up a total of 113 data points showing various levels of toxicity (LC50 or EC50) for each taxon at a range of temperatures from 4-30°C. Most of these studies evaluated the effects of ammonia, metals (e.g., zinc, copper and chromium) and dissolved oxygen concentrations on a wide range of invertebrate groups. There were no data for the same invertebrate species in different independent studies. Only 10 toxicity values in the entire database (i.e., 8.9%) were recorded at temperatures <10°C. These low-temperature data were for various species of crustaceans and molluscs, but data for aquatic stream insects were lacking. Fifty-five percent of the data included toxicity values for more than 2 temperatures; the remainder were for only 2 temperatures. The greatest range in temperatures tested for a taxon varied from 2.5-20.9°C.

Based on the above summary of the data, it is clear that the database has limited use in determining the

sensitivity of aquatic invertebrates, especially insect larvae, to toxins at temperatures representative of Alberta rivers in early spring, late fall and winter. General relationships between toxicity and water temperature, were evident for some taxonomic groups, but not for others and some results were inconclusive.

Results for different species of Daphnidae (Cladocera) showed contrasting patterns for the relationship between temperature and toxicity (Table 2). Neonates of *Ceriodaphnia dubia* were more sensitive to ammonia at 7°C (lowest LC50 values) than at 25°C in two separate tests using river and wastewater to dilute the test solutions. In contrast to *Ceriodaphnia dubia* neonates, adults of *Ceriodaphnia vetulus* were more sensitive to ammonia at 20.4°C than at 17°C. The range in temperatures tested was much less (3.4°C) for *Ceriodaphnia vetulus* than that for *Ceriodaphnia dubia* (18°C). Neonates of *Daphnia magna,* another Daphnidae, showed greater sensitivity to water-soluble fractions of coal at greater temperatures (20 and 25°C) compared to the lowest temperature of 10°C. The lowest toxicity level was at the intermediate temperature (20°C) than at the other test temperatures indicating a non-linear response. Similar to *Daphnia magna*, sensitivity of *Crangonyx* spp. (Amphipoda) to ammonia was found at the highest temperatures (20 and 25°C) compared to lower temperatures (4, 12 and 13 °C) in two studies (Table 2).

Data for six other species of Crustacea (Anostraca, Isopoda and Decapoda: crayfish) showed similar levels of toxicity to various toxins (including ammonia and metals) over relatively wide ranges of temperatures (4-30°C) (Table 3). One exception to this pattern was for immature crayfish (*Orconectes immunis*) that showed increased sensitivity to ammonia at 17 °C compared to 5°C (Table 3).

Toxicity tests for three species of snail and a clam (Mollusca) showed similar sensitivity to ammonia at a range of temperatures (4-25°C) (Table 4). The only exception to this pattern was the marine clam, *Mya arenia*, that was more sensitive to zinc and copper in separate tests at 22°C than at 17.5 and 4°C (Table 4).

The most comprehensive data set from a single study was for 12 species of aquatic insect larvae (Table 5). These data show the responses of aquatic insects, predominantly Ephemeroptera species, to different combinations of water temperature and dissolved oxygen concentrations (Table 5). Interpretation of LC50 ("toxicity") levels for concentrations of dissolved oxygen, however, is different from LC50s derived from responses to known toxicants. In contrast to toxins, dissolved oxygen is an important environmental condition and it is required by most biota at relatively high concentrations. Under the same atmospheric conditions, low temperature water will hold more dissolved oxygen than warmer water.

For six families of Ephemeroptera, the lowest LC50 values were always found at the lowest temperatures beginning at 12°C compared to higher temperatures, up to 30°C (Table 5). Single species of Plecoptera (*Nemoura cinerea*) and Anisoptera (*Sympecma fusca*) also showed the same pattern of lower LC50 values at lowest temperatures. The remaining two species, Trichoptera (*Silo pallipes*) and Anisoptera (*Onychogomphus forcipatus*), showed similar toxicity at the temperatures tested, 25°C and 30°C. The lower LC50 values at lowest temperatures for the aquatic insects indicated that these larvae required less dissolved oxygen at low temperatures. But these findings also indicate that the larvae were more sensitive to low dissolved oxygen concentrations at lower temperatures because fewer organisms survive at these temperatures, based on the LC50 values.

Results for other aquatic insect larvae from smaller data sets showed various patterns (Table 6). For responses to ammonia, two species in different taxa, Ephemeroptera (*Callibaetis skokianus)* and Anisoptera (*Pachydiplax longipennis*), showed greatest levels of toxicity at the lowest temperatures. The third species, Trichoptera (*Philartcus queries)*, tested with ammonia, however, showed similar responses at the temperatures tested. Two species of midge (Chironomidae) showed different responses in separate tests. One species, *Chironomus riparius*, showed greatest sensitivity to a pesticide (carbaryl) at the lowest temperature (10°C compared to 20 and 30°C). Whereas the other species, *Chironomus tentans* was more sensitive to a liquid coal extract at

Table 2. Effects of water temperature on the toxicity of ammonia and liquid coal extract on Cladocera and Amphipoda. Values in parentheses indicate water pH during the experiment. Sources: Nimmo et al. 1989[1], Arthur et al. 1987[2], Becker et al. 1983[3], Diamond et al. 1993[4].

Crustacea	**Test**	**Toxin**	**Temperature (^{0}C)**											
			4	**7**	**10**	**12**	**12.1**	**13**	**13.3**	**17**	**20**	**20.4**	**24.9**	**25**
Cladocera														
Ceriodaphnia dubia [1]	48-h EC50	NH_3 (mg/L) in wastewater		0.3 (8.2)										1.1 (7.8)
Ceriodaphnia dubia [1]	48-h EC50	NH_3 (mg/L) in St. Vrain River		0.5 (8.2)										>1.4 (7.8)
Ceriodaphnia vetulus [2]	48-h LC50	NH_3 (mg/L)								2.3 (8.3)		1.3 (8.1)		
Daphnia magna [3]	48-h LC50	Liquid coal Extract (mg/L)			12.3 (7.3-8.2)						3.0 (7.3-8.2)			4.6 (7.3-8.2)
Amphipoda														
Crangonyx spp.[4]	96-h LC50	NH_3 (mg/L)				2.1 (8.0)					0.9 (8.0)			
Crangonyx pseudogracillis [2]	96-h LC50	NH_3 (mg/L)	2.8 (8.0)				5.6 (8.0)	3.6 (8.2)	3.3 (8.0)				1.6 (8.0)	

Table 3. Effects of water temperature on the toxicity of various compounds on Anostraca, Isopoda and Decapoda (crayfish). Values in parentheses indicate water pH during the experiment. Sources: Centeno et al. 1993[1], Arthur et al. 1987[2], Diamond et al. 1993[3]. Hardness ($CaCO_3$) of water used in experiments by Centeno et al. 1993 ranged from 71–110 mg/L.

Crustacea	Test	Toxin	Temperature (°C)									
			4	4.6	10	12	15	17.1	20	22	25	30
Anostraca												
Streptocephalus proboscideus [1]	24-h LC50	$K_2Cr_20_7$ (mg/L)			1.7 (7.6-7.8)		2.2 (7.6-7.8)		1.6 (7.6-7.8)		0.9 (7.6-7.8)	0.6 (7.6-7.8)
Streptocephalus proboscideus [1]	24-h LC50	$CuSO_45H2O$ (mg/L			0.1 (7.6-7.8)		0.2 (7.6-7.8)		0.2 (7.6-7.8)		0.2 (7.6-7.8)	0.2 (7.6-7.8)
Streptocephalus proboscideus [1]	24-h LC50	NaPCP (mg/L)			0.5 (7.6-7.8)		0.6 (7.6-7.8)		0.7 (7.6-7.8)		0.7 (7.6-7.8)	0.5 (7.6-7.8)
Isopoda												
Asellus racovitzai [2]	96-h LC50	NH_3 (mg/L)	5.0 (8.0)							5.1 (7.8)		
Crayfish												
Procamberus clarkii [3]	96-h LC50	NH_3 (mg/L)				>2.0 (8.0)			1.2 (8.0)			
Orconectes immunis [2]	96-h LC50	NH_3 (mg/L)		22.8 (8.2)				14.7 (7.9)				

Table 4 Effects of water temperature on the toxicity of ammonia, zinc and copper on Gastropoda and Pelecypoda. Values in parentheses indicate water pH during the experiment. Sources: Hickey and Vickers 19941, Arthur et al. 19872, Eisler 19773. Hardness (CaCO3) of water used in experiments by Eisler 1977 ranged from 112-206 mg/L.

Mollusca	**Test**	**Toxin**	**Temperature (°C)**													
			4	**5.4**	**5.5**	**12.1**	**12.8**	**12.9**	**13.3**	**14.6**	**15**	**17.5**	**20**	**20.5**	**22**	**24.9**	**25**
Gastropoda																	
Potamopyrgus antipodarum [1]	96-h EC50	NH_3 (mg/L)									0.4 (8.2)		0.3 (8.2)				0.4 (8.2)
Heliosoma trivolvis [2]	96-h LC50	NH_3 (mg/L)						2.8 (8.2)							2.0 (7.9)		
Physa gyrina [2]	96-h LC50	NH_3 (mg/L)	1.6 (8.0)		2.1 (8.2)	2.5 (8.1)	2.2 (8.0)		1.8 (8.0)							1.7 (8.0)	
Pelecypoda																	
Musculium transversum [2]	96-h LC50	NH_3 (mg/L)		0.9 (8.2)						1.3 (8.1)				1.1 (8.6)			
Mya arenaria [3]	168-h LC50	Zn (mg/L)	>25.0 (8.0))									>10.0 (8.0)			1.5 (8.0)		
Mya arenaria [3]	168-h LC50	Cu (mg/L)	>3.0 (8.0)									0.1 (8.0)			0.0 (8.0)		

Table 5. Temperature specific toxicities (2-5 h LC50) of dissolved oxygen concentrations (mg/L) for Ephemeroptera, Trichoptera, Plecoptera and Anisoptera. Source: Jacob et al. (1984).

Insecta	**Temperature (^{o}C)**				
	12	**15**	**20**	**25**	**30**
Ephemeroptera					
Baetis alpinus	7.50	7.60			
Cloeon simile		1.03	1.90	2.40	3.45
Epeorus sylvicola	7.40	7.40	8.10		
Ephemera danica		0.04	0.32		
Ephemera vulgata		0.30	0.37	0.45	
Leptophlebia marginata		0.10	0.12	0.85	1.80
Siphlonurus asesivalis			0.43	0.50	
Siphlonurus lacustris		2.60	2.90	3.15	
4					
Trichoptera					
Silo pallipes	7.20	7.30			
Plecoptera					
Nemoura cinerea	1.02			2.40	
Anisoptera					
Onychogomphus forcipatus				1.08	1.05
Sympecma fusca			0.73	1.18	1.55

Table 6. Effects of water temperature on the toxicity of ammonia, carbaryl, liquid coal extract (LCE), and minimum dissolved oxygen concentrations on Ephemeroptera, Trichoptera, Chironomidae and Anisoptera. Sources: Arthur et al. 1987[1], Jacob and Walther 1981[2], Nebeker 1972[3], Arthur et al. 1987[4], Becker et al. 1983[5], Lohner and Fisher 1990[6], Diamond et al. 1993[7]. Values in parentheses indicate water pH during the experiment. Water pH values were not available for Jacob and Walther (1981) and Lohner and Fisher (1990).

Insecta	**Test**	**Toxin**	**Temperature (^{o}C)**											
			10	**10.8**	**12**	**13.3**	**15**	**17**	**18.5**	**20**	**21**	**21.9**	**25**	**30**
Ephemeroptera														
Callibaetis skokianus [1]	96-h LC50	NH_3 (mg/L)		3.2 (7.7)		4.8 (7.9)								
Ephemerella muronata [2]	2-5-h LC50	DO (% saturation)					12.2			19.2			49.5	
Trichoptera														
Hydropsyche betteni [3]	96-h LC50	DO (mg/L)	1.0 (7.5-7.8)					2.3 (7.5-7.8)	2.6 (7.5-7.8)		2.9 (7.5-7.8)			
Philartcus queries [4]	96-h LC50	NH_3 (mg/L)				10.2 (7.8)						10.1 (7.8)		
Chironomidae														
Chironomus tentans [5]	48-h LC50	LCE (mg/L)								14.2 (7.3-8.2)			13.4 (7.3-8.2)	
Chironomus riparius [6]	24-h EC50	Carbaryl (ug/L)	96.0							128.0				107.0
Anisoptera														
Pachydiplax longipennis [7]	96-h LC50	NH_3 (mg/L)			>2.0 (8.0)					>3.5 (8.0)				

25 °C than at 20 °C. The two remaining aquatic insect studies examined relationships between dissolved oxygen and LC50 values (Table 6). Similar to the other aquatic insect studies (Table 5), both Ephemeroptera (*Ephemerella mucronata)* and Trichoptera (*Philartcus queris*) showed lowest LC50 values at lowest temperatures.

In summary, there did not appear to be a consistent pattern of sensitivity for different Crustacea taxa to various toxins at temperatures ranging from 4-30°C. In contrast, different species of freshwater Mollusca, including three snail species and a fingernail clam, showed similar levels of sensitivity from 4-25°C. Finally, aquatic insect taxa showed different patterns in response to various toxins at temperatures ranging from 10-30°C. Responses to dissolved oxygen, an environmental condition rather than a toxin, however, showed the most consistent pattern for aquatic insects. For most taxa tested, the lowest LC50 values were found at lowest temperatures indicating the larvae were more sensitive to low dissolved oxygen concentrations at lower temperatures because less organisms survive at the lowest temperatures.

Some temperature-toxicity patterns for aquatic invertebrates were found, however, generalisation of these findings to other wastewaters, such as pulp mill effluents, especially under winter conditions, is not recommended because of various concerns with the data of this review.

Concerns with the database include the following:

1. Different or unreported life history stages were used in some studies making comparisons of results among studies difficult. For example, neonates of Daphnidae are generally considered to be more sensitive to toxins than adults. Both of these life stages were used in the Daphnidae tests. Also for some studies, it was not clear what life history stage was actually used because it was not reported.
2. The temperature-specific response to toxins may vary substantially for individual taxa. For example, the toxicity responses of the marine clam (*Mya arenia*) were different between two toxins in different tests.
3. No studies provided statistical estimates of means and variability for the toxicity-temperature responses, which would provide more confidence in the observed patterns and allow more valid and statistical comparisons among studies.
4. Temperatures used in the tests generally were not directly comparable among studies. For example, each study used different ranges of temperatures. The largest ranges in temperatures of the studies varied from 2.5-18°C. Also, for about half of the studies examined, only two temperatures were tested. As a result predicting the response of invertebrates at intermediate temperatures is problematic because the relationship between toxicity and water temperature may not be linear, as demonstrated by some taxa.
5. No data were found for the same species in independent studies. These data would be especially useful in determining if a species showed a consistent temperature-toxicity relationship.
6. Test conditions varied among studies.
7. Few studies determined the sensitivity of aquatic invertebrates to a wide range of seasonal temperatures and no studies examined effects of contaminants at water temperatures close to 0°C, typical of those during winter months in Alberta.

Conclusions

Based on the findings of this review of data and earlier reviews (Cairns 1975, Mayer and Ellersieck 1986), there are few data on the responses of aquatic invertebrates to toxins at low temperatures. In the extensive review of toxicity data, Mayer and Ellersieck (1986) recommended more research on temperature and toxicity of chemicals to invertebrates. They also suggested that toxicity tests should be done under the environmental conditions of interest because of inherent variation and frequent exceptions found in acute toxicity data.

Extrapolation of the findings from the earlier studies to determine the sensitivity of freshwater invertebrates to toxins at temperatures <5°C is

difficult because of the lack of data. In addition, it is not known if the toxicity response of most taxa is linear. Sensitivity of invertebrates to toxins also will likely be affected by the activity and metabolism of the organisms at near-freezing temperatures compared to warmer seasons. Data on the effects of pulp mill effluents on freshwater invertebrates at different temperatures were not found. Therefore, data from earlier studies have limited use for predicting the sensitivity of aquatic invertebrates to pulp mill effluents under conditions that occur in northern Alberta rivers during winter.

In conclusion, experiments designed to determine the effect of pulp mill effluent on pertinent aquatic invertebrates at seasonal temperatures and under controlled environmental conditions would appear to be a reasonable approach for obtaining data to fill a significant information gap.

LABORATORY EXPERIMENTS

Materials and Methods

Laboratory Animals

Neonatal *Daphnia magna* used in Experiments 1 and 2 were taken from a healthy laboratory culture kept under controlled environmental conditions at the Alberta Research Council (ARC), Vegreville. The culture originated from stock cultures from Environment Canada (Edmonton) and was maintained following standard culturing procedures established by Environment Canada and U.S. Environmental Protection Agency (Environment Canada 1990a, Environment Canada 1990b, Peltier and Weber 1985, Biesinger et al. 1987). The culture was kept in reconstituted water at 21°C and 16-h light and 8-h darkness regime. *Daphnia magna* were maintained on a 1:1 diet of the algae (*Selenastrum* sp.) and a mixture of yeast, crushed cereal leaves and trout chow. Individuals used in all experiments were removed from the stock populations and randomly assigned to each of the treatments. An additional 40 randomly selected individuals were removed and their body lengths measured under 25 times magnification to estimate initial body lengths (Overall mean length ±1 standard deviation = 1.3±0.15 mm). Unless otherwise stated body length and weights are shown as means ±1 standard deviation (SD).

Larval *Taenionema* used in Experiment 3 were collected from a site upstream of the Weyerhaeuser Canada bleached kraft pulp mill on the Wapiti River near Grande Prairie, Alberta in November 1998. Larvae were maintained in the laboratory for about 1-week prior to experimental trials. The use of *Taenionema* rather than *Baetis* larvae represents a departure from the initial proposal (Casey 1997, 1998). This departure was based on the extremely low densities of *Baetis* larvae present at four sites upstream of the Weyerhaeuser pulp mill observed in October, 1998 that precluded their use in toxicological experiments. In contrast, *Taenionema* were considerably more abundant and are known to represent an abundant taxa upstream and downstream of the Weyerhaeuser mill (Scrimgeour et al. 1995). Lastly, the presence of two cohorts of *Taenionema* in the Wapiti River during November 1998 provided the opportunity to evaluate size-specific responses to water temperature and pulp mill effluents.

Prior to use in experiments, both sizes of larvae were maintained in the laboratory for about 1 week prior to trials and fed an ad-libitum supply of algae and leaf material. Larvae used in laboratory trials were selected at random from the laboratory stock tanks. An additional 40 randomly selected individuals were removed and measured under 6 (large larvae) and 25 times (small larvae) magnification to quantify initial lengths (mm) and weights (mg dry weight) of small and large larvae. These measurements showed that small larvae were about half the length (small larvae: 2.1±0.6, large larvae 5.6±1.0) and weight (mg dry weight) of the larger cohort (small larvae: 2.1±0.4, large larvae: 3.8±1.0).

General Laboratory Procedures

Laboratory experiments evaluating the effects of: i) NaCL on *Daphnia magna* (Experiment 1), ii) water temperature and pulp mill effluent on *Daphnia magna* (Experiment 2), and iii) water temperature and pulp mill effluent on *Taenionema* (Experiment 3) were completed in April 1998 (Experiments 1 and 2) and December 1998 (Experiment 3).

Experiments were performed in three large, walk-in temperature controlled environments where air temperatures and light regimes could be automated. For experiments 1 and 2, air temperatures were set at 1°C (Chamber 1), 9°C (Chamber 2) and 17°C (Chamber 3) (±1°C). While chambers were set at these temperature regimes, heating of the experimental microcosms by the lighting increased water temperatures to 7°C, 13°C and 20°C (±1°C). For Experiment 3 air temperatures were set at 3°C (Chamber 1), 9°C (Chamber 2) and 15°C (Chamber 3) (±1°C) and water temperatures closely matched air temperatures largely because tanks contained greater amounts of water and thus were less

sensitive to heat inputs from the lights. Lighting was provided by a bank of 16 cool white fluorescent lights and a total of twenty-four 40 W incandescent lights that were cycled to produce a 16-h light and 8-h dark regime. Each temperature controlled chamber contained large shelves and benches which housed the experimental microcosms (i.e., beakers or tanks).

Laboratory experiments to evaluate the interactive effects of water temperature and sodium chloride (Experiment 1), and water temperature and pulp mill effluent (Experiment 2) on *Daphnia magna* were completed in 250 ml glass beakers (i.e., static water conditions). In contrast, the effects of water temperature and pulp mill effluent on *Taenionema* were completed within re-circulating 20 L glass tanks. For Experiments 1 and 2, a total of 6 *Daphnia magna* were added to each of the three replicate beakers. In contrast, each of the four replicate re-circulating tanks received between 2-6 small larvae and 2-6 large larvae depending on the abundance of *Taenionema* in benthic samples collected from the Wapiti River. For all three experiments, the experimental chamber (i.e., the beakers and tanks) was the unit of replication. Thus for each beaker or tank, we calculated mean survival, mean body length (Experiments 1 and 2) and/or weight (Experiment 3) for several individuals within each microcosm to produce a value (i.e., replicate) for each treatment. Larval *Taenionema* within the re-circulating tanks were enclosed within 250 *u*m mesh cylindrical enclosures (length = 30 cm. Diameter = 10 cm). Each enclosure contained an *ad-libitum* supply of *Selenastrum* as a food source that had been previously cultured on tiles.

Animals used in all experiments were acclimated to the three water temperatures over a 3-day period such that temperature changes did not exceed 3^{o}C every 12 h. The U.S. Environmental Protection Agency recommended changes of 3^{o}C every 12 h (Peltier and Weber 1985). Neonatal *Daphnia magna* were fed at the start of the acclimation period. Three days prior to the commencement of the experiment (i.e., Day 0), 6 immature *Daphnia magna* were taken from the stock culture and placed in 200 mL of the test solutions, in 250-mL glass beakers. Food (1 ml algae: 1 ml YCT) was added to each beaker.

For experiments 1 and 2, test solutions and food were replaced every 2 days during the experiments (e.g., after Day 2 and 4 for Experiment 1 and on Days 2, 4 and 6 for Experiment 2). A concurrent test was conducted over 10 days at 21^{o}C to determine the effect of 100% effluent on the survival of 2 adults and 2 neonates in 200 ml of effluent. This test was originally initiated on the day before the pulp mill experiment to determine if the effluent was immediately toxic to *Daphnia magna*. No food was added to the test beaker and the test was not replicated. Survival and development (increase in size and presence of neonates) were checked intermittently.

During the experiments, survival of *Daphnia magna* and *Taenionema* was determined at 24-48 h intervals. Survival of *Daphnia magna* (i.e., Experiments 1 and 2 were determined using a 12x magnifier and activity of individuals were categorized as: 1) actively swimming, 2) immobile but responsive organisms (i.e., individuals on the bottom of the beaker who responded to physical disturbance) and 3) immobile and unresponsive. Immobile and unresponsive individuals were removed from beakers and viewed under a microscope to check for visible signs of life (i.e., movement of appendages and the heart). When absent, individuals were identified as being dead.

Dead organisms were preserved and the remaining organisms were preserved in 80% ethanol at the end of the experiment. At the end of each experiment all individuals were removed from beakers and their lengths measured (i.e., distance from the base of the posterior spine to the top of the head) at 25 or 50 times magnification with a linear piece micrometer. The number of eggs visible in the brood chamber of the adults was also recorded using categories of: 1) <5, 2) 5-10, and 3) >10. The number of immature individuals produced by adults was also recorded. Survival of *Taenionema* was also determined at 24-48 h intervals by lifting the mesh bag away from the tank bottom and counting the number of active (i.e., swimming or crawling) larvae.

Treated bleached kraft pulp mill effluent was collected by staff of Weyerhaeuser Canada,

Grande Prairie, and sent to ARC at Vegreville 3 days before beginning the experiment. This effluent receives primary clarification and secondary treatment in a five-day retention, aerated stabilization basin, followed by some secondary clarification in a quiescent zone. Large volumes of 100% effluent were transported in polyethylene containers and effluent was stored at 4ºC in darkness at ARC Vegreville prior to use in laboratory trials. The physical and chemical characteristics of the effluent were determined before and after it was used in experiments. For Experiments 1 and 2, samples of 100% effluent and reconstituted water on Day 0 and 50% effluent taken from each of the three temperatures on Day 8 were analyzed for inorganic parameters and for chlorinated phenols, resin and fatty acids. These analyses were also completed for Experiment 3 except that analyses were completed for Wapiti River water samples on Days 0 and 7 (3^{o}C); 30% treated bleached kraft pulp mill on Day 0 at 3^{o}C; 30% treated bleached kraft pulp mill on Day 7 (i.e., after 7 days of being in tanks) at all three temperatures (3^{o}C, 9^{o}C, 15^{o}C); 30% treated bleached kraft pulp mill that replaced the previously used effluent on Day 7 at (3^{o}C) and 30% effluent that had been in experimental tanks for the final 7 days of the experiment.

The different concentrations of treated bleached kraft mill effluents were created by mixing the treated effluent, provided by staff at the Weyerhaeuser Pulp mill, with varying amounts of river water to create the desired 5-50% treated effluent concentrations. Water required for these dilutions was collected from the Wapiti River at a site located upstream (i.e., O'Brien Park) of where the city of Grande Prairie releases its sewage and upstream of where Weyerhaeuser releases its treated effluent into the river. Water was typically collected 3 days prior to the beginning of laboratory experiments and like effluent, was stored in polyethylene containers at 4ºC in the dark prior to use in laboratory trials.

Water samples were analyzed for numerous chemical compounds including pH, total suspended solids, colour, major ions, NH_3, NO_3, NO_2, total kjeldahl nitrogen (TKN), total dissolved phosphorus (TDP), total phosphorus (TP), dissolved organic carbon, resin and fatty acids, chlorinated phenolics, sulphide and metals (including manganese and zinc). These samples were analyzed by the analytical laboratory at ARC Vegreville. Dissolved oxygen (YSI meter, model 59), water temperature, conductivity, pH (WTW Multiline P4 meter) and hardness (Hach kit) were measured in each test solution at 24-48 h intervals during the experiments.

Experiment 1 - Effects of Water Temperature and Sodium Chloride Concentration on *Daphnia magna*

A two-factorial analysis of variance (ANOVA) design was used to determine whether survival and growth of *Daphnia magna* were significantly affected by water temperature (7^{o}C, 13^{o}C and 20ºC [±1ºC]), sodium chloride concentration (NaCl) and the interaction of these factors. Laboratory trials were replicated three times and lasted 6 days. Concentrations of sodium chloride (0, 5000, 5400 and 5800 NaCl mg/L) were made by mixing NaCl with water produced by a Reverse Osmosis Millipore Polygard water treatment system (Millipore Polygard catalogue number CR0503006 fitted with a Reverse Osmosis Filter). These treatment levels (i.e., concentrations) were selected based on the results of reference tests. Lastly, three reference bioassays, each with 2 replicates, were conducted before, during and after the experiments to confirm the health of the *Daphnia magna* used in the experiments. Ten neonates were exposed to a wide range of NaCl concentrations (4600, 5000, 5400, 5800, 6200, 6600 mg NaCl/L) and NaCl-absent control solutions.

Experiment 2 - Effects of Water Temperature and Pulp Mill Effluent Concentration on *Daphnia magna*

A two-factorial analysis of variance (ANOVA) design was used to determine whether survival and growth of *Daphnia magna* were significantly affected by water temperature (7^{o}C, 13^{o}C and 20ºC [±1ºC]), pulp mill effluent concentration (0%, 5%, 10% and 50% treated pulp mill effluent based on volume) and the interaction of these factors. Laboratory trials were replicated three times and lasted 8 days.

Experiment 3 – Effects of Water Temperature and Pulp Mill Effluent on *Taenionema* spp.

A two-factorial analysis of variance (ANOVA) design was used to determine whether survival and growth of small and large *Taenionema* were significantly affected by water temperature (3°C, 9°C and 15ºC [±1ºC]), pulp mill effluent concentration (0%, 15%, 30% treated pulp mill effluent based on volume) and the interaction of these factors. Laboratory trials were typically replicated four times (range = 2-4, average = 3.5) and lasted 14 days.

Statistical Analyses

Two factorial ANOVA tests were used to determine the effects of temperature and test compound concentration (i.e., NaCl or pulp mill effluent) on survival, body length and/or weight, and number of immature individuals produced by each organism with an alpha of 0.05. When statistically significant effects were found in the ANOVA tests, the Student-Newman-Keuls multiple range test was used to test for differences among treatment means. Analyses were conducted using the SAS/STAT statistical software package (SAS Institute Inc. 1988) and the Spearman-Karber statistical method was used to calculate LC50 values for the reference tests (Hamilton et al. 1977, Stephan 1977, Environment Canada 1990a).

RESULTS

Experiment 1 - Effects of Water Temperature and Sodium Chloride on *Daphnia magna*

Reference tests showed a range of LC50 values (N = 6) from 5491-5736 mg NaCl/L. The choice of NaCl concentrations used in the NaCl experiment was based on 2 replicate reference tests conducted before beginning the main experiments (LC50 = 5547 and 5585 mg NaCl/L).

Survival of *Daphnia magna* was significantly affected by water temperature ($P<0.0001$) and NaCl concentration ($P<0.001$) (Table 7). Significantly fewer *Daphnia magna* survived at 7ºC and 13ºC than at 20ºC whereas greater numbers of individuals survived in the NaCl absent controls and the 5000 mg NaCl/L treatment than that in 5400 and 5800 mg NaCl/L treatment (Table 7).

Lengths of *Daphnia magna* that survived the experiment were not analyzed because the majority of organisms in each concentration of NaCl died during the experiment (Table 8). Statistical analysis of these data at Day 2, 4 or 6 would result in unequal sample sizes and likely reflect time to death, rather than growth rates because in the majority of cases, animals that died relatively early in the experiment were smaller than those that died later in the experimental period. Nevertheless, lengths of *Daphnia magna* that died during the experiment and those that survived until the end of the experiment combined, however, showed that mean lengths decreased with decreasing temperatures from 20-7ºC (Table 8).

Eggs (5-10 eggs/individual) were only found in *Daphnia magna* that survived to Day 6 at 20ºC. Eggs were found in 93% of these organisms in each NaCl concentration, including the single organisms that survived until Day 6 in 5800 mg/L NaCl, the greatest concentration of NaCl used in the experiment.

Experiment 2 - Effects of Water Temperature and Pulp Mill Effluent on *Daphnia magna*

Water temperature and concentration of pulp mill effluent had no detectable effects on survival of *Daphnia magna* (ANOVA: $P=0.383$ and 0.801, respectively; Table 9). The interaction between concentration of effluent and temperature was also not statistically significant ($P=0.448$). Only 3 out of a total of 216 *Daphnia magna* (1.4%) used in the experiment were dead at Day 8. These organisms died between Day 0 and Day 4. The additional test, that was not replicated, showed 100% effluent had no effect on the survival of 2 neonates and 2 adult *Daphnia magna* after 10 days at 20ºC. At the end of this test, all organisms were active and swimming in the effluent; the neonates had grown and >10 neonates were produced by the 2 adults.

Table 7. Effects of water temperature and sodium chloride concentration on mean (±1SD) percent survival of *Daphnia magna* at the end of the 6-day experimental period.

Concentration (mg NaCl/L)	Temperature (°C) 20 Mean	SD	13 Mean	SD	7 Mean	SD
0	100	0	100	0	100	0
5000	100	0	39	26	0	0
5400	44	26	6	10	0	0
5800	6	10	0	0	0	0

Table 8. Effect of water temperature and sodium chloride concentration on mean (±1SD) length (mm) of *Daphnia magna* at the end of the 6-day experimental period.

Concentration (mg NaCl/L)	Temperature (°C) 20 Mean	SD	13 Mean	SD	7 Mean	SD
0	2.24	0.12	1.30	0.09	1.08	0.10
5000	2.04	0.17	1.08	0.12	0.89	0.11
5400	1.98	0.41	0.95	0.05	0.87	0.16
5800	1.36	0.21	0.92	0.06	0.87	0.08

Table 9. Effects of water temperature and pulp mill effluent concentration on mean (±1 SD) percent survival of *Daphnia magna* at the end of the 8-day experimental period.

Concentration Of Pulp Mill Effluent (%)	Temperature (°C) 20 Mean	SD	13 Mean	SD	7 Mean	SD
0	100	0	100	0	94	10
5	100	0	100	0	94	10
10	100	0	100	0	100	0
50	94	10	100	0	100	0

At Day 0, that is, after 3 days of acclimation, *Daphnia magna* maintained at 20ºC were significantly larger than those kept at 7ºC and 13ºC (P<0.01). At the end of the 8-day experimental period, mean body lengths of *Daphnia magna* generally increased with increasing effluent concentration from 0-50%, although these differences were only marginally statistically significant (ANOVA: P=0.08) (Table 10).

Daphnia magna produced neonates at 20ºC in all test concentrations (0-50% effluent) after Days 6 and 8 of the experiment (Table 11). In contrast, *Daphnia magna* did not produce neonates at the lower temperatures of 7ºC and 13ºC. Numbers of immature *Daphnia magna* produced within the 5%, 10% and 50% were statistically greater than produced in the effluent-absent controls (P<0.01; Table 11).

At the end of the experiment (Day 8), the presence of eggs in *Daphnia magna* was strongly affected by water temperature and pulp mill effluent. Our qualitative observations indicated that all *Daphnia magna* produced eggs in the 20ºC treatments whereas eggs were less abundant in individuals reared at 13ºC and absent from all individuals reared at 7ºC. Within the 20ºC treatment, individuals maintained at effluent concentrations of 0% and 10% contained about 5-10 eggs, whereas individuals reared in the 50% effluent treatment typically contained greater than 10 eggs/ individual. In contrast, individuals reared at 13ºC typically contained fewer than 5 eggs and eggs were not visible in *Daphnia magna* maintained at 7ºC.

Table 10. Effects of water temperature and pulp mill effluent concentration on mean (±1SD) length (mm) of *Daphnia magna* at the end of the 8-day experimental period.

Concentration	Temperature (°C)					
Of Pulp Mill	20		13		7	
Effluent (%)	Mean	SD	Mean	SD	Mean	SD
0	2.40	0.11	1.65	0.11	1.35	0.11
5	2.70	0.19	1.74	0.10	1.41	0.13
10	2.73	0.17	1.66	0.15	1.43	0.09
50	2.90	0.16	2.02	0.14	1.44	0.10

Table 11. Effects of pulp mill effluent on mean (±1SD) number of immature *Daphnia magna* produced at 20ºC on Day 6 and 8 of the experimental period.

Concentration	Day of Experiment		Combined average	
Of Pulp Mill	6	8	6+8	
Effluent (%)	Mean	Mean	Mean	SD
0	31	19	50	5
5	47	33	80	15
10	52	46	98	15
50	57	45	103	28

As expected based on the dilution, concentrations of most inorganic parameters in the pulp mill effluent showed approximately two-fold reductions in 50% compared to 100% effluent and few parameters in 50% effluent differed markedly with water temperature (Table 12).

Only 1 of 23 chlorinated phenols (4-chloroguaiacol) and 12 of 19 resin and fatty acids measured were found in the pulp mill effluent (Table 13). For most compounds in the 50% effluent concentration, the greatest concentrations were observed at the lower temperatures (Table 13). Nevertheless, increased concentrations at lower temperatures were generally small (i.e., linoleic acid, linolenic acid and dehydroabietic acid), and only exceeded detection limits by small amounts (Table 13). They were not associated with adverse effects on survival or growth of *Daphnia magna* (Tables 9 and 10).

Similar to the inorganic compounds, concentrations of most organic compounds decreased by about two-fold in 50% compared to 100% effluent (Table 13). None of the chlorinated phenols or resin and fatty acids were detectable in the reconstituted water (Table 13). Thus, difference in chemical composition of the bleached kraft pulp mill effluent treatments did not translate into significant effects (i.e., i) statistically non-significant pulp mill effluent treatment effect, and ii) statistically non-significant interaction terms of pulp mill effluent treatment and water temperature) on survival and growth of *Daphnia magna*.

Monitoring of general physical and chemical parameters in the test solutions every 2 days showed only minor changes in pH, dissolved oxygen, conductivity, water temperature and hardness. Conductivity, pH and hardness measured in dummy beakers of each test concentration at the start and end of each 2-day period generally showed similar or identical levels; dissolved oxygen saturation typically ranged from 80-100% throughout the experiments.

Experiment 3 - Effects of Water Temperature and Pulp Mill Effluent on *Taenionema* spp.

All *Taenionema* larvae survived the 14-day experimental trials (Table 14). Thus, survival of both small and large larvae were not significantly affected by water temperature, pulp mill effluent or the interaction of these terms ($P=1.0$).

While neither body length nor weight of small larvae were affected ($P>0.05$) by pulp mill effluent concentration, body lengths ($P=0.054$) and weights ($P<0.0001$) were significantly affected by water temperatures. Larvae maintained at 9°C and 15°C were longer ($P=0.07$) and heavier ($P< 0.05$) than those kept at 3°C and 9°C (Table 15). The interaction between water temperature and pulp mill effluent was not significant ($P=0.22$).

In contrast with small larvae, body length of large larvae was significantly affected by pulp mill effluent concentration ($P<0.002$) but not water temperature ($P=0.075$) or the interaction of temperature and pulp mill effluent ($P=0.37$). Larvae maintained in 30% effluent were significantly longer than those from 0% and 15% effluent treatments (Table 16). Body weights of large larvae were also significantly affected by water temperature ($P<0.0001$) but not pulp mill effluent concentration ($P=0.93$) or the interaction between water temperature and pulp mill effluent concentration ($P=0.20$). Larvae maintained at 9°C and 15°C were significantly heavier than those kept at 3°C (Table 16).

Concentrations of most inorganic parameters and fractions of nitrogen and phosphorus did not differ greatly among the three water temperature treatments (3, 9, 15 °C) (Tables 17 and 18). In contrast, concentrations of nickel and chromium increased at least three-fold between days 0 and 14 (Table 18). While the cause of these increases were not examined, it is unlikely that such increases resulted from inputs from glass aquaria.

Table 12. Concentrations of inorganic parameters in Wapiti River water, and treated bleached kraft pulp mill effluent at different water temperatures and dilutions used to evaluate the effects of water temperature and treated bleached kraft pulp mill effluent on *Daphnia magna* (Experiment 2). Zeros represent values lower than analytical detection levels. Treated bleached kraft pulp mill effluent and water from the Wapiti River were collected from the Weyerhaeuser pulp mill and the Wapiti River about 3 days prior to commencing experiments in April 1997.

Parameter	Units	Detection Limit	Day 0 Wapiti River Water 100% 4°C	Day 0 Effluent 100% 4°C	Day 0 Effluent 50% 20°C	Day 8 Effluent 50% 13°C	Day 8 Effluent 50% 7°C
PH	units	N/A	8.18	7.38	7.87	7.87	7.88
CONDUCTIVITY	*u*S/cm	2.0	411.0	1870.0	1051.0	1017.0	1022.0
P-ALKALINITY	mg CaCO3/L	4.0	0.0	0.0	0.0	0.0	0.0
T-ALKALINITY	mg CaCO3/L	4.0	67.0	271.0	173.0	170.0	172.0
T.SUSP.SOLIDS	mg/L	10.0	0.0	11.0	6.0	6.0	6.0
COLOUR, TRUE	TCU	2.0	0.0	686.0	344.0	326.0	329.0
SULPHIDE	mg/L	0.004	0.000	0.043	0.019	0.017	0.027
CHLORIDE	mg/L	0.30	6.7	192.4	100.4	97.1	99.3
FLUORIDE	mg/L	0.04	0.0	0.07	0.03	0.05	0.04
POTASSIUM	mg/L	0.10	6.7	17.5	9.5	9.2	9.4
SODIUM	mg/L	0.50	29.6	314.5	182.5	176.5	180.3
SILICA	mg/L	0.10	0.0	7.5	3.7	3.7	3.7
SULFATE	mg/L	6.00	115.0	326.0	235.0	231.0	241.0
Metals							
ALUMINUM	mg/L	0.03	0.03	0.46	0.24	0.23	0.27
BARIUM	mg/L	0.002	0.001	0.286	0.147	0.145	0.146
BERYLLIUM	mg/L	0.001	0.000	0.000	0.000	0.000	0.000
CALCIUM (ICP)	mg/L	0.015	28.070	64.370	46.550	45.280	45.420
CADMIUM	mg/L	0.002	0.000	0.000	0.000	0.000	0.000
COBALT	mg/L	0.001	0.000	0.000	0.000	0.000	0.001
CHROMIUM	mg/L	0.002	0.000	0.002	0.001	0.001	0.001
COPPER	mg/L	0.002	0.001	0.003	0.002	0.013	0.005
IRON	mg/L	0.010	0.003	0.262	0.136	0.133	0.140
MAGNESIUM (ICP)	mg/L	0.002	12.430	11.080	11.630	11.470	11.320
MANGANESE	mg/L	0.003	0.000	0.439	0.234	0.222	0.234
MOLYBDENUM	mg/L	0.002	0.000	0.001	0.000	0.000	0.000
NICKEL	mg/L	0.004	0.000	0.002	0.002	0.001	0.001
LEAD	mg/L	0.010	0.000	0.014	0.000	0.000	0.016
VANADIUM	mg/L	0.002	0.000	0.000	0.000	0.000	-0.002
ZINC	mg/L	0.001	0.002	0.033	0.019	0.017	0.018
Nutrients (N, P, C)							
(NO2+NO3)-N	mg/L	0.02	0.00	0.28	0.34	0.20	0.18
NO2-N	mg/L	0.016	0.001	0.257	0.280	0.164	0.153
N03-N	mg/L	0.32	0.00	0.03	0.06	0.03	0.02
NH3-N	mg/L	0.32	0.00	2.64	1.18	1.26	1.31
TKN	mg/L	0.05	0.02	4.50	2.31	2.29	2.33
PHOSPHOR DISS	mg/L	0.001	0.004	0.695	0.337	0.337	0.326
T-PHOSPHORUS	mg/L	0.008	0.001	0.771	0.383	0.384	0.384
DOC	mg/L	0.6	0.0	126.6	65.9	61.2	65.4

Table 13. Concentrations of chlorinated phenols, resin acids and fatty acids in Wapiti River water and treated pulp mill effluent at different dilutions and temperatures used to evaluate effects of water temperature and treated bleached kraft pulp mill effluent on *Daphnia magna* (Experiment 2). Chemical analyses are shown for samples on Days 0 and 8. Pulp mill effluent and water from the Wapiti River were collected from the Weyerhaeuser pulp mill and the Wapiti River about 3 days prior to commencing the experiments in April 1997.

Compound	Method Detection Limit (ug/L)	Day 0 Wapiti River Water 100% 4°C	Day 0 Effluent 100% 4°C	Day 8 Effluent 50% 20°C	Day 8 Effluent 50% 13°C	Day 8 Effluent 50% 7°C
Chlorinated Phenolics						
4-Chlorophenol	0.02	0.00	0.00	0.00	0.00	0.00
2,4-Dichlorophenol	0.02	0.00	0.00	0.00	0.00	0.00
4-Chloroguaiacol	0.02	0.00	0.67	0.31	0.46	0.40
2,4,6-Trichlorophenol	0.02	0.00	0.00	0.00	0.00	0.00
4,5-Dichloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00
4-Chlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
4,6-Dichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00
4,5-Dichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00
3,5-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
2,3,4,6-Tetrachlorophenol	0.02	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00
3,4,6-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00
3,4-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
4,5-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00
Tetrachloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00
4,5,6-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00
3,4,6-Trichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
Pentachlorophenol	0.02	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
Tetrachloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00
4,5,6-Trichlorosyringol	0.02	0.00	0.00	0.00	0.00	0.00
Tetrachlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00
Resin and Fatty Acids						
Myristic acid	2.00	0.00	0.00	0.00	0.00	0.00
Palmitic acid	10.00	0.00	1.70	0.00	0.00	0.00
Linoleic acid	1.00	0.00	1.60	0.40	0.60	0.90
Oleic acid	2.00	0.00	1.00	0.70	6.80	10.60
Linolenic acid	1.00	0.00	0.50	0.20	0.50	0.30
Stearic Acid	5.00	0.00	0.00	0.00	0.00	0.00
Pimaric acid	0.20	0.00	1.70	0.90	0.80	1.00
Sandaracopimaric acid	0.30	0.00	0.00	0.00	0.00	0.00
Isopimaric acid	0.20	0.00	0.00	0.00	0.00	0.00
Palustric acid	10.00	0.00	0.00	0.00	0.00	0.00
Levopimaric acid	2.00	0.00	0.00	0.00	0.00	0.00
Arachidic acid	1.00	0.00	0.50	0.10	0.30	0.30
Dehydroabietic acid	0.20	0.00	2.10	0.90	1.10	1.10
Abietic acid	1.00	0.00	1.60	0.70	0.60	0.90
Neoabietic acid	2.00	0.00	0.00	0.00	0.00	0.00
9,10-Dichlorostearic acid	1.00	0.00	0.60	0.10	0.20	0.20
14-Chlorodehydroabietic acid	0.20	0.00	0.30	0.10	0.10	0.20
12-Chlorodehydroabietic acid	0.20	0.00	0.60	0.30	0.30	0.40
12,14-Dichlorodehydroabietic	0.20	0.00	0.40	0.20	0.20	0.30

Table 14. Effects of water temperature and pulp mill effluent concentration on mean (± 1SD) percent survival of *Taenionema* at the end of the 14-day experimental period (Experiment 3).

Concentration of Pulp Mill Effluent (%)		Water temperature (°C) 3 Mean	SD	9 Mean	SD	15 Mean	SD
Small larvae	0	100	0	100	0	100	0
	15	100	0	100	0	100	0
	30	100	0	100	0	100	0
Large larvae	0	100	0	100	0	100	0
	15	100	0	100	0	100	0
	30	100	0	100	0	100	0

Table 15. Effects of water temperature and pulp mill effluent concentration on mean (± 1SD) body lengths (mm) of small and large *Taenionema* at the end of the 14-day experimental period (Experiment 3).

Concentration of Pulp Mill Effluent (%)		Water temperature (°C) 3 Mean	SD	9 Mean	SD	15 Mean	SD
Small	0	2.08	0.42	2.00	0.07	2.49	0.48
	15	2.06	0.61	2.63	0.91	2.58	0.34
	30	2.13	0.61	3.36	0.72	2.53	0.20
Large	0	5.79	0.47	5.91	0.46	5.39	0.95
	15	6.23	0.34	6.30	0.32	6.04	0.17
	30	6.33	0.29	7.08	0.50	6.39	0.11

Table 16. Effects of water temperature and pulp mill effluent concentration on mean (± 1SD) dry weight (mg) of small and large *Taenionema* at the end of the 14-day experimental period (Experiment 3).

Concentration of Pulp Mill Effluent (%)		Temperature (°C) 3 Mean	SD	9 Mean	SD	15 Mean	SD
Small							
	0	1.98	0.19	2.00	0.07	2.49	0.48
	15	1.87	0.27	2.78	0.34	2.66	0.27
	30	2.04	0.40	3.00	0.42	2.49	0.34
Large							
	0	3.46	0.76	4.34	0.37	5.40	0.95
	15	3.74	0.77	5.01	0.42	4.70	0.48
	30	3.52	0.30	5.20	1.03	4.70	0.56

Table 17. Concentrations of inorganic parameters in Wapiti River water, and treated bleached kraft pulp mill effluent at different water temperatures and dilutions used to evaluate the effects of water temperature and treated bleached kraft pulp mill effluent on *Taenionema* (Experiment 3). Zeros represent values lower than analytical detection levels. Treated bleached kraft pulp mill effluent and water from the Wapiti River were collected from the Weyerhaeuser pulp mill and the Wapiti River about 3 days prior to commencing in December 1998. Detection limits are shown as mg/L. Concentrations are shown in both *u*g and mg/L

Parameter	Units	Method	Day 0		Day 7 (used)			Day 7 (new)			Day 14 (used)	
		Detection Limit	Wapiti River Water 3°C	Effluent 30% 3°C	Effluent 30% 3°C	Effluent 30% 9°C	Effluent 30% 15°C	Wapiti River water 3°C	Effluent 30% 3°C	Effluent 30% 3°C	Effluent 30% 9°C	Effluent 30% 15°C
Major Ions and Other Characteristics												
CONDUCTIVITY	uS/cm	2.000	413.000	955.000	977.000	954.000	973.000	354.000	927.000	1106.00	1084.000	1030.000
P-ALKALINITY	mgCaCO3/L	4.000	5.000	0.000	0.000	0.000	0.000	5.000	0.000	10.000	11.000	25.000
T-ALKALINITY	mgCaCO3/L	4.000	192.000	237.000	247.000	243.000	245.000	191.000	253.000	263.000	261.000	251.000
T.SUSP.SOLIDS	mg/L	10.000	1.000	5.000	5.000	4.000	3.000	2.000	13.000	5.000	7.000	3.000
COLOUR, TRUE	TCU	2.000	7.000	418.000	403.000	403.000	403.000	7.000	432.000	432.000	432.000	432.000
SULPHIDE	mg/L	0.004	0.000	0.010	0.007	0.006	0.004	0.000	0.014	0.010	0.009	0.007
CHLORIDE	mg/L	0.300	1.200	72.800	76.500	74.400	76.100	1.100	90.300	92.500	89.900	85.800
FLUORIDE	mg/L	0.040	0.090	0.090	0.100	0.100	0.100	0.100	0.110	0.110	0.110	0.110
POTASSIUM	mg/L	0.100	0.800	7.100	7.500	7.200	7.500	0.800	8.400	8.900	8.700	9.000
SODIUM	mg/L	0.500	9.100	129.800	140.600	133.900	138.900	9.000	162.800	168.500	166.200	157.100
SILICA	mg/L	0.100	3.900	4.900	4.800	4.400	4.800	4.000	4.900	3.800	2.800	0.300
SULFATE	mg/L	6.000	36.000	140.000	146.000	140.000	146.000	36.000	172.000	176.000	169.000	160.000
Metals												
BARIUM	ug/L	0.002	134.208	210.483	218.738	211.488	211.381	135.362	213.987	224.172	220.630	213.724
BERYLLIUM	ug/L	0.001	0.049	0.011	0.082	0.018	0.032	0.000	0.048	0.027	-0.074	0.012
CALCIUM (ICP)	mg/L	0.005	60.550	66.070	67.880	67.420	67.580	60.430	69.680	72.220	72.100	68.220
CADMIUM	ug/L	0.002	0.161	0.459	0.669	0.932	1.210	0.248	0.456	0.494	0.768	0.586
COBALT	ug/L	0.001	0.033	0.096	0.109	0.082	0.062	0.016	0.083	0.174	0.102	0.124
CHROMIUM	ug/L	0.002	0.212	1.902	2.011	1.955	3.139	0.429	6.419	10.235	6.253	7.168
COPPER	ug/L	0.002	0.939	1.896	2.890	3.200	2.644	1.955	2.863	2.655	2.545	2.417
IRON	ug/L	0.010	73.139	146.868	110.840	103.574	83.962	11.827	154.682	158.835	114.592	103.873
MAGNESIUM (ICP)	mg/L	0.002	2.343	3.947	3.349	3.213	2.767	0.000	5.299	5.220	3.610	3.067
MANGANESE	ug/L	0.003	10.838	307.370	272.659	251.306	139.705	8.270	307.008	308.518	278.476	47.683
MOLYBDENUM	ug/L	0.002	0.883	0.921	1.139	1.033	1.218	0.867	1.126	1.119	1.150	1.105
NICKEL	ug/L	0.004	0.078	0.559	0.681	0.567	1.411	0.155	1.906	4.399	2.858	3.612
LEAD	ug/L	0.010	0.101	0.790	1.380	1.275	1.042	0.200	0.779	0.866	0.754	0.632
VANADIUM	ug/L	0.002	0.403	1.041	0.982	0.987	0.976	0.206	1.244	1.268	1.132	1.055
ZINC	ug/L	0.001	2.019	18.168	19.223	18.700	20.467	3.645	22.855	24.792	20.581	14.960
Nutrients												
(NO2+NO3)-N	mg/L	0.020	0.050	0.150	0.190	0.180	0.330	0.050	0.240	0.250	0.240	0.060
NO2-N	mg/L	0.016	0.001	0.042	0.0570	0.063	0.144	0.001	0.083	0.067	0.038	0.015
N03-N	mg/L	0.320	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
NH3-N	mg/L	0.320	0.000	0.280	0.130	0.120	0.140	0.010	0.360	0.490	0.340	0.060
TKN	mg/L	0.050	0.100	1.190	1.100	0.960	1.230	0.310	1.320	1.600	1.420	0.990
PHOSPHOR DISS	mg/L	0.001	0.010	0.179	0.104	0.128	0.121	0.011	0.193	0.232	0.239	0.202
T-PHOSPHORUS	mg/L	0.008	0.001	0.244	0.160	0.169	0.165	0.006	0.251	0.283	0.295	0.241
DOC	mg/L	0.600	2.700	46.700	49.600	47.000	47.000	2.900	59.200	62.500	62.300	58.100

Chemical analyses showed that the pulp mill effluent did not contain detectable levels of chlorinated phenolics (Table 18). While numerous resin and fatty acids were present in detectable concentrations, levels were low and not strongly affected by water temperature (Table 18).

Our qualitative comparisons showed differences in chemical composition of treated bleached kraft pulp mill effluent used to evaluate the effects of water temperature and pulp mill effluent on *Daphnia magna* (Experiment 2: effluent collected in April 1997) compared with that used to evaluate the effects of water temperature and pulp mill effluent on *Taenionema* (Experiment 3: effluent collected in November 1998). Such variation in effluent likely arises from differences in wood supplies, chemical recovery during washing and bleaching processes and efficiency of the primary and secondary treatment processes.

Monitoring of general physical and chemical parameters in the test solutions every 2 days during the experiments showed no parameters with major changes. Dissolved oxygen concentrations and pH ranged between 8 and 10 mg/L and 7.3 and 8.9, respectively.

Table 18. Concentrations of chlorinated phenols, resin acids and fatty acids in water from the Wapiti River and treated bleached pulp mill effluent at different temperatures and dilutions used to evaluate the effects of water temperature and treated bleached kraft pulp mill effluent on *Taenionema* (Experiment 3). Zeros represent values lower than analytical detection levels. Treated bleached kraft pulp mill effluent and water from the Wapiti River were collected from the Weyerhaeuser pulp mill and the Wapiti River about 3 days prior to commencing in December 1998.

		Day 0				Day 7(used)	Day 7(new)		Day 14(used)		
Compound	Detection Limit (ug/L)	Wapiti River Water 3°C	Effluent 30% - 3°C	Effluent 30% - 3°C	Effluent 30% - 15°C	Effluent 30% - 3°C	Wapiti River Water 3°C	Effluent 30% - 3°C	Effluent 30% - 3°C	Effluent 30% - 9°C	Effluent 30% - 15°C
Chorinated Phenolics											
4-Chlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dichlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Chloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4,6-Trichlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5-Dichloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Chlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,6-Dichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5-Dichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,5-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,3,4,6-Tetrachlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,6-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tetrachloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5,6-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,6-Trichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pentachlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tetrachloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5,6-Trichlorosyringol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tetrachlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Chlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4-Dichlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Chloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,4,6-Trichlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5-Dichloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Chlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,6-Dichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Compound	Detection Limit (ug/L)	Day 0				Day 7(used)	Day 7(new)		Day 14(used)		
		Wapiti River Water 3°C	Effluent 30% - 3°C	Effluent 30% - 3°C	Effluent 30% - 15°C	Effluent 30% - 3°C	Wapiti River Water 3°C	Effluent 30% - 3°C	Effluent 30% - 3°C	Effluent 30% - 9°C	Effluent 30% - 15°C
4,5-Dichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,5-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2,3,4,6-Tetrachlorophenol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,6-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5-Dichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,5-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tetrachloroveratrole	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,5,6-Trichloroguaiacol	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,4,6-Trichlorocatechol	0.02	0.00	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00
Resin and Fatty Acids											
Myristic acid	2.00	0.00	0.30	0.00	0.00	0.00	0.00	0.00	13.90	0.70	0.00
Palmitic acid	10.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.30	0.00	0.00
Linoleic acid	1.00	0.00	0.60	0.10	0.40	0.60	0.00	0.10	7.00	0.00	0.00
Oleic acid	2.00	0.00	1.30	0.00	0.30	0.50	0.00	0.20	0.70	0.00	0.00
Linolenic acid	1.00	0.00	0.00	0.00	0.50	0.60	0.00	0.30	0.70	0.30	0.70
Stearic Acid	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pimaric acid	0.20	0.00	2.50	2.00	1.50	2.20	0.00	3.50	3.00	2.60	2.30
Sandaracopimaric acid	0.30	0.00	0.40	0.40	0.30	0.40	0.00	0.60	2.00	0.70	0.50
Isopimaric acid	0.20	0.50	2.70	2.10	1.50	2.20	0.00	3.70	3.30	2.90	2.40
Palustric acid	10.00	0.00	0.90	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
Levopimaric acid	2.00	0.00	0.20	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Arachidic acid	1.00	0.00	0.70	0.20	0.20	0.40	0.00	0.30	0.00	0.00	0.00
Dehydroabietic acid	0.20	0.00	4.30	3.60	2.70	3.90	0.00	6.60	6.40	5.70	4.80
Abietic acid	1.00	0.00	5.90	4.30	3.30	4.90	0.00	10.30	8.60	7.70	6.40
Neoabietic acid	2.00	0.00	0.60	0.00	0.00	0.00	0.00	0.50	0.20	0.00	0.00
9,10-Dichlorostearic acid	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14-Chlorodehydroabietic acid	0.20	0.00	0.20	0.10	0.10	0.10	0.00	0.40	0.20	0.20	0.20
12-Chlorodehydroabietic acid	0.20	0.00	0.30	0.20	0.10	0.20	0.00	0.50	0.30	0.30	0.30
12,14-Dichlorodehydroabietic	0.20	0.00	0.10	0.00	0.00	0.10	0.00	0.10	0.10	0.10	0.10

DISCUSSION

Experimental Design and Departures From Proposed Techniques

Our evaluation of the interactive effects of water temperature on toxic effects on NaCl and pulp mill effluent on two aquatic invertebrates required several departures from the initially proposed techniques (Casey 1997, 1998). The majority of these departures improved the experimental design or resulted from minor changes in laboratory conditions that did not adversely affect the overall study objectives.

While the initial research proposal identified the use of *Ceriodaphnia dubia* (Crustacea: Daphnidae) as one of the two study animals, experiments were completed using *Daphnia magna* (Crustacea: Daphnidae), a closely related genus. *Daphnia magna* were selected as one of the study organisms based on recommendations from several specialists who have extensive experience in raising and completing bioassays using both *Daphnia magna* and *Ceriodaphnia dubia*. In general, *Daphnia magna* can be cultured more readily than *Ceriodaphnia dubia*, and results of bioassays using *Ceriodaphnia dubia* appear to be more variable than tests with *Daphnia magna* (DeGraeve and Cooney 1987; personal communications: David Poirier – Ontario Department of Environment, Ken Doe, Nancy Cooper – Environment Canada, Hydroqual Ltd.). Thus, the use of *Daphnia magna* improves the statistical robustness of the experimental design by reducing the within-subject source of variation. In fact, our results from Experiments 1 and 2 revealed low within-replicate variation in terms of the responses of *Daphnia magna* to variation in water temperature, NaCl and pulp mill effluents. Low variability could also arise because our laboratory populations of *Daphnia magna* were healthy and that variation among individual body condition was low. This contention is consistent with Gersich et al. (1985), who demonstrated low variability in survival and reproduction of *Daphnia magna* when individuals were well nourished.

Lastly, numerous studies have also shown both *Daphnia magna* and *Ceriodaphnia dubia* are highly responsive to a broad range of toxins that has lead to their frequent use in various toxicity tests, including standard acute and chronic tests of Environment Canada and U.S. Environmental Protection Agency (Environment Canada 1990b, Peltier and Weber 1985). Both species are widely distributed throughout North America and native to Alberta; daphnids occur in river systems including slow flowing, backwater and vegetated habitats (Peltier and Weber 1985, Pennak 1989, Environment Canada 1990b).

The effects of water temperature and pulp mill effluent on a benthic macroinvertebrate were completed using larvae of *Taenionema* (Plecoptera: Taeniopterygidae) rather than *Baetis* (Ephemeroptera: Baetidae). While both genera are widely distributed in streams throughout the Wapiti-Smoky (Terrestrial and Aquatic Environmental Managers Ltd. 1992) and Peace-Athabasca Basins (Scrimgeour et al. 1995), *Baetis* were not abundant upstream of the Weyerhaeuser pulp in October-November 1998. In addition, maintenance of *Baetis* larvae in laboratory streams is considerably more difficult compared with *Taenionema* (Scrimgeour unpubl. data). Thus, the use of *Taenionema* was preferable for use in laboratory bioassays because of its availability and robustness compared with *Baetis*.

Actual water temperatures used in Experiments 1 and 2 with *Daphnia magna* (7^{o}C, 13^{o}C and 20^{o}C) were greater than those originally proposed (1^{o}C, 9^{o}C and 17^{o}C) (Casey 1997). Increased water temperatures arose from unforeseen effects of heating from the overhead light system. These increases in water temperature however, were not thought to adversely affect the integrity of the study because they are still well within the range observed within the river. Further, our results showed low survival of *Daphnia magna* at the lowest temperature of 7^{o}C after 4 days in all of the NaCl solutions. After 2 days at 7^{o}C, all *Daphnia magna* (with the exception of 1 organism) were immobile or dead in each NaCl solution. Thus, the use of lower temperatures such as those that were initially proposed would have likely lead to the same overall conclusion, that is, survival of *Daphnia magna* is strongly affected by water temperature and NaCl concentration.

While concentrations of pulp mill effluent used in Experiment 2 were initially set at 0, 10, 20 and 30% effluent (Casey 1997), we eventually selected a range of effluent concentrations that more closely approximated the full range of conditions present downstream of the effluent diffuser in the Wapiti River (i.e., 0, 5, 10 and 50% pulp mill effluent). The highest concentration of 50% effluent was chosen to increase the probability of showing toxicity caused by the effluent, should they occur.

Effects of Water Temperature and NaCl on *Daphnia magna*

Results of our bioassay experiments showed that survival of *Daphnia magna* was strongly affected by NaCl concentrations and water temperature. In general, the ability of *Daphnia magna* to tolerate (i.e., survive) NaCl declined with declining water temperature. For example, while all individuals survived the 6-day exposure to 5000 mg NaCl/L treatment at 20°C (i.e., 100% survival), the proportion of individuals surviving the same NaCl concentration declined at lower water temperatures (i.e., 39% survival at 13°C and 0% survival at 7°C). We attribute the reduced survival to differences in water temperature and NaCl concentrations because dissolved oxygen, pH, and hardness did not differ markedly among treatments throughout the 6-day trials.

Temperature-specific responses of Daphnids have been reported elsewhere (Becker et al. 1983, Nimmo et al. 1989). For example, *Ceriodaphnia dubia* neonates were more sensitive to NH_3 at 7°C than at 25°C (Nimmo et al. 1989) whereas *Daphnia magna* neonates were more sensitive to a solvent refined coal liquid at 25°C and 20°C than at 10°C (Becker et al. 1983).

Effects of Water Temperature and Pulp Mill Effluent on *Daphnia magna* and *Taenionema*

Survival of both *Daphnia magna* and *Taenionema* were unaffected by water temperature and pulp mill effluent concentrations despite large differences in concentrations of most inorganic and organic compounds present in the treated pulp mill effluent relative to reference water from the Wapiti River. These results are consistent with laboratory trials completed between 1995-1998 on *Daphnia magna* and *Ceriodaphnia dubia* exposed to 100% effluent from the Weyerhaeuser pulp (Ms. Elaine Wasylenchuk, Industrial Waste and Wastewater Branch, Alberta Environment, unpubl. data).

The absence of significant effects of pulp mill effluent on survival is not surprising given the low concentrations of contaminants present within the treated effluent. For example, the greatest concentration for organic contaminants in 100% effluent was 2.1 ug/L dehydroabietic acid. However, toxicity at this level is unlikely because the concentrations were well below previous toxicity levels reported for organic contaminants in the review of aquatic toxicity of pulp and paper mill effluents by McLeay (1987). For example, 96-h LC50 levels for resin acids using the static rainbow trout bioassays ranged from 400-1740 ug/L (McLeay 1987). In earlier studies comparing the sensitivity of rainbow trout and *Daphnia* spp. to pulp and paper mill effluents, most data showed similar sensitivities of trout and *Daphnia* or trout were more sensitive to effluents (See Table 2.2 in McLeay 1987). Concentrations of organic contaminants reported by McLeay (1987) were expected to be higher than those found in our study because since that review, there have been several major improvements in the reduction of chlorinated phenols and resin acids, the main toxic contaminants in bleached kraft pulp mill effluent. Concentrations of these compounds in 100% effluent also appeared to be well below those levels reported previously in effluent from the same pulp mill used in our study. For example, concentrations of chlorinated phenols and dehydroabietic acid from 1990-1994 ranged 0.01-0.14 and 0-1.1 mg/L, respectively (Alberta Environmental Protection 1996). In contrast, our results for the single chlorinated phenol detected and dehydroabietic acid were 3 orders of magnitude less at 2.1 and 0.67 ug/L, respectively. The almost complete absence of chlorinated phenolics in the effluent used in our study was likely caused by the 100% substitution of chlorine gas with chlorine dioxide in the bleaching process (McLeay 1987).

In contrast to survival, growth of both *Daphnia magna* and *Taenionema* were affected by water temperature and pulp mill effluent concentrations. *Daphnia magna* maintained at 20°C in both pulp mill effluent and water from the Wapiti River were larger than those maintained at 7°C and 13°C. Similarly, small *Taenionema* larvae maintained at the higher temperatures were longer ($P=0.054$) and heavier ($P<0.01$) after the 14 day trials than those kept at 3°C. Increased size at higher water temperatures likely reflects the positive relationships between growth and metabolic rate (i.e., increased growth at higher temperatures).

Our analyses of final body lengths of *Daphnia magna* and *Taenionema* provide some evidence that higher pulp mill effluent concentrations may have stimulated growth of *Daphnia magna* and *Taenionema* and egg production by *Daphnia magna*. For example, body lengths of *Daphnia magna* were affected by pulp mill effluent concentration ($P=0.078$) whereas body lengths, of *Taenionema* larvae were strongly affected ($P<0.01$) by pulp mill effluent concentration (growth in 30% treated pulp mill effluent was greater than that in both the 15% treated pulp mill effluent and effluent-absent control). While our experiment was not designed to identify causal mechanisms, increased growth of *Daphnia magna* and *Taenionema* likely arises from the increased quantity and quality of algal and bacterial food resources with increasing pulp mill effluent concentration.

At 20°C, *Daphnia magna* maintained at the highest effluent concentration of 50% produced more eggs than at lower effluent concentrations (i.e., 0, 5 and 10% effluent). At the next lowest temperature of 13°C, eggs were only found in *Daphnia magna* in 50% effluent. Greatest numbers of immature *Daphnia magna* were also found at higher rather than lower effluent concentrations, especially for the 5-50% effluent treatments compared with that at 0% effluent. Enhanced growth and development of *Daphnia magna* with increasing effluent concentration likely arises because the effluent contains greater densities of bacteria and algae that are consumed by daphnids.

Recent studies examining the effects of treated bleached kraft pulp mill effluent using artificial streams alongside the Thompson River (British Columbia) and Athabasca River have also failed to show adverse acute or sub-lethal effects on aquatic insects and periphyton. Rather, there is evidence that treated effluents rich in nitrogen and phosphorus may enhance growth and development of grazing chironomids and mayflies by increasing the quantity, and perhaps quality, of their algal prey (Lowell et al. 1995, Culp and Podemski 1996, Dubè et al. 1997).

Project rationale and Summary

The present study was implemented to address adverse effects of pulp mill effluents on benthic communities during lengthy low-flow winter conditions. These conditions raised serious concerns as to whether biota in Alberta rivers would be more sensitive to treated bleached pulp mill effluent during winter months of November-April when water temperatures approach 0°C.

While our results are based on only two macroinvertebrate taxa, the results of our study suggest that benthic macroinvertebrates may not be increasingly vulnerable to pulp mill effluents at low temperatures. The lack of significant adverse effects are noteworthy given that our experiments were completed using high concentrations of treated bleached kraft pulp mill effluents that greatly exceed that typically found in most Alberta rivers. The lack of detectable effects, however, is consistent with results obtained from monitoring of benthic communities during winter months at sites located upstream and downstream of the bleached kraft pulp mill in the Wapiti River in 1991-1992 (L. Noton, Terrestrial and Aquatic Environmental Managers 1992). Overall, our work provides some assurance that river biota are not necessarily more sensitive to pulp mill effluent during winter than at other seasons, and that the toxic effects from these effluents during winter may not be occurring as initially contended.

Conclusions

We designed and completed three sets of experiments to evaluate the interactive effects of water temperature, pulp mill effluent, and sodium

chloride on survival and growth of *Daphnia magna* (Experiments 1 and 2) and *Taenionema* spp. (Experiment 3). The first set of experiments evaluated the effects of water temperature (i.e., 7, 13, 20ºC) and varying concentrations of sodium chloride (NaCl) (0, 5000, 5400 and 5800 mg NaCl/L) on survival and growth *Daphnia magna* over a 6-day period. Survival of *Daphnia magna* was significantly affected by water temperature with greater number of individuals surviving at 20^{o}C compared with 13^{o}C and 7^{o}C, respectively. Similarly, significantly greater number of individuals survived at 0 and 5000 mg NaCl/L compared with 5400 and 5800 mg NaCl/L.

The second set of experiments evaluated the effects of water temperature (i.e., 7, 13, 20ºC) and varying concentrations of treated bleached kraft pulp mill effluent (i.e., of 0, 5, 10, 50%) on survival and growth of *Daphnia magna.* While survival of *Daphnia magna* was not significantly affected by either water temperature or pulp mill effluent concentration, body length (i.e., growth) and the production of neonates increased significantly with increasing water temperature and pulp mill effluent concentration over the 8-day period.

The third set of experiments evaluated the effects of water temperature (i.e., 3, 9, 15ºC) and varying concentrations of treated bleached kraft pulp mill effluent (i.e., of 0, 15, 30%) on survival and growth of small and large larval *Taenionema* over a 14-day period. Survival of *Taenionema* was not significantly affected by water temperature and pulp mill effluent and all individuals survived the 14-day trials. In contrast, growth of both small and large larvae was significantly affected by water temperature and pulp mill effluent concentration. Larval growth was highest under high water temperatures and high concentrations of pulp mill effluent. Taken together, our data indicate that both *Daphnia magna* and *Taenionema* can grow and develop in even moderately high concentrations of pulp mill effluent. These two taxa did not show greater sensitivity to effluent under colder temperatures.

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