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STIMULATION OF INCREASED SHORT-TERM GROWTH AND DEVELOPMENT OF MAYFLIES BY PULP MILL EFFLUENT

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Abstract – To determine the food-dependent effects of biologically treated, bleached-kraft pulp mill effluent on mayfly (*Baetis tricaudatus* Dodds) growth and development, mayflies were exposed to effluent for 2 weeks within artificial streams arranged in a 2 × 3 factorial design (two periphyton food levels – low, high; three concentrations – control river water, 1% effluent, 10% effluent). Although survival was not affected, the effluent had a significant stimulatory effect on growth (resulting in 20 to 50% greater body weights) and development of the wing pads. Furthermore, the initial growth and development trajectories suggest that effluent-exposed mayflies may emerge sooner and at a larger size than nonexposed individuals. A key result was that the stimulatory effects occurred within both the low- and high-food treatments. Thus, the effluent-exposed mayflies grew faster than even the high-food control animals, which already had access to more food than they could eat throughout the experiment. This shows that the stimulatory effect of the effluent on the mayflies involved more than just an increase in food availability due to enhanced algal growth in response to nutrients in the effluent. Three possible mechanisms for this growth-enhancement effect are that the effluent (a) increased the nutritive value of the food, (b) enhanced the palatability of the periphyton, inducing increased mayfly feeding, and/or (c) directly stimulated increased mayfly growth via hormonal or other growth-stimulation effects. Further work is required to determine whether the growth stimulation occurred at the expense of future reproductive output.

Keywords – Pulp mill effluent Mayfly *Baetis* Periphyton Growth

INTRODUCTION

Pulp mill effluents contain a wide variety of compounds that can have differing effects on aquatic organisms and communities in receiving waters [1]. At high concentrations, many of these compounds are toxic and can cause mortality or a variety of sublethal effects. Some of the better studied toxicants include the chlorophenolics, resin acids, and metals. On the other hand, pulp mill effluents usually contain high levels of the algal nutrients, phosphorus and nitrogen. These nutrients have frequently been shown to have an enrichment effect leading to enhanced productivity in some parts of the receiving water ecosystem [2–4]. Before 1980, regulatory intervention tended to focus on organic and nutrient loading, together with oxygen depletion and suspended solids. More recent regulations have emphasized toxicity [5]. Setting regulatory guidelines can be difficult because nutrient-enhancement effects can sometimes mask the toxic effects of pulp mill effluents, and little data is available to disentangle these two effects [6].

Weyerhaeuser Canada Ltd. operates a bleached-kraft pulp mill at Kamloops, British Columbia, on the Thompson River just below the confluence of the North and South Thompson rivers (Fig. 1). Upstream of Kamloops, the Thompson River system is phosphorus limited [7]. In 1972, the pulp mill expanded its operations, leading to increased nutrient loadings to the river. Together with loadings from the city of Kamloops' municipal sewage plant, which have since decreased, the increased nutrient availability led to pronounced algal blooms in the Thompson River. Algal biomass in the river at present appears to be lower than in the mid-1970s, but still remains a concern [7]. The combined effects of nutrient loading and potential toxicity of the pulp mill effluent are of particular relevance to the management of water use in this river system.

The mayfly *Baetis tricaudatus* (Ephemeroptera: Baetidae) is one of the more abundant benthic macroinvertebrates in the Thompson River system. This widespread, multivoltine species is found in moderate to swift current streams and rivers throughout northwestern North American [8,9]. *Baetis tricaudatus* grazes on periphyton [10], making it a good candidate for evaluating the combined effects of nutrient loading (via effects on food availability to the mayfly) and toxicity. As a first attempt to tease out the relative contribution and nature of these two effects, we conducted a factorial experiment designed to measure the survival, growth, and developmental responses of *B. tricaudatus* to different concentrations of pulp mill effluent under two different feeding regimes.

MATERIALS AND METHODS

Experimental design

The food-dependent effects of pulp mill effluent on the mayfly *Baetis tricaudatus* Dodds were determined by exposing the mayflies for 2 weeks to three effluent concentrations and two food levels within artificial streams. The study was conducted in an outdoor experimental stream enclosure located beside the Thompson River adjacent to the Kamloops Weyerhaeuser mill. The enclosure contained a series of 250-L

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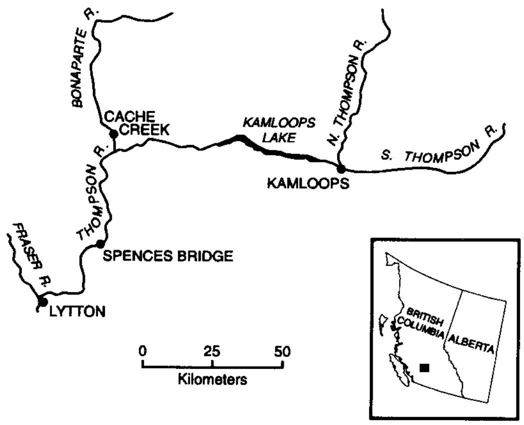

Fig. 1. Location map of the Thompson River where the study was conducted in south-central British Columbia.

flow-through mixing reservoirs to provide different concentrations of pulp mill effluent to the arrays of artificial streams. River water was pumped into each reservoir at 0.9 to 1.0 L/min (depending on effluent inflow) so that, when combined with the inflow of effluent, each reservoir had a total flow-through rate of 1.0 L/min. The river water intake was located upstream of the outfalls for both the mill effluent and the city's sewage treatment plant.

Effluent was pumped into each noncontrol reservoir using peristaltic pumps drawing from a single effluent holding tank. The holding tank was refilled each day with fresh effluent collected from the mill outfall, just beyond the secondary (biological) treatment ponds. The effluent pumping rate was adjusted to maintain a fixed concentration within each reservoir. Air-stone bubblers, together with the return flow from the artificial streams, provided mixing and aeration within the reservoirs; this also maintained dissolved oxygen at near saturation levels within all of the treatment streams. Temperature was also kept at an equal level (approx. 9°C) among the different treatments by using thermostatically controlled aquarium heaters in the reservoirs.

The experiment was arranged in a 2 × 3 factorial design: Mayflies were offered two food levels (low, high) at each of three concentrations (control river water, 1% effluent, 10% effluent; v/v). The 1% treatment simulated effluent concentrations in the Thompson River at complete mix during periods of low flow (November–March) [11,12]. The 10% treatment simulated effluent concentrations closer to the mill before complete mix.

The experimental test chambers were circular Plexiglas artificial streams (diameter = 8.8 cm; stream bottom area = 50 cm^2) [13]. Current was produced in each stream by small water jets driven by pumps drawing water from the mixing reservoirs described above; water returned to the reservoirs via a central standpipe drain in each stream. Seven replicate streams were used for each of the six experimental treatments, for a total of 42 streams.

To provide the mayflies with a stream-like substratum on which to rest, move, and feed, six 2.4 × 2.4 × 0.5-cm roughened ceramic blocks were placed onto the bottom of each stream. The mayflies spent most of their time among these blocks. Inflow to the water jets was adjusted so that the current velocity within 1.5 cm of the substratum was approximately 6 cm/s (measured with a low-velocity propeller probe; Nixon Instrumentation, Cheltenham, UK). This velocity is typical of the intrasubstratum velocity among the stones where the mayflies are found [14], and is more relevant to invertebrates in the benthic microenvironment than the mainstream velocity usually reported for real and artificial streams. For comparative purposes, the mainstream velocity in our streams was approximately 25 cm/s, which is similar to the faster mainstream velocities reported for other toxicity studies [15].

Food was provided during the experiment by periphyton cultured on ceramic blocks identical to those used for the stream substrata. The periphyton was grown beforehand on the blocks within 2.0 × 0.19-m Plexiglas flumes through which river water was pumped at approximately 20 cm/s (see Bothwell [11] for further information on the use of these flumes for growing periphyton). The high velocity ensured that the periphyton that grew on the blocks would remain firmly attached after transfer to the 6-cm/s streams. A 500-g bag of slow-release fertilizer (Nutricoat; ratio of nutrients 14 N: 14 P: 14 K) was placed in a mixing chamber at the head of each flume to elevate the concentration of phosphorus to increase algal growth rate, yielding a dense algal growth on the blocks after about 3 weeks. Phosphorus has been shown to be the limiting nutrient to benthic algae in the Thompson River above the pulp mill and sewage outlets at Kamloops [7].

Low- versus high-food treatment levels were set by how long the mayflies were allowed to feed on the periphyton-covered blocks. At the beginning of the experiment, two periphyton-covered blocks were transplanted from the flumes into each stream after removing two corresponding periphyton-bare blocks. In the high-food treatments, the blocks were left in place for 1 week, whereas in the low-food treatments, the two food blocks were removed after 3 d and replaced again with bare blocks for the remainder of the week. During this latter part of the week, a small amount of periphyton was available to the mayflies in the low-food treatments because it was not possible to remove all food particles that accumulated on some of the bare surfaces of the streams.

At the beginning of the second week, the same procedure was followed; the old periphyton-covered blocks were taken out of the high-food treatments and two fresh periphyton-covered blocks from the flumes were placed into each stream for both food treatments. Again, the periphyton-covered blocks in the low-food treatment were replaced with bare ones after 3 d. This procedure varied food availability to the mayflies by controlling the length of time during which they could feed at high rates, without varying the community assemblage of periphyton on the blocks (since both low- and high-food treatments received periphyton cultured under identical conditions and for the same length of time).

Several hundred *B. tricaudatus* were collected a day prior to the experiment from the Bonaparte River, a tributary of the Thompson River, near the town of Cache Creek approximately 8 km from the confluence with the Thompson River. Thus, although the mayflies were sampled close enough to the Thompson River to be part of the same interbreeding population of aerial adults, the individual aquatic larvae used in the experiment had no previous exposure to pulp mill effluent and, consequently, were not pre-acclimated to the effluent. Only mayflies of a uniform size class (approximately 3.5 mm in length) were used in the experiment, and these presorted animals were held overnight in aerated aquaria at ambient river temperature. On the first day of the experiment, the *B. tricaudatus* were randomly allocated to one of the 42 streams until each stream contained 10 animals. Fifty additional mayflies were preserved in 10% formalin and then transferred to 80% ethanol for later determination of animal weight and morphometric dimensions at the beginning of the experiment.

During the experiment, the facility was monitored each day to ensure that the effluent concentrations and the stream velocities remained at their nominal values and to remove depositing seston from the streams. In addition, water samples were taken once at the beginning and once at the end of the experiment and sent to Zenon Environmental Laboratories (Burnaby, British Columbia) for all determinations of levels of contaminants and other water quality variables in the (a) river water, (b) full-strength effluent, and (c) river water/effluent mixture from the 10% effluent treatment. These measured variables (listed in Tables 1–6) included metals, chlorophenolics, resin acids, polycyclic aromatic hydrocarbons, and algal nutrients (phosphorus, nitrogen). The high cost of contaminant analysis prevented more frequent analyses. Ceramic food blocks with periphyton in earlier (3 weeks) and later (5 weeks) successional stages were also collected during the experiment. These were either (a) preserved in Lugol's solution for later determination of the relative abundances of the dominant species of algae growing on the blocks or (b) frozen for determination of the amount of periphyton on the blocks (as measured by ash-free dry mass and chlorophyll-*a* content per square centimeter of block surface area; the chlorophyll-*a* measurements were corrected for pheophytin-*a*).

Biological end points and statistical analysis

Survival, growth, molting, and morphological development were measured during and at the end of the experiment to determine the response of *B. tricaudatus* to the effluent under the two feeding regimes. During the experiment, the streams were monitored daily and all molts and dead mayflies were counted and removed. At the end of the experiment, surviving mayflies were counted and preserved in 10% formalin and later transferred to 80% ethanol before final measurements were taken. The proportion of mayflies surviving in each replicate stream was arcsine–square root transformed before analysis to normalize the data and homogenize the variances [16]. Growth and development end points for each replicate stream were determined from the means for all the measured animals from that stream.

The effect of the effluent on mayfly growth was determined by comparing dry body weights of the preserved animals from the end of the experiment. The number of molts during the first and second weeks of the experiment provided additional measures of the effect on growth. Relative wing pad length is a measure of the degree of development of aquatic insect larvae as they mature toward the final adult instar [17,18]. This was measured as the ratio of length to spread of the wing pads. Wing pad length was measured from the posterior edge of the right wing pad to the point where the medial edge of the right wing pad joined the thorax as

Table 1. Metals present in the control river water, full-strength effluent, and 10% effluent mixture at the beginning and end of the experiment

	Measured concns. (mg/L)						
	Control		Effluent		10%		
	Begin	End	Begin	End	Begin	End	WQG[a]
Aluminum	0.1	0.18	0.39	0.82	0.12	0.2	0.1
Barium	0.009	0.011	0.105	0.158	0.021	0.028	1.0
Calcium	12.7	13.8	105	126	23.3	28.9	
Chromium	nd[b]	nd	0.012	0.013	nd	0.003	0.002
Copper	nd	nd	0.003	0.011	nd	0.002	0.002
Iron	0.2	0.33	0.8	0.84	0.23	0.31	0.3
Magnesium	2.25	2.56	4.23	5.11	2.5	2.89	
Manganese	0.009	0.016	0.573	0.707	0.072	0.12	
Potassium	1.1	1.0	8.3	7.5	1.7	1.9	
Sodium	1.7	2.0	264	288	33.3	39.2	
Strontium	0.076	0.082	0.162	0.198	0.088	0.098	
Titanium	0.007	0.011	0.016	0.031	0.006	0.01	0.1
Vanadium	nd	nd	0.003	0.007	nd	nd	
Zinc	0.01	0.02	0.03	0.09	0.01	nd	0.03

[a]WQG = Canadian Federal (or British Columbia Provincial—barium, titanium) Water Quality Guidelines (mg/L) [21,22].
[b]nd = not detected: concentrations that were below the detection limit of 0.002 mg/L for chromium and copper, 0.003 mg/L for vanadium, or 0.01 mg/L for zinc.

Table 2. Chlorophenolics and AOX present in the full-strength effluent and 10% effluent mixture at the beginning and end of the experiment

	Measured concentrations (μg/L)					
	Effluent		10%			
	Begin	End	Begin	End	WQG[a]	LC50[b]
Monochlorophenols	nd[c]	nd	nd	nd	7.0	
Dichlorophenols	nd	nd	nd	nd	0.2	~2,800
Trichlorophenols	nd	nd	nd	nd	18.0	~1,500
Tetrachlorophenols	nd	nd	nd	nd	1.0	
Pentachlorophenol	nd	nd	nd	nd	0.5	
Monochloroguaiacols	0.2	0.43	nd	0.1		
Dichloroguaiacols	nd	0.17	nd	nd		~2,300
Trichloroguaiacols	nd	nd	nd	nd		~850
Tetrachloroguaiacols	nd	nd	nd	nd		~950
Monochlorocatechols	nd	nd	nd	nd		
Dichlorocatechols	nd	1.1	nd	nd		~750
Trichlorocatechols	nd	0.1	nd	nd		~1,300
Tetrachlorocatechols	nd	0.3	nd	nd		~950
Monochlorovanillins	nd	nd	nd	0.2		
AOX	4,400	5,700	500	800		

[a]WQG = Canadian Federal Water Quality Guidelines (μg/L) [21].
[b]LC50 (μg/L) for 96-h toxicity tests with salmonids [1].
[c]nd = not detected: concentrations that were below the detection limit of 0.1 μg/L (0.05 μg/L for mono- through pentachlorophenols).

viewed dorsally. Wing spread was measured as the distance between the points where the medial edges of the right and left wing pads joined the thorax as viewed dorsally.

Results for each of the end points were analyzed in a 2 × 3 factorial analysis of variance (ANOVA; two food levels by three effluent concentrations) with streams as replicates using SYSTAT® [19]. Each ANOVA was then broken down into single-degree-of-freedom contrasts to compare concentration effects within each food level (see [19] for a discussion of the statistical rationale and procedures). Specifically, survival, growth, and development of the control animals were compared to the averaged effect of the 1% and 10% effluent treatments on these end points. In addition, the 1% effluent effect was compared to the 10% effect. Planned comparisons of this sort have the advantage of maximizing statistical power while focusing on comparisons of particular interest [16,20]. The number of comparisons is limited by the number of degrees of freedom available, and these specific comparisons were chosen to test whether the effluent had a significant overall effect relative to the controls and to test for differences between the 1% and 10% effluent effects. In addition, the relationship of size (body weight) to the rate of development (relative wing length) was examined with analysis of covariance (ANCOVA).

Table 3. Resin acids present in the full-strength effluent and 10% effluent mixture at the beginning and end of the experiment

	Measured concentrations (μg/L)					
	Effluent		10%			
	Begin	End	Begin	End	WQG[a]	LC50[b]
Abietic acid	nd[c]	nd	nd	nd		~1,100
Dehydroabietic acid	nd	nd	nd	nd	12	~1,300
Isopimaric acid	nd	11	12	2		~700
Levopimaric acid	7	nd	nd	nd		~850
Neoabietic acid	nd	nd	nd	nd		~650
Pimaric acid	nd	45	nd	2		~950
Sandaracopimaric acid	18	nd	nd	nd		~350
Total resin acids	25	56	12	4	45	

[a]WQG = British Columbia Provincial Water Quality Guidelines (μg/L) [22].
[b]LC50 (μg/L) for 96-h toxicity tests with salmonids [1].
[c]nd = not detected: concentrations that were below the detection limit of 1.0 μg/L.

Table 4. Polycyclic aromatic hydrocarbons present in the full-strength effluent and 10% effluent mixture at the beginning and the end of the experiment

	Measured concns. (μg/L)				
	Effluent		10%		
	Begin	End	Begin	End	WQG[a]
Naphthalene	0.06	nd[b]	nd	nd	1.0
Acenaphthylene	nd	nd	nd	nd	
Acenaphthene	nd	nd	nd	nd	6.0
Fluorene	0.01	0.01	nd	nd	12.0
Phenanthrene	0.06	0.05	nd	nd	0.3
Anthracene	0.01	0.01	nd	nd	0.1
Fluoranthene	0.05	nd	nd	nd	0.2
Pyrene	0.05	nd	nd	nd	0.02
Benz[*a*]anthracene	0.01	nd	nd	nd	0.1
Chrysene	0.01	nd	nd	nd	
Benzo[*b* + *k*]fluoranthene	nd	nd	nd	nd	
Benzo[*a*]pyrene	nd	nd	nd	nd	0.01
Indeno[1,2,3-*cd*]pyrene	nd	nd	nd	nd	
Dibenz[*a, h*]anthracene	nd	nd	nd	nd	
Benzo[*g,h,i*]perylene	nd	nd	nd	nd	

[a]WQG = British Columbia Provincial Water Quality Guidelines (μg/L) [22].
[b]nd = not detected: concentrations that were below the detection limit of 0.01 μg/L.

RESULTS

Contaminants and other water quality variables

When referenced to water quality guidelines levels, contaminant levels in the diluted pulp mill effluent were low (Tables 1-4). Fourteen metals were present at detectable levels in the control river water, the full strength pulp mill effluent, and/or the river water/effluent mixture from the 10% effluent treatment (Table 1). Values for each of these are given for both the beginning and the end of the experiment. Federal or British Columbia provincial water quality guidelines are also given for those metals for which guidelines were available [21,22]. These are general guidelines based on past research; they recommend postdilution contaminant levels for rivers and other water bodies that are low enough to protect the health of freshwater organisms. Following dilution, metal concentrations were about at or below guideline levels.

Table 5. Phosphorus and nitrogen present in the control river water, full-strength effluent, and 10% effluent mixture at the beginning and end of the experiment

	Measured concns. (μg/L)						
	Control		Effluent		10%		
	Begin	End	Begin	End	Begin	End	WQG[a]
P-SRP[b]	6	nd[c]	1,051	1,180	126	155	
P-dissolved	4	6	1,330	1,520	151	230	
P-total	7	9	1,430	2,240	172	249	
N-organic	110	110	1,630	5,300	400	920	
Ammonia-total	13	nd	2,300	2,160	262	142	~2,000
N-Kjeldahl	123	110	3,930	7,460	662	1,062	
$NO_3 + NO_2$	30	70	130	50	40	170	
N-total	153	180	4,060	7,510	702	1,232	

[a]WQG = Canadian Federal Water Quality Guideline (μg/L) [21].
[b]SRP = soluble reactive phosphorus.
[c]nd = not detected: concentrations that were below the detection limit of 1.0 μg/L for P-SRP or 5.0 μg/L for ammonia.

On average, metal concentration in the 10% effluent treatment was about equal to the control river water concentration plus 1/10 the concentration in the full-strength effluent, providing evidence that the 10% mixing ratio was maintained during the experiment. Concentrations of most metals in the effluent were higher in samples taken at the end of the experiment than at the beginning. Metal concentrations in the river water also followed this trend, but to a lesser degree. Note that the metals and other contaminants and water quality variables may have shown further variations in concentration during the experiment, in addition to the concentration differences that we measured at the beginning versus the end of the experiment, due to the potentially changing nature of the chemically complex effluent that is produced by pulp mills.

Three classes of chlorophenolics were present at low levels in the full-strength effluent or 10% mixture: guaiacols, catechols, and vanillins (Table 2; because chlorophenolics, resin acids, and polycyclic aromatic hydrocarbons were all below detection limits in the control river water, river water concentrations are not included in Tables 2–4). Federal water quality guidelines are not yet available for the chlorophenolic classes that were present, but Table 2 gives guideline values for the mono- through pentachlorophenols for comparison. In addition, 96-hour LC50 values for salmonids are listed for the chlorophenols, guaiacols, and catechols; these values show that the acute toxicities of the different classes of chlorophenolics are roughly similar. If this similarity between classes also applies to chronic toxicity, then the levels of the chlorophenolics following dilution would be roughly at or below guidelines levels. Similar to metal concentrations, levels of chlorophenolics in the effluent were higher at the end of the experiment than at the beginning. Adsorbable organic halogens (AOX), which provide a general indication of the overall level of chlorinated organic compounds present, were also higher at the end of the experiment.

Total concentration of resin acids in the diluted effluent was below the British Columbia provincial guideline level (Table 3). In addition, the total concentration in the full-strength effluent was higher at the end of the experiment than at the beginning, although the composition changed as well. Further study is required to determine the cause for the higher concentration of isopimaric acid measured in the 10% mixture as compared to the full-strength effluent at the beginning of the experiment. This led to the higher than expected beginning level of total resin acids in the 10% mixture relative to the full-strength effluent.

Although several polycyclic aromatic hydrocarbons (PAHs) were present (Table 4), concentrations were near

Table 6. Other water quality variables for the control river water, full-strength effluent, and 10% effluent mixture at the beginning and end of the experiment (concn. in mg/L, except where otherwise noted)

	Measured values					
	Control		Effluent		10%	
	Begin	End	Begin	End	Begin	End
Alkalinity	37.8	40.5	176	214	55.8	63.4
BOD[a]	nd[b]	nd	11	nd	nd	nd
Color (TAC)[c]	4	4	524	587	71	89
Conductance (μS/cm)	97	103	1,740	2,070	310	371
Hardness	41.0	45.0	280	336	68.5	84.1
pH (pH units)	7.8	7.4	7.7	7.3	7.8	7.5
Suspended solids	7	14	12	60	4	13
Boron	nd	0.04	nd	nd	nd	nd
Chloride	0.5	0.6	174	219	21.2	20.8
Silicon	2.5	3.3	3.9	3.7	2.7	2.7
Sulfur	2.3	2.6	138	159	18.4	21.9
Chlorate	nm[d]	0.57	0.13	0.16	0.43	0.38

[a]BOD = biochemical oxygen demand.
[b]nd = not detected: concentrations that were below the detection limit of 10.0 mg/L for BOD or 0.04 mg/L for boron.
[c]TAC = total absorbance color.
[d]nm = not measured.

detection limits (0.01 μg/L) in the full-strength effluent and below detection limits in the 10% effluent treatments. Concentrations in the diluted effluent were below British Columbia guideline levels. In contrast to the other contaminants, more PAHs were present (within detection limits) in samples collected at the beginning of the experiment.

Because phosphorus is a limiting nutrient in the Thompson River [7], the higher concentrations of phosphorus in the effluent (Table 5) were great enough to stimulate increased algal growth. This increased growth was apparent on previously bare substrata in the artificial streams toward the end of the experiment, even for the 1% effluent treatments. Of the three forms of phosphorus listed, soluble reactive phosphorus most closely approximates the orthophosphates that are actually available to algae as nutrients. Similar to the contaminants discussed earlier, levels of phosphorus and nitrogen were higher at the end of the experiment (Table 5). Concentrations of ammonia and ammonium in the diluted effluent were below the federal guideline level.

Concentrations of most of the remaining water quality variables were also higher at the end of the experiment (Table 6). Chlorate differed markedly from the other contaminants in that it was present at a higher level in the river than in the effluent (analytical procedure allowed detection down to 0.01 mg/L). This compound is produced in mills using high chlorine dioxide substitution, like the Kamloops Weyerhaeuser mill, but is reduced to chloride during the secondary biological treatment process [6,23]. The source of the slightly higher levels of chlorate in the upstream river water is not known.

Species composition and biomass of periphyton on the experimental food blocks

The dominant species of algae on the experimental food blocks after both 3 and 5 weeks of growth were the pennate diatom *Synedra rumpens* (order Pennales; 30 to 34% by number of individuals for the common algal species present) and the blue-green alga *Oscillatoria prolifica* (order Oscillatoriales; 30 to 66% by number). The pennate diatoms *Synedra ulna* (21% by number) and *Tabellaria fenestrata* (19% by number) were also abundant at the 3-week stage. This species composition is similar to that reported in previous studies on the Thompson River [24].

The amount of periphyton on the blocks after 3 weeks was 1.060 ± 0.109 mg/cm^2 ash-free dry mass and 9.664 ± 0.481 μg/cm^2 chlorophyll-*a* (mean ± 1 SE; $N = 5$). After 5 weeks, these increased to 2.663 ± 0.369 mg/cm^2 ash-free dry mass and 25.688 ± 3.046 μg/cm^2 chlorophyll-*a*. These values are similar to those for natural rock substrata in the Thompson River between Kamloops Lake and the mouth of the Bonaparte River downstream of the pulp mill and sewage outfall [12]. While the blocks were in the streams, these amounts far exceeded what the mayflies could consume (personal observation) [10].

Response of Baetis tricaudatus *to the experimental effluent treatments*

Survival. The pulp mill effluent had no significant effect on *B. tricaudatus* survival during the course of the experiment (Fig. 2; Table 7). The basic ANOVA (Table 7) shows that the main effects (food level and concentration) and interaction term were not significant at $p \leq 0.05$. The ANOVA with contrasts table (Table 7) breaks the basic ANOVA down into single-degree-of-freedom contrasts to test for the significance of effluent concentration effects within each food level. Within food levels, there were, again, no significant differences between the 1% and 10% treatments, nor between the controls versus the mean of the 1% and 10% treatments.

Growth. The pulp mill effluent had an overall stimulatory effect on *B. tricaudatus* growth. This effect can be seen most

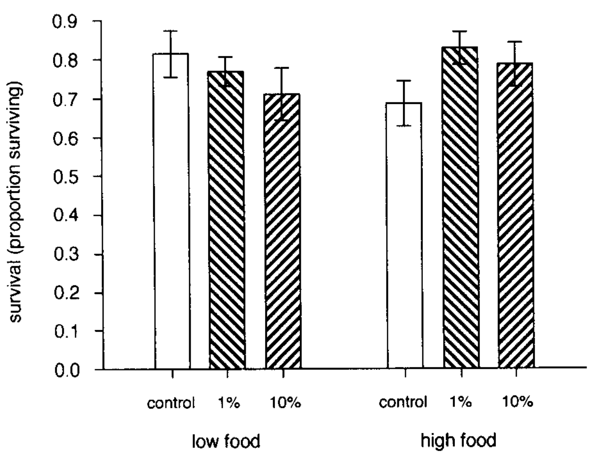

Fig. 2. Survival (proportion surviving) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent) (±1 SE).

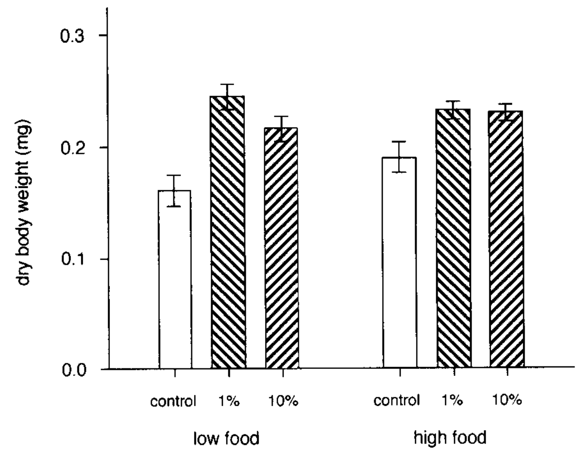

Fig. 3. Dry body weight (mg) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent) (±1 SE).

readily for the standard measure of size, dry body weight, which was 20 to 50% greater for mayflies exposed to the effluent (Fig. 3; Table 8). The basic ANOVA for dry body weight shows a significant main effect for concentration; greater body weights were observed for the 1% and 10% effluent treatments than for the control river water treatment. Food level and interaction effects were not significant. This suggests that the difference between the food levels was not great enough to produce a significant main food effect during the 2-week course of the experiment.

A key result was that a significant increase in body weight was induced by the 1% and 10% effluent treatments, as compared to the controls, within both the low- and high-food treatments (ANOVA with contrasts, Table 8). Thus, the effluent-treated mayflies grew to a greater weight than observed even for the high-food control animals, which already had access to more food than they could consume throughout the experiment. No significant differences between the 1% and 10% treatments were found within food levels.

The effect of the effluent treatments on molting changed from week 1 to week 2 of the experiment. During the first week, no significant differences were observed (Fig. 4; Table 9). During the second week, concentration had a significant main effect and the averaged effect of the 1% and 10% treatments was a significant increase in molting, relative to the controls, but only within the high-food treatment (Fig. 5; Table 10). In addition, 1% effluent caused more frequent molting than did 10% effluent within both the low- and high-food treatments. We have also noted this general tendency for the 1% treatment to account for more of the stimulatory effect of the effluent than the 10% treatment when considering other measures of growth (total body length, thorax length, and head width) [25]. This suggests that, at a 10% concentration, the inhibitory effects of the effluent may have

Table 7. Survival (proportion surviving) analysis of variance at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent)

Source of variation	SS	*d.f.*	MS	*F*	*p*
Basic ANOVA					
Food level	0.000009	1	0.000009	<0.001	0.987
Concentration	0.024571	2	0.012286	0.366	0.696
Food × concentration	0.132495	2	0.066247	1.973	0.154
Error	1.208910	36	0.033581		
ANOVA with contrasts					
Food level	0.000009	1	0.000009	<0.001	0.987
Within low food					
Control vs. mean of 1% and 10%	0.045164	1	0.045164	1.345	0.254
1% vs. 10%	0.010852	1	0.010852	0.323	0.573
Within high food					
Control vs. mean of 1% and 10%	0.091339	1	0.091339	2.720	0.108
1% vs. 10%	0.009711	1	0.009711	0.289	0.594
Error	1.208910	36	0.033581		

SS = sum of squares; MS = mean squares.

Table 8. Dry body weight analysis of variance at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent)

Source of variation	SS	*d.f.*	MS	*F*	*p*
Basic ANOVA					
Food level	0.001094	1	0.001094	1.252	0.271
Concentration	0.030211	2	0.015106	17.286	<0.001
Food × concentration	0.003115	2	0.001557	1.782	0.183
Error	0.031459	36	0.000874		
ANOVA with contrasts					
Food level	0.001094	1	0.001094	1.252	0.271
Within low food					
Control vs. mean of 1% and 10%	0.022662	1	0.022662	25.933	<0.001
1% vs. 10%	0.002783	1	0.002783	3.185	0.083
Within high food					
Control vs. mean of 1% and 10%	0.007867	1	0.007867	9.003	0.005
1% vs. 10%	0.000014	1	0.000014	0.016	0.901
Error	0.031459	36	0.000874		

SS = sum of squares; MS = mean squares.

Table 9. Week-1 molt analysis of variance at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent)

Source of variation	SS	*d.f.*	MS	*F*	*p*
Basic ANOVA					
Food level	6.095238	1	6.095238	2.220	0.145
Concentration	2.047619	2	1.023810	0.373	0.691
Food × concentration	0.619048	2	0.309524	0.113	0.894
Error	98.857143	36	2.746032		
ANOVA with contrasts					
Food level	6.095238	1	6.095238	2.220	0.145
Within low food					
Control vs. mean of 1% and 10%	0.214286	1	0.214286	0.078	0.782
1% vs. 10%	1.785714	1	1.785714	0.650	0.425
Within high food					
Control vs. mean of 1% and 10%	0.595238	1	0.595238	0.217	0.644
1% vs. 10%	0.071429	1	0.071429	0.026	0.873
Error	98.857143	36	2.746032		

SS = sum of squares; MS = mean squares.

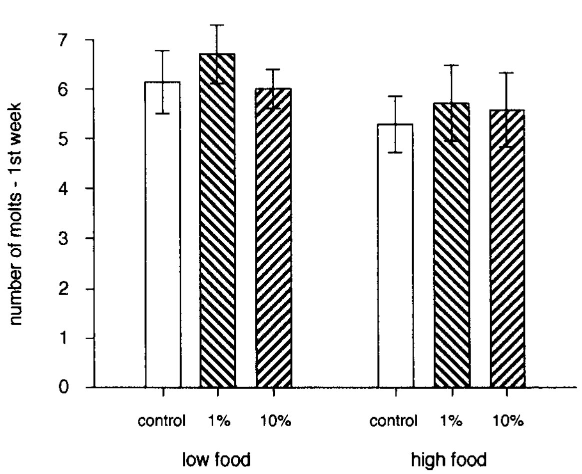

Fig. 4. Number of molts produced during the first week at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent) (±1 SE).

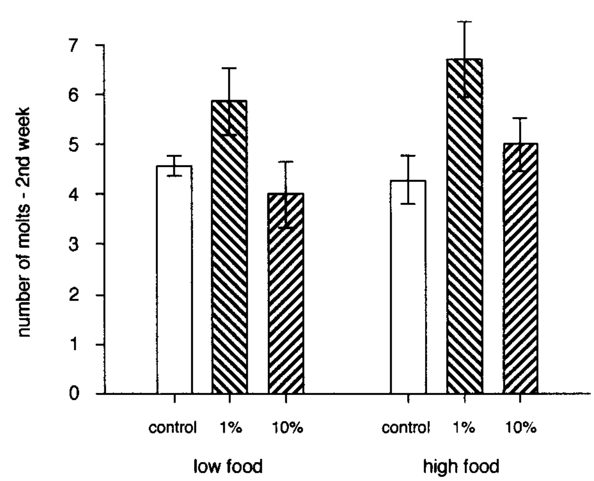

Fig. 5. Number of molts produced during the second week at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent) (±1 SE).

Table 10. Week-2 molt analysis of variance at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent)

Source of variation	SS	*d.f.*	MS	*F*	*p*
Basic ANOVA					
Food level	2.880952	1	2.880952	1.243	0.272
Concentration	31.000000	2	15.500000	6.688	0.003
Food × concentration	3.476190	2	1.738095	0.750	0.480
Error	83.428571	36	2.317460		
ANOVA with contrasts					
Food level	2.880952	1	2.880952	1.243	0.272
Within low food					
Control vs. mean of 1% and 10%	0.595238	1	0.595238	0.257	0.615
1% vs. 10%	12.071429	1	12.071429	5.209	0.028
Within high food					
Control vs. mean of 1% and 10%	11.523810	1	11.523810	4.973	0.032
1% vs. 10%	10.285714	1	10.285714	4.438	0.042
Error	83.428571	36	2.317460		

SS = sum of squares; MS = mean squares.

been great enough to begin to mask the stimulatory effects observed at the lower concentration.

Development. The pulp mill effluent also had a stimulatory effect on the degree of development of *B. tricaudatus* as measured by the relative length of the wing pads. The concentration main effect was significant, and the average effect of the 1% and 10% effluent was a significant increase in relative wing development within the low-food treatment, due primarily to the stimulatory effect of the 1% treatment; the p value was nearly significant at the 0.05 level for the high-food treatment as well (Fig. 6, Table 11).

Because the effluent stimulated increases in the rates of both growth and development, this raises the question of whether effluent-exposed *B. tricaudatus* would emerge at a different size than nonexposed individuals. This question was addressed with analysis of covariance (ANCOVA) for plots of size (body weight) versus degree of development (wing length/spread). Each regression line used in the ANCOVAs showed growth and development from the beginning to the end of the experiment; that is, for each regression, the appropriate treatment group of mayflies from the end of the experiment (control, 1%, or 10%) was pooled with the mayflies preserved at the beginning of the experiment.

Within the low-food treatment, the slopes for the 1% (Fig. 7) and 10% (Fig. 8) treatments were not significantly different from the control slope (ANCOVA; $p > 0.2$), but the 1% and 10% regression lines were significantly elevated above the controls (adjusted means significantly different; ANCOVA; $p \leq 0.05$). Within the high-food treatment, the slopes for the 1% (Fig. 9) and 10% (Fig. 10) treatments were significantly greater than for the controls (ANCOVA; $p < 0.02$). The 1% and 10% treatments did not differ from each other in either slope or elevation for either food treatment (ANCOVA; $p > 0.3$). Thus, if they continued on this growth and development trajectory, the effluent-exposed mayflies would not only be expected to emerge sooner, but

Table 11. Wing pad length/spread analysis of variance at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent)

Source of variation	SS	*d.f.*	MS	*F*	*p*
Basic ANOVA					
Food level	0.000326	1	0.000326	0.061	0.806
Concentration	0.049904	2	0.024952	4.664	0.016
Food × concentration	0.018901	2	0.009451	1.767	0.186
Error	0.181889	34	0.005350		
ANOVA with contrasts					
Food level	0.000326	1	0.000326	0.061	0.806
Within low food					
Control vs. mean of 1% and 10%	0.026106	1	0.026106	4.880	0.034
1% vs. 10%	0.020457	1	0.020457	3.824	0.059
Within high food					
Control vs. mean of 1% and 10%	0.019607	1	0.019607	3.665	0.064
1% vs. 10%	0.002271	1	0.002271	0.425	0.519
Error	0.181889	34	0.005350		

SS = sum of squares; MS = mean squares.

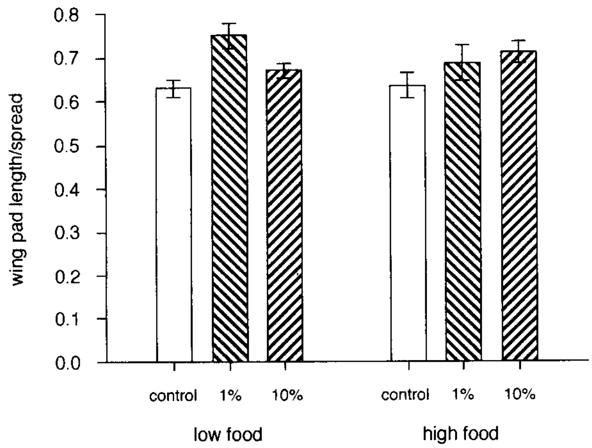

Fig. 6. Wing pad length/spread at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1%, and 10% effluent) (±1 SE).

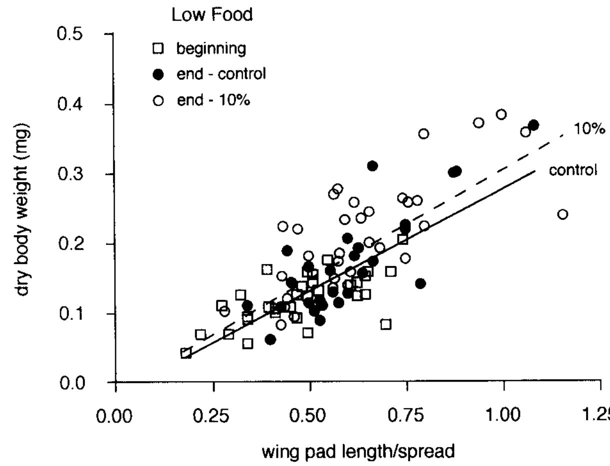

Fig. 8. Body size (dry weight, mg) versus degree of development (wing pad length/spread) for individual mayflies at the beginning (pretreatment) and end (control river water and 10% effluent treatments) of the experiment at the low-food level. Solid line indicates least-squares regression for pooled beginning and end-control mayflies. Dashed line indicates least-squares regression for pooled beginning and end-10% mayflies.

also at a larger size than the nonexposed animals. This conclusion is tentative, however, because of the restricted time frame of the experiment and the degree of data overlap.

DISCUSSION

Levels of contaminants in the effluent

The stimulation of increased growth (resulting in 20 to 50% greater body weights) and development of *B. tricaudatus* occurred in response to what were low levels of contaminants in the effluent. Levels were particularly low for the organic components that were measured: chlorophenolics, resin acids, and PAHs.

During the bleaching process, chlorine and chlorine dioxide react with lignin via different chemical processes [6,23]. Increased chlorine dioxide substitution for chlorine during the bleaching process, such as practiced by the Kamloops mill, can reduce organochlorine discharge five- to tenfold. This was reflected in the low concentrations of chlorophenolics measured during our study. Previous measurements at other mills of the chlorophenolic content of bleached-kraft mill effluent (BKME) that has received biological secondary treatment range from 2 to 51 μg/L for dichlorophenol to

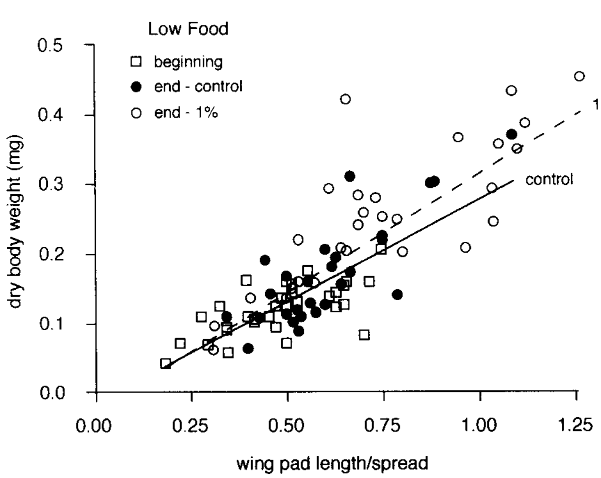

Fig. 7. Body size (dry weight, mg) versus degree of development (wing pad length/spread) for individual mayflies at the beginning (pretreatment) and end (control river water and 1% effluent treatments) of the experiment at the low-food level. Solid line indicates least-squares regression for pooled beginning and end-control mayflies. Dashed line indicates least-squares regression for pooled beginning and end-1% mayflies.

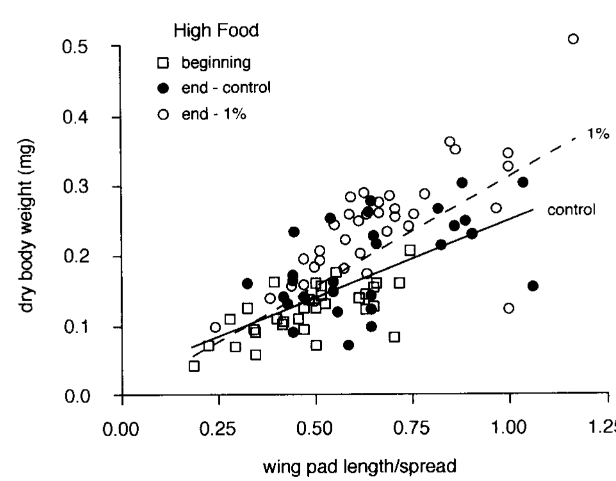

Fig. 9. Body size (dry weight, mg) versus degree of development (wing pad length/spread) for individual mayflies at the beginning (pretreatment) and end (control river water and 1% effluent treatments) of the experiment at the high-food level. Solid line indicates least-squares regression for pooled beginning and end-control mayflies. Dashed line indicates least-squares regression for pooled beginning and end-1% mayflies.

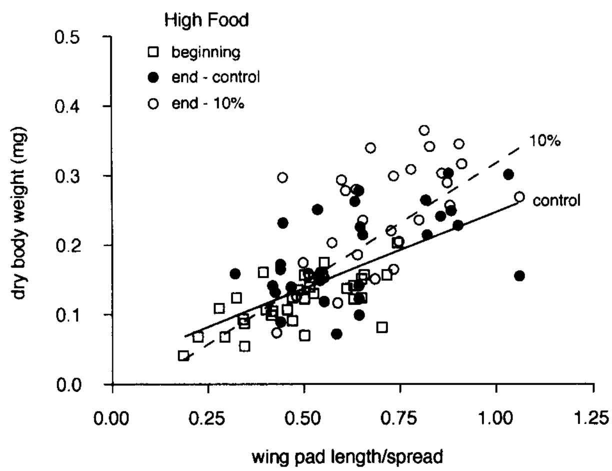

Fig. 10. Body size (dry weight, mg) versus degree of development (wing pad length/spread) for individual mayflies at the beginning (pretreatment) and end (control river water and 10% effluent treatments) of the experiment at the high-food level. Solid line indicates least-squares regression for pooled beginning and end-control mayflies. Dashed line indicates least-squares regression for pooled beginning and end-10% mayflies.

2 to 280 μg/L for trichlorocatechol, with the ranges for the other classes of chlorophenolics falling between these two [1]. In contrast, the greatest concentration that we measured was 1.1 μg/L for dichlorocatechol (Table 2). Data suggest that the potentials for bioconcentration of chlorophenolics within aquatic organisms rank as follows: tetrachloroveratrole > trichloroveratrole > trichloroguaiacol > tetrachloroguaiacol > di/trichlorophenol > the chlorocatechols [1]. Of these, only the chlorocatechols were above detection limits during our study.

Resin acids occur naturally in the wood of trees used by pulp mills [23]. Most of the acute toxicity of pulp mill effluents is due to resin and fatty acids [5], with the fatty acids being rapidly degraded during biological treatment of effluent [1]. Previous measures of resin acid levels in biotreated BKME range from <1 to 150 μg/L for neoabietic acid to <1 to 2,140 μg/L for dehydroabietic acid, with the ranges for the other resin acids falling between [1]. In comparison, the maximum concentration measured during our study was 45 μg/L (Table 3). Dehydroabietic acid, the most persistent of the naturally occurring resin acids that are often present in high concentrations [1], was below detection limits during our study.

Relative to chlorophenolics and resin acids, PAHs have received little attention in studies of pulp mill effluents. The PAHs are often formed by processes involving the incomplete combustion of organic material [21]. They can bioaccumulate and are sometimes acutely toxic at low concentrations (e.g., 12 μg/L for fish exposed to anthracene in the presence of sunlight [26]). Naphthalene has been shown to stimulate increased growth of blue-green algae (*Anabaena flos-aquae*) [27]. Several PAHs were present at low levels in the Kamloops effluent.

Metals can enter pulp mill effluent via the wood, chemicals, and water added during processing [23]. For example, cadmium, copper, mercury, and zinc are accumulated during growth by trees that are exposed to these metals. Aluminum is sometimes added during processing to reduce chemical oxygen demand (COD) and AOX. In contrast to the low levels of organic contaminants, metal concentrations during our study were fairly similar to levels that have been reported for other BKME mills [23].

Response of Baetis tricaudatus *to the experimental effluent treatments*

The majority of previous studies of the direct effects of biotreated BKME on aquatic organisms (mostly fish) have demonstrated either deleterious sublethal effects or no effect at all, with a few notable exceptions discussed below. Acutely lethal effects are uncommon after biotreatment and the consequent degradation or removal of toxic contaminants [6]. In a review of studies using BKME receiving secondary treatment, McLeay [1] noted mostly either deleterious sublethal effects or a lack of any effect on fish blood composition, as well as on the condition of several fish organs including the gill, liver, spleen, gonad, heart, pancreas, kidney, muscle, and brain. In some cases, the incidence of gill parasites increased. Little effect on fish behavior was observed. Several cases of reduced or abnormal growth of larval fish were also described. Growth, development, and reproduction of the cladoceran *Daphnia magna* were not significantly affected.

In contrast to these mostly deleterious or neutral direct effects on fish and invertebrates, biotreated BKME can have indirect growth-enhancing effects due to nutrient addition and increased food availability. For example, biotreated BKME added to experimental streams in the northwestern United States increased nutrient levels, leading to an increase in periphyton production and the macroinvertebrates that fed on the periphyton [4]. Mean weight of rainbow trout in the effluent-addition streams also increased. This pattern of effluent-induced nutrient enrichment resulting in increased food availability to invertebrates and the fish that feed on them has been described in several reviews of pulp mill effluent effects in North America and Europe [1,5,6]. These previously described results agree with our observation of increased algal growth in the 1% and 10% effluent treatments.

In addition to this increase in food availability, however, we measured an increase in the growth (and possibly development) of effluent-treated *B. tricaudatus* relative even to the high-food control animals, which already had access to an ad libitum food supply throughout the experiment. Thus, the stimulatory effect of the effluent on the mayflies involved more than just an increase in food availability due to nutrient-enhanced algal growth. Possible mechanisms for the growth-enhancing effect of the pulp mill effluent include (a) an increase in the nutritive value of the food, (b) an increase in feeding rate due to a palatability enhancer in the effluent, and/or (c) an increase in growth of the mayflies due to direct stimulation by one or more of the compounds within the effluent.

At present, few data are available to evaluate mechanisms (a) or (b). The nutritive content of the food consumed by *B.*

tricaudatus could potentially be increased either in the algal cells themselves, or in the detritus and microbial community coating the algae. Stable isotopic analyses of aquatic biota in the Thompson River suggest that the effluent may be an important source of carbon for insects grazing on the biofilm [28]. Previous studies of the effects of biotreated BKME on the growth of coho salmon have provided indirect evidence that effluent may stimulate an increase in feeding rate [29,30]. These studies measured an increase in growth rate for salmon exposed to the effluent and provided with an excess of food pellets. This growth-enhancement effect was reduced, however, when the food pellets available to the salmon were restricted to 70% of their satiation level, thus preventing the salmon from exhibiting an increased feeding rate. The authors also suggested that the dark effluent could provide a form of "cover," thereby reducing aggressive interactions and enhancing growth.

Several lines of evidence lend support to the hypothesis that one or more of the diverse array of compounds in pulp mill effluent could directly stimulate increased growth or otherwise affect development. For example, several insect hormones, antihormones, and their pharmacobiological mimics are known to occur in plants [31]. Two of the most important, juvabione-type compounds (juvenile hormone) and ecdysone-type compounds (molting hormone), are particularly common in woody plants, including trees used in pulp mills. In particular, juvabione, juvabiol, and dehydrojuvabione can be major components of the neutral fractions of effluents derived from pine, fir, and spruce [32]. The pulpwood used by the mill during our experiment was derived primarily from lodgepole pine (35%), Douglas fir (25%), Engelmann spruce (20%), and cedar (10%); smaller amounts of balsam fir and hemlock were also used (W. Pehowich, personal communication). Juvenile hormone inhibits morphogenesis and differentiation of reproductive organs, allowing continued somatic growth. Molting hormone stimulates development from one molting cycle to the next [31].

A variety of other plant compounds can have antihormonal effects that can interfere with the hormonal control of growth and development in insects. For example, diterpenes related to abietic acid, a resin acid, can have marked antijuvenile hormone effects [31]. During our experiment with *B. tricaudatus*, an increase in both growth and molting occurred following exposure to the effluent, and it is possible that the mayflies were exhibiting a combined response to several compounds within the effluent.

Pulp mill effluent has been shown to disrupt the metabolic capabilities and alter the energy allocation of fish [33]. Specific effects include higher condition factor (weight relative to length) and lower growth rate and commitment to reproduction. Biochemically, these effects are associated with lower steroid levels and increased mixed-function oxidase (MFO) activity. The MFOs are found in virtually all animal phyla, including the arthropods/insects, as well as in plants and aerobic microorganisms [34]. Their primary function is the conversion of lipophilic, potentially toxic compounds to water-soluble metabolites that can be excreted. It appears that the effect of pulp mill effluent on the fish hormonal system is not via direct metabolism of the sex steroids by the MFOs [5]. The specific effluent compound(s) responsible for disrupting metabolism in fish has not yet been identified (K.R. Munkittrick, personal communication).

When contaminants are present at low levels, such as during our experiment, pulp mill effluent may also have direct effects on growth via a phenomenon known as *hormesis*, a term first proposed by Southham and Ehrlich [35] to describe the tendency for low levels of toxic chemicals or other stressors to have a stimulatory effect leading to, for example, increased growth. Hormesis has since been found to be a very general phenomenon observed within many taxa, including bacteria, yeast, protists, algae, higher plants, nematodes, insects, and vertebrates [36]. Observed hormetic responses include increases in growth, development, reproductive success, disease resistance, and longevity following exposure to low concentrations of a variety of compounds that are toxic at higher concentrations, including inorganic salts and acids, heavy metals, and organic compounds. For example, several pesticides have been shown to stimulate increased growth of crickets when applied in doses ranging from 0.1 to 0.001 of the LD100 for the particular insecticide being tested [37]. As yet, the mechanisms responsible for hormesis are not well understood.

One possible mechanism may involve the association between growth stimulation and increased protein turnover that has been observed in aquatic invertebrates such as *Daphnia* following exposure to low levels of contaminants (D.J. Baird, personal communication) [38]. As protein turnover is increased to repair potential damage caused by the contaminant, a secondary consequence may be a shift in resource allocation so that more resources go into structural materials, leading to an increase in growth. The potential trade-off is that, as a result, fewer resources may go into energy storage and future reproductive output.

Thus, care must be taken in interpreting our results with *B. tricaudatus*. The data show that the effluent treatments increased the growth and development of the mayflies during the 2-week course of the experiment. It is possible that a longer exposure time would result in increased final adult size and/or decreased time required to reach maturity, followed by increased reproductive success. Alternatively, the metabolic changes caused by the effluent may ultimately result in a decreased investment in successful reproduction (e.g., fewer or less viable eggs). Further study is needed to determine the generality of these effects within the benthic macroinvertebrate community and to estimate the potential for indirect effects on the periphyton food supply and on the fish that feed upon the benthic invertebrates.

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