Fraser River Action Plan

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Stimulation of Increased Short-Term Growth and Development of the Mayfly *Baetis Tricaudatus* from the Thompson River Basin Following Exposure to Biologically Treated Pulp Mill Effluent

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Environment Canada Environnement Canada

STIMULATION OF INCREASED SHORT-TERM GROWTH AND DEVELOPMENT OF THE MAYFLY *BAETIS TRICAUDATUS* FROM THE THOMPSON RIVER BASIN FOLLOWING EXPOSURE TO BIOLOGICALLY TREATED PULP MILL EFFLUENT

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TABLE OF CONTENTS

TABLES

FIGURES

APPENDICES

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EXECUTIVE SUMMARY

As part of the Fraser River Action Plan, this report summarizes a portion of the ongoing investigations of pulp mill effluent effects on aquatic life in the Fraser River and its tributaries. We report here the results of a toxicity experiment using bleached kraft mill effluent from the Weyerhaeuser pulp mill on the Thompson River in Kamloops, British Columbia. This effluent has the potential for both nutrient enrichment and toxic effects on aquatic communities in the river. These two effects can mask one another, making it difficult to make predictions about the future impacts of pulp mill effluent on the Fraser River system. The experiment was designed as a first attempt to tease out the relative nature of these two effects as determined by the response to the effluent of the mayfly *Baetis tricaudatus*, an abundant benthic macroinvertebrate in the river and its tributaries. *B. tricaudatus* grazes on periphyton and is, therefore, potentially sensitive to both nutrient enrichment (via effects on food availability to the mayfly) and to the direct toxic effects of the effluent.

The food-dependent effects of the pulp mill effluent on *B. tricaudatus* were determined by exposing the mayflies to the effluent for two weeks within arrays of artificial streams. The streams were arranged in a 2x3 factorial design consisting of two periphyton food levels (low, high) at each of three concentrations (control river water, 1% effluent, 10% effluent). The 1% treatment simulated effluent concentrations in the Thompson River at complete mix during periods of low flow. The effect of the effluent on mayfly growth was determined from five end point measures of size (body weight, total body length, thorax length, head width, wing pad length) and from the frequency of molting. The effect of the effluent on the rate of development toward maturity was determined from a measure of wing

length relative to spread. Head width can also be used as an indicator of relative development.

Following biotreatment, the levels of contaminants in the effluent were fairly low. Present in the effluent were several metals, chlorophenolics, resin acids, and polycyclic aromatic hydrocarbons. Nutrients (phosphorus and nitrogen) were at high enough levels to cause increased algal growth within the streams. Phosphorus, in particular, is otherwise a limiting nutrient in the Thompson River system. In general, levels of both contaminants and nutrients were higher in samples collected at the end of the experiment.

Although survival was not affected, the effluent had a significant stimulatory effect on *B. tricaudatus* growth (resulting in 20-50% greater body weights) and development. Furthermore, the initial growth and development trajectories suggest that effluent exposed mayflies may emerge sooner and at a larger size than nonexposed individuals. We stress, however, that the restricted time frame of the experiment makes this conclusion tentative. In addition, the stimulatory effects tended to be greater at the 1% effluent concentration, suggesting that the net stimulatory effect may have been partially offset by a slight inhibitory effect at the 10% effluent concentration.

Interestingly, the stimulatory effects occurred within both the low and high food treatments. Thus, the effluent exposed mayflies grew faster than observed even for the high food control animals, which already had access to more food than they could eat throughout the experiment. This indicates that the stimulatory effect of the effluent on the mayflies involved more than just an increase in food availability due to nutrient enhanced algal growth.

Three possible mechanisms for this growth enhancement effect are that the effluent 1) increased the nutritive value of the food, 2) enhanced the palatability of the periphyton so as to induce an increase in mayfly feeding rate, and/or 3)

directly stimulated increased mayfly growth through the action of one or more compounds within the effluent. 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. Direct stimulation of increased growth or development could potentially be caused by one or a combination of several insect hormones and their analogues that have been found in pulp mill effluent. Growth stimulation may also have been caused by a phenomenon known as hormesis, whereby compounds that are normally toxic at high concentrations can cause increases in growth and development when present at low concentrations, such as observed during our experiments. The mechanisms responsible for hormesis are not yet well understood, but may be related to the increase in protein turnover accompanying the damage-repair response to contaminants.

Care must be taken in interpreting the short-term stimulation in growth and development that we observed for *B. tricaudatus*. Although this stimulation may lead to earlier maturity and/or increased body size and egg production, it could also entail a trade-off against reproductive development and energy storage resulting in decreased reproductive output. Further work is needed to determine the generality of these effects within the benthic invertebrate community. This information on the potential for changes in invertebrate grazer abundance is needed to estimate indirect effects on periphyton biomass or on the abundance of fish that feed upon benthic invertebrates.

1.0 INTRODUCTION

The effluent produced by pulp mills contains a wide variety of compounds which can have differing effects on aquatic organisms and communities in receiving waters (McLeay, 1987). When at high enough 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 effluent usually contains fairly 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 (Hansson, 1987; Feder and Pearson, 1988; Hall et al., 1991). 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 (Owens, 1991). Setting regulatory guidelines can be difficult because nutrient enhancement effects can sometimes mask the toxic effects of pulp mill effluents and little data is yet available to disentangle these two effects (Solomon et al., 1993).

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. Upstream of Kamloops, the Thompson River system is phosphorus limited (Bothwell et al., 1992). 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 Lower Thompson River. Algal biomass in the river at present appears to be lower than in the mid 1970's, but still remains a concern (Bothwell

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The mayfly *Baetis tricaudatus* 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 America (Hubbard and Peters, 1978; Robinson et al., 1992). *B. tricaudatus* grazes on periphyton (Scrimgeour et al., 1991), 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 response of *B. tricaudatus* to different concentrations of pulp mill effluent under two different feeding regimes.

2.0 METHODS

2.1 Experimental Design

The food-dependent effects of pulp mill effluent on the mayfly *Baetis tricaudatus* were determined by exposing the mayflies for two weeks to three effluent concentrations and two food levels within artificial streams. The study was conducted at the National Hydrology Research Institute's outdoor experimental stream enclosure located on the Lower Thompson River beside the Weyerhaeuser bleached kraft pulp mill in Kamloops, British Columbia. The enclosure contained a series of 250 L 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 non-control reservoir using Masterflex peristaltic pumps (Cole-Parmer, Niles, Illinois) drawing from an 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.

The experiment was arranged in a 2x3 factorial design: the mayflies were offered two food levels (low, high) at each of three concentrations (control river water, 1% effluent, 10% effluent). The 1% treatment simulated effluent concentrations in the Thompson River at complete mix during periods of low flow (November-March; Bothwell, 1992; Nordin and Holmes, 1992). The 10% treatment simulated effluent concentrations closer to the mill before complete mix.

The experimental test chambers were circular Plexiglas artificial streams, previously described by Walde and Davies (1984) (diameter = 8.8 cm, stream bottom area = 50 cm^2). 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 2.4x2.4x0.5 cm roughened ceramic blocks were placed onto the bottom of each stream. The mayflies spent most of their time amongst these blocks. Inflow to the water jets was adjusted so that the current velocity at the level of the stream bottom's substratum (within 1.5 cm) was approximately 6 cm/s (measured with a low velocity propeller probe; Nixon Instrumentation, Cheltenham, United Kingdom). This velocity is typical of the intrasubstratum velocity among the stones where the mayflies are found (Culp et al., 1983), 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 (Kosinski, 1989).

Food was provided during the experiment by algae cultured on ceramic blocks identical to those used for the stream substrata. The algae was grown beforehand on the blocks within 2.0x0.19 m Plexiglas flumes through which river water was pumped at approximately 20 cm/s [see Bothwell (1992) for further information on the use of these flumes for growing algae]. The high velocity ensured that the algae 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 14N:14P:14K) was placed in a mixing chamber at the head of each flume to elevate the concentration of phosphorus so as to increase algal growth rate, yielding a dense algal growth on the blocks after approximately 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 (Bothwell et al., 1992).

The low versus high food treatment levels were set by how long the mayflies were allowed to feed on the algae-covered blocks. At the beginning of the experiment, two algae-covered blocks were transplanted from the flumes into each stream after removing two corresponding algae-bare blocks. In the high food treatments, the blocks were left in place for one week, while in the low food treatments, the two food blocks were removed after 3 days and replaced again with bare blocks for the remainder of the week. During this latter part of the week, a small amount of algae 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 algae-covered blocks were taken out of the high food treatments and two fresh algae-covered blocks from the flumes were placed into each stream for both food treatments. Again, the algae-covered blocks in the low food treatment were replaced with bare ones after 3 days. This procedure was employed to vary 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 algae on the blocks (since both low and high food treatments received algae cultured under identical conditions and for the same length of time).

Several hundred *B. tricaudatus* were collected prior to the experiment from the Bonaparte River, a tributary of the lower Thompson River, near the town of Cache Creek. Thus, although the mayflies were sampled close enough to the Lower Thompson River to be part of the same interbreeding population, they 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 within the compound. On the first day of the experiment, the *B. tricaudatus* were randomly allocated to one of the 42 streams until each stream contained ten animals. Fifty additional mayflies were preserved in 10% formalin and then transferred to 80% ethanol for later determination of animal size and dimensions at the beginning of the experiment (Appendix 1).

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 the seston from the streams. In addition, water samples were taken at the beginning and end of the experiment and sent to Zenon Environmental Laboratories (Burnaby, British Columbia) for determination of the levels of several contaminants and other water quality variables in the 1) river

water, 2) full strength effluent, and 3) river water/effluent mixture from the 10% effluent treatment. These measured variables included metals, chlorophenolics, resin acids, polycyclic aromatic hydrocarbons, and algal nutrients (phosphorus, nitrogen). Ceramic food blocks with algae in earlier (3 weeks) and later (5 weeks) successional stages were also collected during the experiment. These were either 1) preserved in Lugol's solution for later determination of the relative abundances of the dominant species of algae growing on the blocks or 2) frozen for determination of the amount of periphyton on the blocks (as measured by ash-free dry mass and chlorophyll *a* content per cm^2 of block surface area).

2.2 Biological End Points and Statistical Analysis

Several survival, growth, and development parameters were measured during and at the end of the experiment to determine the response of the *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, the surviving mayflies were counted and preserved in 10% formalin and later transferred to 80% ethanol before the 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 (Sokal and Rohlf, 1981). The 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 from five measures of the size of the preserved animals from the end of the experiment. Total body length was measured as the distance from the anterior edge of the head to the posterior edge of the last abdominal segment, exclusive of the cerci. Thorax

length was measured along the medial dorsal line of the thorax. Head width was determined at the widest part of the head capsule as viewed dorsally. 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 viewed dorsally. After the length measurements were taken, all but two mayflies from each replicate stream were dried to a constant weight for dry body weight measurements; the latter two animals were reserved for future reference. The number of molts during the first and second weeks of the experiment provided a sixth measure of the effluent effects on growth.

Head width has also been used as a measure of the relative degree of development of aquatic insect larvae as they mature toward the final adult instar (Baker, 1986). Another measure of the degree of development is the relative length of the wing pad (Clifford, 1970; Clifford et al., 1979). Relative wing pad length was measured as the ratio of wing length to wing spread. 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.

The results for each of the end points were analyzed in a 2x3 factorial analysis of variance (ANOVA; two food levels by three effluent concentrations) with streams as replicates using SYSTAT (Macintosh version 5.2, Evanston, Illinois; Wilkinson et al., 1992). Each ANOVA was then broken down into single degree of freedom contrast statements to compare concentration effects within each food level. 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. The relationship of size (body weight) to the rate of development (relative wing length) was examined with analysis of covariance (ANCOVA).

3.0 RESULTS

3.1 Contaminants and Other Water Quality Variables

For the most part, levels of contaminants in the 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 (CCREM, 1987; Nagpal and Pommen, 1994). These are general guidelines based on past research; they recommend contaminant levels for rivers and other water bodies that are low enough to protect the health of freshwater organisms. For barium, titanium, and zinc, concentrations in the full strength effluent were approximately at or below the guidelines. For aluminum, chromium, copper, and iron, concentrations in the full strength effluent were higher than the guidelines; dilution to 10% reduced these concentrations to guideline levels.

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1. Metals present in the pulp mill effluent experimental treatments

Table 1. Metals present in the control river water, full strength effluent, and 10% effluent mixture at the beginning and end of the experiment (concentration in mg/L). Missing values indicate concentrations that were below detection limits. WQG = Canadian Federal (or British Columbia Provincial - barium, titanium) Water Quality Guidelines (mg/L; CCREM, 1987; Nagpal and Pommen, 1994).

	control		effluent		10%		WQG
	begin	end	begin	end	begin	end	
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			0.012	0.013		0.003	0.002
copper			0.003	0.011		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			0.003	0.007			
zinc	0.01	0.02	0.03	0.09	0.01		0.03

On average, metal concentration in the 10% effluent treatment was approximately equal to the control river water concentration plus 1/10 the concentration in the full strength effluent. This indicates that there was not a buildup of metals in the mixing reservoir water supply to the streams over the course of the experiment. Thus, the 10% mixing ratio was maintained through the experiment. The concentrations of most metals in the effluent were higher in samples taken at the end of the experiment. Metal concentrations in the river water also followed this trend, but to a lesser degree.

Three classes of chlorophenolics were present at low levels in the full strength effluent or 10% mixture: guaiacols, catechols, and vanillins (Table 2; chlorophenolics, resin acids, and polycyclic aromatic hydrocarbons were all below detection limits in the control river water, 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 LC_{50}'s for salmonids are listed for the chlorophenols, guaiacols, and catechols; these values suggest 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 in the full strength effluent were roughly at or below guideline levels. Again, the levels of chlorophenolics in the effluent were higher at the end of the experiment. AOX (adsorbable organic halogens), which gives a general indication of the overall level of chloronated organic compounds present, was also higher at the end of the experiment.

The total concentration of resin acids in the full strength effluent was close to 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, although the composition changed as well.

Table 2. Chlorophenolics and AOX present in the full strength effluent and 10% effluent mixture at the beginning and end of the experiment (concentration in µg/L). Missing values indicate concentrations that were below detection limits. WQG = Canadian Federal Water Quality Guidelines (µg/L; CCREM, 1987). LC_{50} (µg/L) for 96 hr toxicity tests with salmonids (McLeay, 1987).

	effluent		10%		WQG	LC_{50}
	begin	end	begin	end		
monochlorophenols					7.0	
dichlorophenols					0.2	~2800
trichlorophenols					18.0	~1500
tetrachlorophenols					1.0	
pentachlorophenol					0.5	
monochloroguaiacols	0.2	0.43		0.1		
dichloroguaiacols		0.17				~2300
trichloroguaiacols						~850
tetrachloroguaiacols						~950
monochlorocatechols						
dichlorocatechols		1.1				~750
trichlorocatechols		0.1				~1300
tetrachlorocatechols		0.3				~950
monochlorovanillins				0.2		
AOX	4400	5700	500	800		

Table 3. Resin acids present in the full strength effluent and 10% effluent mixture at the beginning and end of the experiment (concentration in µg/L). Missing values indicate concentrations that were below detection limits. WQG = British Columbia Provincial Water Quality Guidelines (µg/L; Nagpal and Pommen, 1994). LC_{50} (µg/L) for 96 hr toxicity tests with salmonids (McLeay, 1987).

	effluent		10%		WQG	LC_{50}
	begin	end	begin	end		
abietic acid						~1100
dehydroabietic acid					12	~1300
isopimaric acid		11	12	2		~700
levopimaric acid	7					~850
neoabietic acid						~650
pimaric acid		45		2		~950
sadaracopimaric acid	18					~350
Total resin acids	25	56	12	4	45	

Although several polycyclic aromatic hydrocarbons (PAH) were present (Table 4), concentrations were near detection limits (0.01 µg/L) in the full strength effluent and, therefore, were below detection limits in the 10% effluent treatments. The concentrations in the full strength effluent were at or below British Columbia guideline levels. In contrast to the other contaminants, more PAH's were present in samples collected at the beginning of the experiment.

Since phosphorus is a limiting nutrient in the Thompson River (Bothwell et al., 1992), the 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. As for the above contaminants, levels of phosphorus and nitrogen were higher at the end of the experiment (Table 5). The concentration of ammonia and ammonium in the full strength effluent was approximately at the federal guideline level.

The 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. 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 (McCubbin and Folke, 1993; Solomon et al., 1993).

Table 4. Polycyclic aromatic hydrocarbons present in the full strength effluent and 10% effluent mixture at the beginning and end of the experiment (concentration in µg/L). Missing values indicate concentrations that were below detection limits. WQG = British Columbia Provincial Water Quality Guidelines (µg/L; Nagpal and Pommen, 1994).

	effluent		10%		WQG
	begin	end	begin	end	
naphthalene	0.06				1.0
acenaphthylene					
acenaphthene					6.0
fluorene	0.01	0.01			12.0
phenanthrene	0.06	0.05			0.3
anthracene	0.01	0.01			0.1
fluoranthene	0.05				0.2
pyrene	0.05				0.02
benz(a)anthracene	0.01				0.1
chrysene	0.01				
benzo(b+k)fluoranthene					
benzo(a)pyrene					0.01
indeno(1,2,3-cd)pyrene					
dibenz(a,h)anthracene					
benzo(g,h,i)perylene					

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 (concentration in µg/L). Missing values indicate concentrations that were below detection limits. WQG = Canadian Federal Water Quality Guideline (µg/L; CCREM, 1987). SRP = soluble reactive phosphorus.

	control		effluent		10%		WQG
	begin	end	begin	end	begin	end	
P-SRP	6		1051	1180	126	155	
P-dissolved	4	6	1330	1520	151	230	
P-total	7	9	1430	2240	172	249	
N-organic	110	110	1630	5300	400	920	
ammonia-total	13		2300	2160	262	142	~2000
N-kjeldahl	123	110	3930	7460	662	1062	
NO3+NO2	30	70	130	50	40	170	
N-total	153	180	4060	7510	702	1232	

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 (concentration in mg/L, except where otherwise noted). Missing values indicate concentrations that were below detection limits. BOD = biochemical oxygen demand. TAC = total absorbance color. nm = not measured.

	control		effluent		10%	
	begin	end	begin	end	begin	end
alkalinity	37.8	40.5	176	214	55.8	63.4
BOD			11			
color (TAC)	4	4	524	587	71	89
conductance (µS/cm)	97	103	1740	2070	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		0.04				
chloride	0.5	0.6	174	219	21.2	20.8
silicon	2.5	3.3	3.9	3.7	2.7	2.7
sulphur	2.3	2.6	138	159	18.4	21.9
chlorate	nm	0.57	0.13	0.16	0.43	0.38

3.2 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) and the blue-green alga *Oscillatoria prolifica* (order Oscillatoriales). The pennate diatoms *Synedra ulna* and *Tabellaria fenestrata* were also abundant at the 3 week stage. This species composition is similar to that reported in previous studies on the Thompson River (Bothwell, 1988).

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 ±1SE; 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 Lower Thompson River at Savona and Walhachin downstream of the pulp mill and sewage outfall (Nordin and Holmes, 1992). While the blocks were in the streams, these amounts far exceeded what the mayflies could consume (personal observation; Scrimgeour et al., 1991).

3.3 Response of *Baetis tricaudatus* to the Experimental Effluent Treatments

3.3.1 Survival

The pulp mill effluent had no significant effect on *B. tricaudatus* survival during the course of the experiment (Figure 1; Table 7). The basic ANOVA (first part of Table 7) shows that the main effects (food level and concentration) and interaction term were not significant at $P \leq 0.05$ (the main effect means for all of

Figure 1. 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).

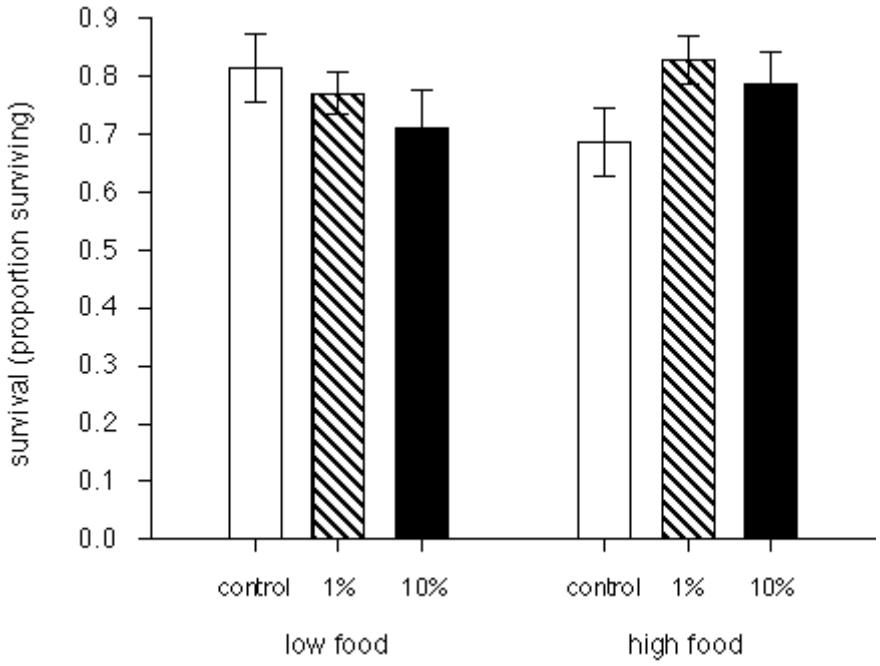

survival (proportion surviving)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
control
1%
10%
control
1%
10%
low food
high food

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). Data arcsine square root transformed before analysis.

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.000009	1	0.000009	<0.001	0.987
Concentration	0.024571	2	0.012286	0.366	0.696
Food x Concentration	0.132495	2	0.066247	1.973	0.154
Error	1.208910	36	0.033581		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
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		

the end points are tabulated in Appendix 2). The ANOVA with contrasts table (second part of Table 7) breaks the basic ANOVA down into single degree of freedom contrasts. This statistical technique tests 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.

3.3.2 Growth

In general, the pulp mill effluent had a stimulatory effect on *B. tricaudatus* growth. This effect can be seen most readily for the standard measure of overall size, dry body weight, which was 20-50% greater for mayflies exposed to the effluent (Fig. 2; 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. The food level and interaction effects were not significant. Although the main effect means for the high food treatment were greater than those for the low food treatment for four of the five measures of size (Appendix 2), the differences were not significant (Tables 8, 10-13). This suggests that the difference between the food levels was not great enough to produce a significant main food effect during the two week course of the experiment.

Interestingly, 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

Figure 2. 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).

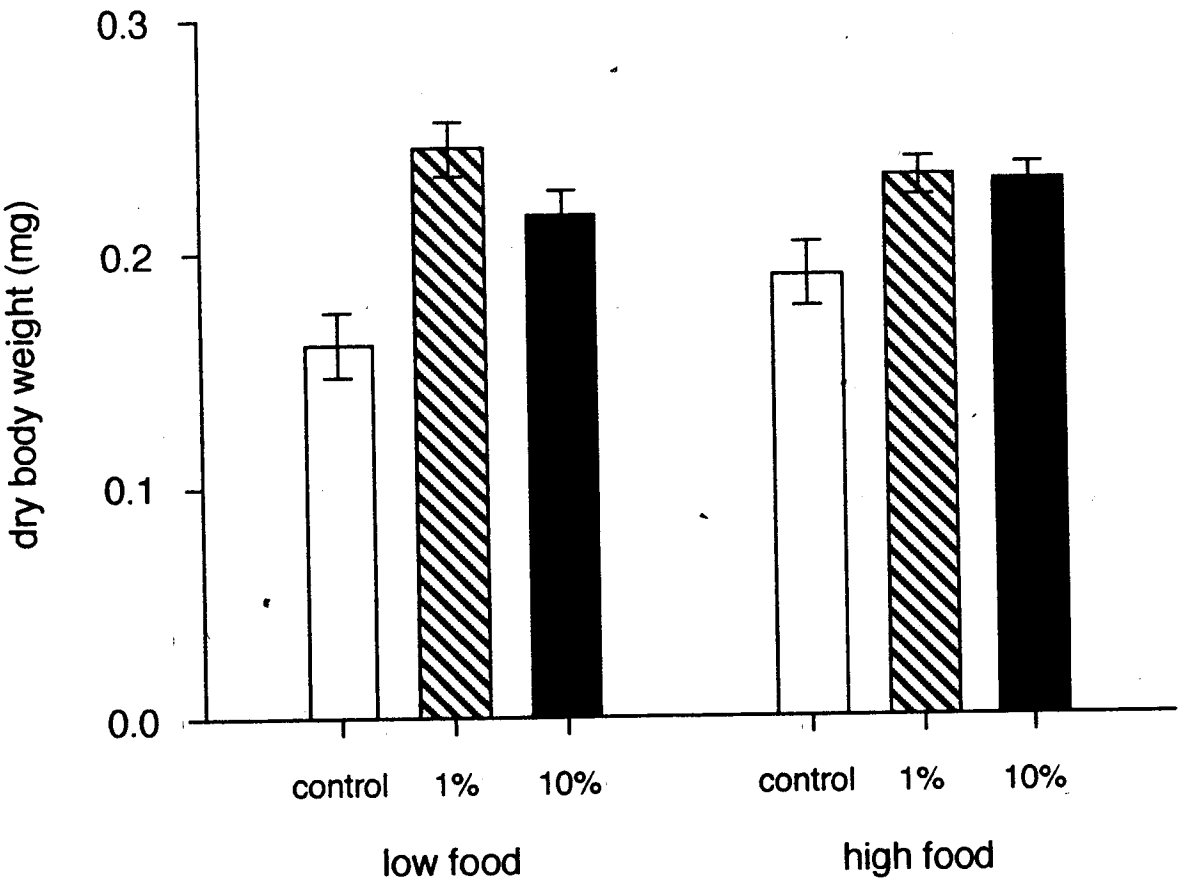

0.3
0.2
0.1
0.0
dry body weight (mg)
control
1%
10%
control
1%
10%
low food
high food

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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.001094	1	0.001094	1.252	0.271
Concentration	0.030211	2	0.015106	17.286	<0.001
Food x Concentration	0.003115	2	0.001557	1.782	0.183
Error	0.031459	36	0.000874		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
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		

throughout the experiment. No significant differences between the 1% and 10% treatments were found within food levels .

The effects of the effluent were not as pronounced for the linear measures of size, which are expected to increase in proportion to the cube root of weight for an isometrically growing animal. The ANOVA results for the linear measures were, however, similar to the results for body weight. As for weight, 1% and 10% effluent induced a significant increase in thorax length within both the low and high food treatments, while no significant difference was observed between the 1% and 10% effluent effects (Tables 9 and 10). The effluent affected total body length and wing pad length in a similar manner (Tables 9, 11-12). Concentration had a significant main effect and, as for weight and thorax length, the averaged effect of the 1% and 10% effluent treatments was to cause a significant increase in total body and wing lengths, relative to the controls, within both the low and high food treatments. In addition, 1% effluent induced greater total body and wing length increases than 10% effluent within the low food treatment; this was reflected in the significant main effect interaction terms. Several of the measures of growth and development showed this general tendency for the 1% treatment to account for most of the stimulatory effects of the effluent. This suggests that, at a 10% concentration, the inhibitory effects of the effluent may have been great enough to begin to mask the stimulatory effects observed at the lower concentration.

Head width followed this same trend, although it showed a slightly different response pattern (Tables 9 and 13). As with the other measures, there was a significant concentration main effect. For head width, however, a significant stimulation of increased growth by 1% and 10% effluent was observed only within the low food treatment.

Table 9. Body size and development measures (mm; ±1 SE) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

	Low Food			High Food		
	control	1%	10%	control	1%	10%
thorax length	0.927 ±0.0311	1.012 ±0.0122	0.973 ±0.0266	0.931 ±0.0255	1.006 ±0.0336	1.061 ±0.0232
total body length	3.395 ±0.1199	3.807 ±0.0898	3.463 ±0.0619	3.290 ±0.0958	3.673 ±0.1042	3.771 ±0.0759
wing pad length	0.206 ±0.0067	0.253 ±0.0122	0.220 ±0.0075	0.206 ±0.0088	0.227 ±0.0116	0.242 ±0.0088
head width	0.665 ±0.0091	0.724 ±0.0123	0.688 ±0.0059	0.684 ±0.0221	0.705 ±0.0156	0.703 ±0.0087
wing pad spread	0.328 ±0.0054	0.325 ±0.0036	0.328 ±0.0050	0.326 ±0.0042	0.331 ±0.0074	0.340 ±0.0039
wing length/spread	0.630 ±0.0207	0.750 ±0.0305	0.670 ±0.0178	0.635 ±0.0295	0.687 ±0.0397	0.712 ±0.0245

Table 10. Thorax length 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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.008715	1	0.008715	1.804	0.188
Concentration	0.066541	2	0.033271	6.887	0.003
Food x Concentration	0.018710	2	0.009355	1.936	0.159
Error	0.173918	36	0.004831		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
Food Level	0.008715	1	0.008715	1.804	0.188
Within Low Food					
Control vs. Mean of 1% and 10%	0.020277	1	0.020277	4.197	0.048
1% vs. 10%	0.005496	1	0.005496	1.138	0.293
Within High Food					
Control vs. Mean of 1% and 10%	0.048975	1	0.048975	10.138	0.003
1% vs. 10%	0.010502	1	0.010502	2.174	0.149
Error	0.173918	36	0.004831		

Table 11. Total body length 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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.005591	1	0.005591	0.097	0.757
Concentration	1.103269	2	0.551635	9.584	<0.001
Food x Concentration	0.422349	2	0.211175	3.669	0.036
Error	2.014447	35	0.057556		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
Food Level	0.005591	1	0.005591	0.097	0.757
Within Low Food					
Control vs. Mean of 1% and 10%	0.242031	1	0.242031	4.205	0.048
1% vs. 10%	0.412825	1	0.412825	7.173	0.011
Within High Food					
Control vs. Mean of 1% and 10%	0.870192	1	0.870192	15.119	<0.001
1% vs. 10%	0.033632	1	0.033632	0.584	0.450
Error	2.014447	35	0.057556		

Table 12. Wing pad length 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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.000016	1	0.000016	0.025	0.876
Concentration	0.007849	2	0.003924	6.198	0.005
Food x Concentration	0.004075	2	0.002037	3.218	0.052
Error	0.022160	35	0.000633		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
Food Level	0.000016	1	0.000016	0.025	0.876
Within Low Food					
Control vs. Mean of 1% and 10%	0.003728	1	0.003728	5.888	0.021
1% vs. 10%	0.003752	1	0.003752	5.926	0.020
Within High Food					
Control vs. Mean of 1% and 10%	0.003600	1	0.003600	5.687	0.023
1% vs. 10%	0.000836	1	0.000836	1.321	0.258
Error	0.022160	35	0.000633		

Table 13. Head width 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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.000231	1	0.000231	0.184	0.671
Concentration	0.010688	2	0.005344	4.240	0.022
Food x Concentration	0.003021	2	0.001510	1.198	0.314
Error	0.044113	35	0.001260		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
Food Level	0.000231	1	0.000231	0.184	0.671
Within Low Food					
Control vs. Mean of 1% and 10%	0.007194	1	0.007194	5.708	0.022
1% vs. 10%	0.004392	1	0.004392	3.485	0.070
Within High Food					
Control vs. Mean of 1% and 10%	0.001830	1	0.001830	1.452	0.236
1% vs. 10%	0.000010	1	0.000010	0.008	0.929
Error	0.044113	35	0.001260		

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. 3; Table 14). During the second week, concentration had a significant main effect and the averaged effect of the 1% and 10% treatments was to cause a significant increase in molting, relative to the controls, but only within the high food treatment (Fig. 4; Table 15). In addition, 1% effluent caused more frequent molting than 10% effluent within both the low and high food treatments.

3.3.3 Development

The pulp mill effluent also had a stimulatory effect on the relative development of *B. tricaudatus* as measured both by head width and by the relative length of the wing pads. Taken by itself, wing spread was uniform amongst the different treatments (no significant differences; Tables 9 and 16). This occurred because, as the mayflies grew and developed and the body became wider, the left and right wing pads grew toward one another, thus maintaining the same spread between the wings. But the ratio of wing length to spread (relative length) showed the same response pattern as observed for head width. The concentration main effect was significant and the averaged effect of the 1% and 10% effluent was to induce 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 (Tables 9 and 17).

Since 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

Figure 3. 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).

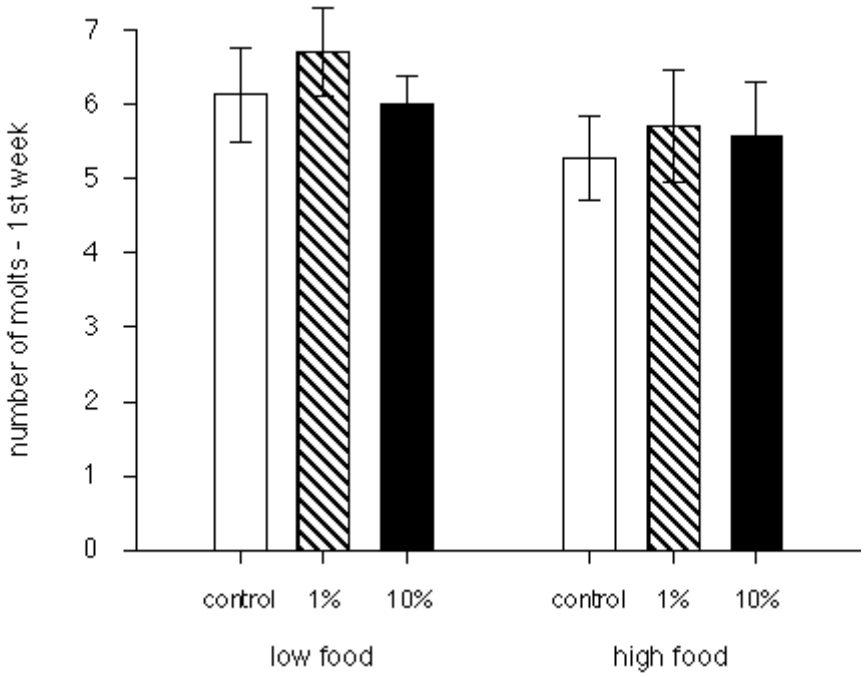

number of molts - 1st week
0
1
2
3
4
5
6
7
control
1%
10%
control
1%
10%
low food
high food

Table 14. Week one molts analysis of variance at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	6.095238	1	6.095238	2.220	0.145
Concentration	2.047619	2	1.023810	0.373	0.691
Food x Concentration	0.619048	2	0.309524	0.113	0.894
Error	98.857143	36	2.746032		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
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		

Figure 4. 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).

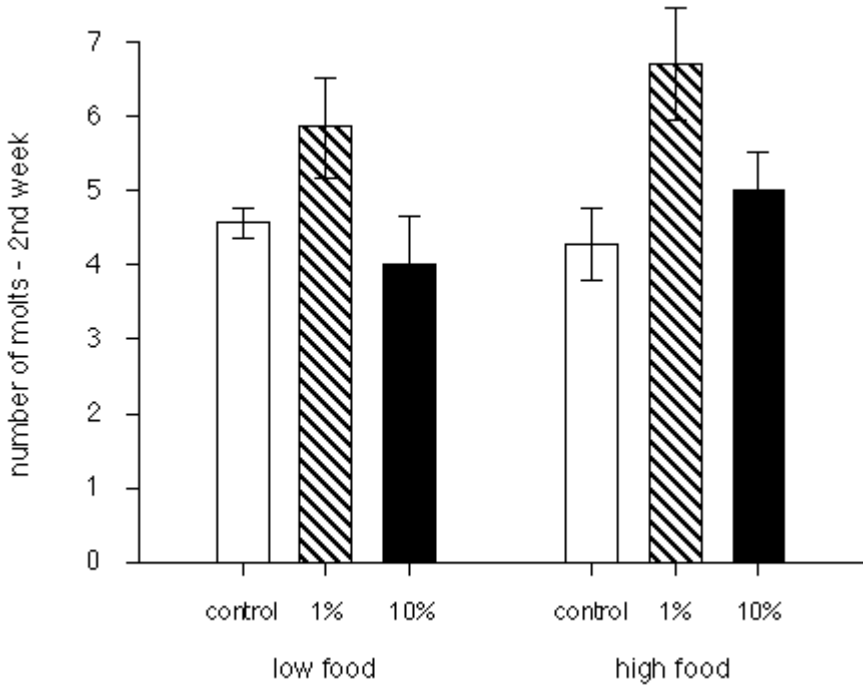

number of molts - 2nd week
0
1
2
3
4
5
6
7
control
1%
10%
control
1%
10%
low food
high food

Table 15. Week two molts analysis of variance at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	2.880952	1	2.880952	1.243	0.272
Concentration	31.000000	2	15.500000	6.688	0.003
Food x Concentration	3.476190	2	1.738095	0.750	0.480
Error	83.428571	36	2.317460		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
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		

Table 16. Wing pad 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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.000305	1	0.000305	1.730	0.197
Concentration	0.000367	2	0.000183	1.038	0.365
Food x Concentration	0.000325	2	0.000163	0.921	0.408
Error	0.006003	34	0.000177		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
Food Level	0.000305	1	0.000305	1.730	0.197
Within Low Food					
Control vs. Mean of 1% and 10%	0.000006	1	0.000006	0.034	0.855
1% vs. 10%	0.000020	1	0.000020	0.113	0.739
Within High Food					
Control vs. Mean of 1% and 10%	0.000438	1	0.000438	2.480	0.125
1% vs. 10%	0.000251	1	0.000251	1.422	0.241
Error	0.006003	34	0.000177		

Table 17. 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).

Basic ANOVA

Source of Variation	SS	df	MS	F	P
Food Level	0.000326	1	0.000326	0.061	0.806
Concentration	0.049904	2	0.024952	4.664	0.016
Food x Concentration	0.018901	2	0.009451	1.767	0.186
Error	0.181889	34	0.005350		

ANOVA with Contrasts

Source of Variation	SS	df	MS	F	P
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		

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 ANCOVA's indicated 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. 5) and 10% (Fig. 6) 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 (ANCOVA; P_0.05). Within the high food treatment, the slopes for the 1% (Fig. 7) and 10% (Fig. 8) 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 emerge sooner, but also at a larger size than the nonexposed animals. This conclusion is tentative, however, due to the restricted time frame of the experiment and the degree of data overlap.

Figure 5. 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.

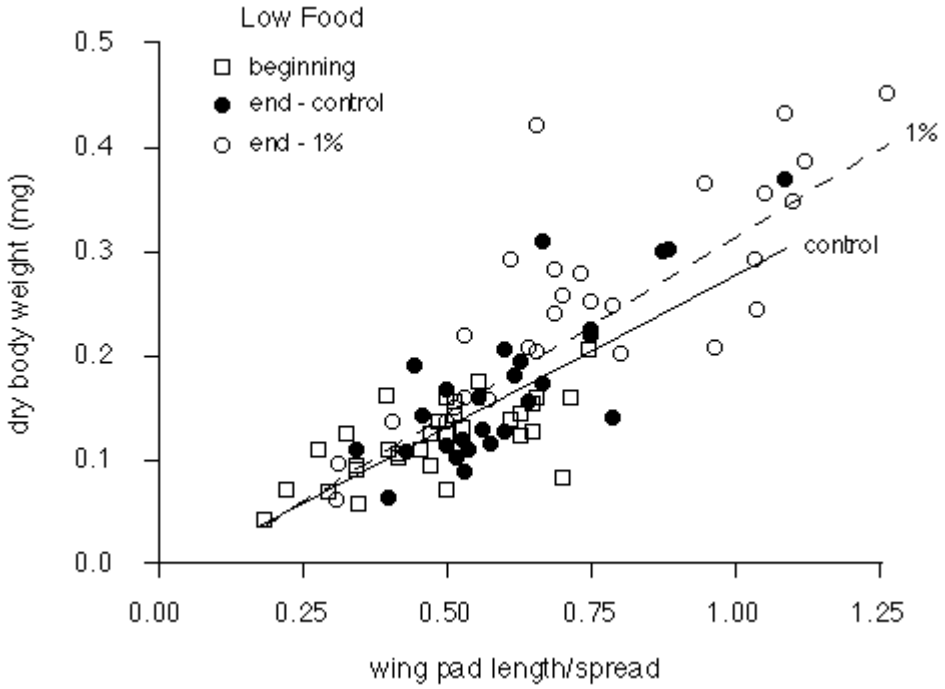

Low Food
beginning
end - control
end - 1%
1%
control
dry body weight (mg)
wing pad length/spread
0.0
0.1
0.2
0.3
0.4
0.5
0.00
0.25
0.50
0.75
1.00
1.25

Figure 6. 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.

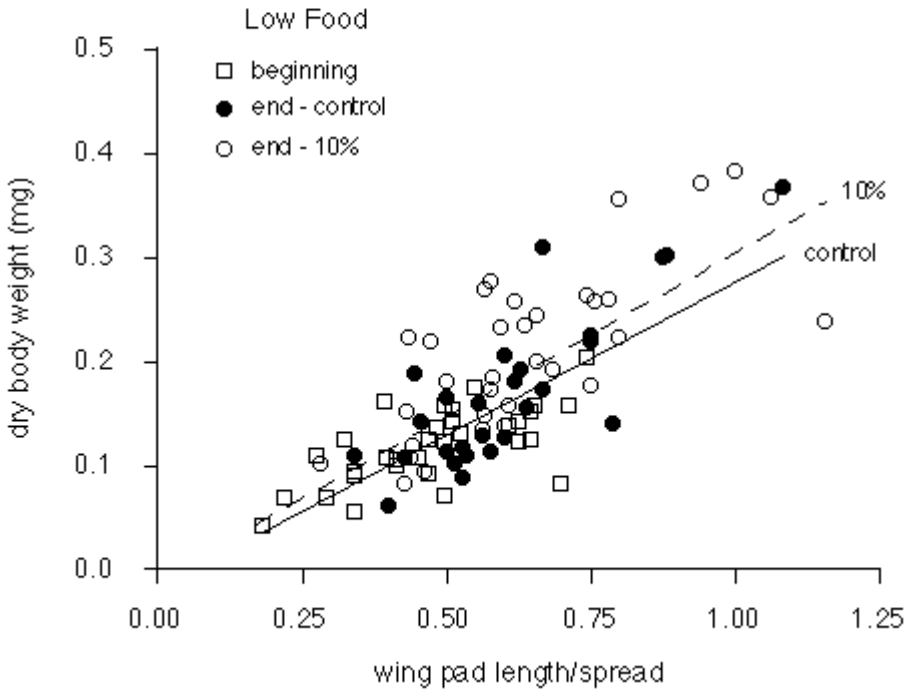

Low Food
beginning
end - control
end - 10%
10%
control
dry body weight (mg)
wing pad length/spread
0.0
0.1
0.2
0.3
0.4
0.5
0.00
0.25
0.50
0.75
1.00
1.25

Figure 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 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.

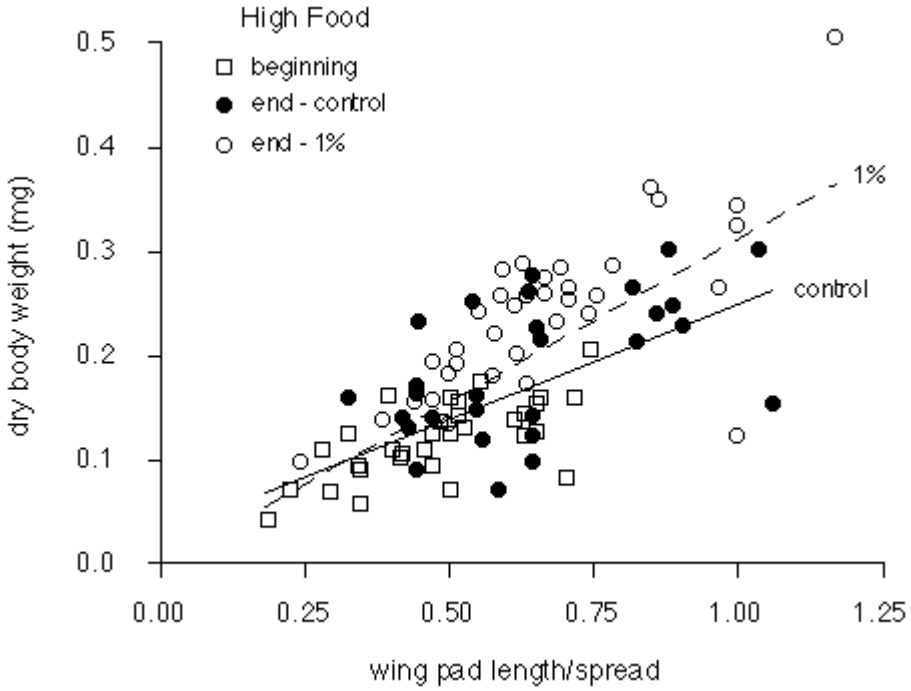
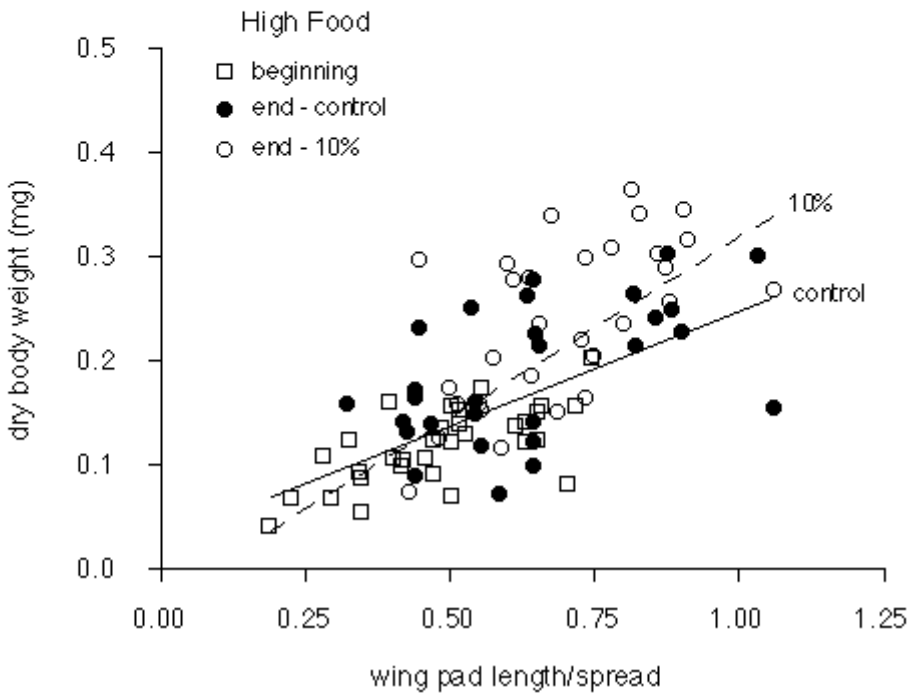

High Food
beginning
end - control
end - 1%
1%
control
dry body weight (mg)
wing pad length/spread
0.0
0.1
0.2
0.3
0.4
0.5
0.00
0.25
0.50
0.75
1.00
1.25

Figure 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 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.


High Food
beginning
end - control
end - 10%
10%
control
dry body weight (mg)
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.25
0.50
0.75
1.00
1.25
wing pad length/spread

4.0 DISCUSSION

4.1 Levels of Contaminants in the Effluent

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

During the bleaching process, chlorine and chlorine dioxide react with lignin via different chemical processes (McCubbin and Folke, 1993; Solomon et al., 1993). Increased chlorine dioxide substitution for chlorine during the bleaching process, such as practiced by the Kamloops mill, can reduce organochlorine discharge five to ten-fold. This was reflected in the low concentrations of chlorophenolics that were 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 2-51 µg/L for dichlorophenol to 2-280 µg/L for trichlorocatechol, with the ranges for the other classes of chlorophenolics falling between these two (McLeay, 1987). In contrast, the greatest concentration that we measured was 1.1 µg/L for dichlorocatechol (Table 2). Data suggests that the potentials for bioconcentration

of chlorophenolics within aquatic organisms rank as follows: tetrachloroveratrole > trichloroveratrole > trichloroguaiacol > tetrachloroguaiacol > di/trichlorophenol > the chlorocatechols (McLeay, 1987). 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 (McCubbin and Folke, 1993). Most of the acute toxicity of pulp mill effluents is due to resin and fatty acids (Owens, 1991), with the fatty acids being rapidly degraded during biological treatment of effluent (McLeay, 1987). Previous measures of resin acid levels in biotreated BKME range from <1-150 µg/L for neoabietic acid to <1-2140 µg/L for dehydroabietic acid, with the ranges for the other resin acids falling between (McLeay, 1987). In comparison, the maximum concentration measured during our study was 45 µg/L (Table 3). Dehydroabietic acid is the most persistent of the naturally occurring resin acids that are often present in high concentrations (McLeay, 1987); it was below detection limits during our study.

Relative to chlorophenolics and resin acids, PAH's have received little attention in studies of pulp mill effluents. PAH's are often formed by processes involving the incomplete combustion of organic material (CCREM, 1987). They can bioaccumulate and are sometimes acutely toxic at quite low concentrations (e.g., 12 µg/L for fish exposed to anthracene in the presence of sunlight; Bowling et al., 1983). Naphthalene has been shown to stimulate increased growth of blue-green algae (*Anabaena flos-aquae*; Bastian and Toetz, 1982). Several PAH's were present at low levels in the Kamloops effluent.

Metals can enter pulp mill effluent via the wood, chemicals, and water added during processing (McCubbin and Folke, 1993). 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 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 (McCubbin and Folke, 1993).

4.2 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 (Solomon et al., 1993). In a review of studies using BKME receiving secondary treatment, McLeay (1987) 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. The growth, development, and reproduction of the cladoceran *Daphnia magna* was 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 (Hall et al., 1991). 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 (McLeay, 1987; Owens, 1991; Solomon et al., 1993). 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 effluent include 1) an increase in the nutritive value of the food, 2) an increase in feeding rate due to a palatability enhancer in the effluent, and/or 3) an increase in growth of the mayflies due to direct stimulation by one or more of the compounds within the effluent.

At present, very little data is available to evaluate mechanisms 1 or 2. 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 (Wassenaar and Culp, 1994). 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 (McLeay and Brown, 1974, 1979). 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 (Slama, 1979). Two of the most important, jubabione-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 (Leach et al., 1975). 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 (Slama, 1979). 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 the resin acid, abietic acid, can have marked antijuvenile hormone effects (Slama, 1979). 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 (Munkittrick et al., 1991). 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. MFO's are found in virtually all animal phyla, including the arthropods/insects, as well as in plants and aerobic microorganisms (Brattsten, 1979). 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 MFO's (Owens, 1991). 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 (1943) 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 (Boxenbaum et al., 1988). 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 LD_{100} for the particular insecticide being tested (Luckey, 1968). 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 which has been observed in aquatic

invertebrates such as *Daphnia* following exposure to low levels of contaminants (D.J. Baird, personal communication; Barber et al., 1990). 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, less resources may go into energy storage and future reproductive output.

Thus, care must be taken in interpreting our results with *B. tricaudatus*. The data shows that the effluent treatments increased the growth and development of the mayflies during the two 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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1. Body dimensions at beginning of experiment

Appendix 1. Body dimensions at beginning of experiment.

	Mean	**SE**
Dry Body Weight (mg)	0.119	0.006
Thorax Length (mm)	0.982	0.020
Total Body Length (mm)	3.478	0.088
Wing Length (mm)	0.173	0.007
Head Width (mm)	0.658	0.009
Wing Spread (mm)	0.345	0.005
Wing Length/Spread	0.508	0.029

Appendix 2a. 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).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.814	0.686	0.750
	1%	0.771	0.829	0.800
	10%	0.710	0.786	0.748
____mean across concentrations		0.765	0.767	

Appendix 2b. 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).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.161	0.190	0.175
	1%	0.245	0.232	0.238
	10%	0.216	0.230	0.223
mean across concentrations		0.207	0.217	

Appendix 2c. Thorax length (mm) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.927	0.931	0.929
	1%	1.012	1.006	1.009
	10%	0.973	1.061	1.017
	mean across concentrations	0.971	0.999	

Appendix 2d. Total body length (mm) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	3.395	3.290	3.342
	1%	3.807	3.673	3.740
	10%	3.463	3.771	3.617
mean across concentrations		3.555	3.578	

Appendix 2e. Wing pad length (mm) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.206	0.206	0.206
	1%	0.253	0.227	0.240
	10%	0.220	0.242	0.231
	mean across concentrations	0.226	0.225	

Appendix 2f. Head width (mm) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.665	0.684	0.674
	1%	0.724	0.705	0.714
	10%	0.688	0.703	0.696
mean across concentrations		0.692	0.697	

Appendix 2g. Number of molts produced during the first week at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	6.143	5.286	5.714
	1%	6.714	5.714	6.214
	10%	6.000	5.571	5.786
mean across concentrations		6.286	5.524	

Appendix 2h. Number of molts produced during the second week at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	4.571	4.286	4.429
	1%	5.857	6.714	6.286
	10%	4.000	5.000	4.500
mean across concentrations		4.810	5.333	

Appendix 2i. Wing pad spread (mm) at the end of the experiment at two food levels (low, high) and three concentrations (control river water, 1% and 10% effluent).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.328	0.326	0.327
	1%	0.325	0.331	0.328
	10%	0.328	0.340	0.334
mean across concentrations		0.327	0.332	

Appendix 2j. 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).

		Food Level		
		low	high	mean across food levels
Effluent Concentration	control	0.630	0.635	0.632
	1%	0.750	0.687	0.718
	10%	0.670	0.712	0.691
mean across concentrations		0.684	0.678	