Effects of the Bacterial Insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) on a Stream Benthic Community

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Commercial formulations of the insecticidal bacterium *Bacillus thuringiensis* var. *kurstaki* (*Btk*) are frequently sprayed over bodies of water. The hypothesis that *Btk* poses a threat to stream communities was tested using streamside, flow-through mesocosms which received water and invertebrate colonists from a second-order stream in southwestern British Columbia; low (50 BIU/ha) and high (≥5000 BIU/ha) treatment levels were contrasted with controls. There were no significant differences in the density or composition of benthos sampled 7 d after *Btk* application. The densities were highest in the high-dose mesocosms. There were no significant differences in emergence rate of adults due to treatment. During the 2.5-h treatment, there were marginally elevated drift rates of the mayfly *Baetis* when exposed to the solution, but these differences were negated during the remainder of the 24-h period. Leaf packs lost significantly more mass in controls than in high-dose mesocosms, but there were no significant differences in the numbers of macroinvertebrates on those leaf packs. There was no evidence that addition of *Btk* to stream mesocosms created adverse effects for this benthic community, even at >100 times expected exposure rates.

Il arrive souvent que les formulations commerciales de la bactérie insecticide *Bacillus thuringiensis* var. *kurstaki* (*Btk*) sont appliquées sur des plans d'eau. Nous avons testé l'hypothèse que le *Btk* constituait une menace pour les communautés lotiques en l'essayant sur des mésocosmes installés au bord de l'eau et alimentés en continu avec de l'eau et des organismes colonisateurs invertébrés qui provenaient d'un cours d'eau de second ordre du sud-ouest de la Colombie-Britannique; on a comparé des cas témoins à des cas à faible dose de traitement (50 BUI/ha) et à forte dose de traitement (≥5 000 BUI/ha). Il n'y a pas eu de différence significative de densité ou de composition du benthos échantillonné sept jours après l'application du *Btk*. Les densités les plus élevées ont été observées dans les mésocosmes soumis à un traitement à forte dose. Quant à l'émergence des adultes, il n'y a pas eu de différence significative attribuable au traitement. Durant les 2,5 h de traitement, il y a eu un taux légèrement supérieur de dérive des éphémères *Baetis* exposées à la solution, mais ces différences n'ont pas été observées durant la suite des 24 h. Les lots de feuilles prélevés dans les stations-témoins ont perdu beaucoup plus de biomasse que ceux des mésocosmes exposés à de fortes doses de *Btk*; cependant, il n'y a pas eu de différence significative dans le nombre de macro-invertébrés observés sur ces lots. Rien n'indique que l'application de *Btk* à des mésocosmes lotiques a eu des effets nuisibles sur cette communauté benthique, même à des concentrations > 100 fois le taux d'exposition attendu.

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Formulations of the insecticidal bacterium *Bacillus thuringiensis* (commonly abbreviated *Bt*) are increasingly being used as highly selective pesticides against a variety of pest organisms. There are several serovars or varieties of this bacterium, each of which has differing toxicity to particular groups of insects. *Bacillus thuringiensis* is a naturally occurring bacterium in the environment and is ubiquitous in soils around the world (Lambert and Peferoen 1992). The insecticidal properties of this bacterium come from the production of a parasporal protein body, also known as crystals, when the organism enters its spore stage (van Frankenhuyzen 1990; Lambert and Peferoen 1992). If a lethal dose of *Bt* spores with crystals is ingested by an insect, and if that insect has the appropriate gut biochemistry for digestion of the particular protein, and if the particular gut epithelial receptors are present, then the insect generally will die within 1–4 d (Lacey and Mulla 1990; van Frankenhuyzen 1990; Lambert and Peferoen 1992). The specificity of the toxin for particular groups of insects means that there are few of the environmental effects often seen with broad-spectrum chemical insecticides, such as nontarget species mortality and residual toxicity. Operational application of the insecticidal *Bt* has been widespread over the past decade in the treatment of forest pests (van Frankenhuyzen 1990) or insect vectors of human disease (Dejoux and Elouard 1990). Several varieties or serovars of *Bt* have been developed for their

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activity against particular insect pests, such as *B. thuringiensis* var. *kurstaki* (hereafter *Btk*) which is toxic to a variety of forest pest Lepidoptera including spruce budworm (*Choristoneura fumiferana*), jack pine budworm (*C. pinus*), gypsy moth (*Lymantria dispar*), and hemlock looper (*Lambdina fiscellaria*) (van Frankenhuyzen 1990). More than 3.2 million ha of forests in Canada were sprayed with *Btk* for control of pest Lepidoptera between 1985 and 1989, and use of *Btk* continues to increase (van Frankenhuyzen 1990).

As the operational use of commercial formulations of *Btk* continues to increase, concerns about potential effects on nontarget organisms, including those in aquatic habitats, have increased. As part of routine *Btk* spraying, stream channels receive direct overspray plus that insecticide which may be lost from the riparian zone. Potential effects on nontarget organisms in streams are considered to be minor because of the specificity of the method of action of *Bt*. Nevertheless, since insects tend to dominate stream communities in terms of biomass, numbers, and species richness, any effect on these nontarget organisms could have implications for other values such as fish habitat capacity. There is evidence that *Btk* may have negative effects on some aquatic insects (Eidt 1985; Kreutzweiser et al. 1992), and thus there is concern for stream food webs, fish productivity, and ecosystem health.

Another variety of *Bt*, *B. thuringiensis* var. *israelensis* (*Bti*), is extensively used in streams to control black flies, and mosquitoes, e.g., as part of the World Health Organization's attempts to reduce densities of vectors of human disease. While *Bti* is fairly specific to black flies and mosquitoes, studies have shown negative effects on nontarget species (Merritt et al. 1989; Dejoux and Elouard 1990; Lacey and Mulla 1990), but these may have been due to trophic responses and food-web effects of black fly mortality (Wipfli 1992).

Much of the knowledge about the nontarget effects of *Btk* is based on acute toxicity laboratory tests. Widespread use of *Btk* in the field has allowed researchers to observe nontarget effects in terrestrial systems, but little is known of effects on aquatic organisms. A few studies have considered the effects of *Btk* on stream organisms, and these have been acute laboratory toxicity tests (Eidt 1985; Kreutzweiser et al. 1992). Eidt (1985) used a logarithmic dose intensity treatment in static toxicity tests with 2 times, 20 times, and 200 times normal spray concentrations and a control. Of the aquatic insects he tested, only larval *Simulium vittatum* showed significantly elevated rates of mortality over the 15-d experiment, and this was only at the highest dose rate. Black flies are often controlled using *Bti*, so perhaps there is some relation here. Larvae of one species of taeniopterygid stonefly had significantly elevated mortality due to *Btk* additions in laboratory toxicity tests at 100 times the expected field concentration (Kreutzweiser et al. 1992). Tests in ponds have shown no significant effect of *Btk* on pond invertebrates (Melin and Cozzi 1990).

Btk spores may persist in freshwater for several weeks. One estimate suggested a half-life for spore viability in a stream at 50 d (Melin and Cozzi 1990), and another study found viable *Btk* spores more than 70 d after addition to lake water (Menon and de Mestral 1985). One observation with *Bti* application in streams and laboratory microcosms is that the spores apparently chelate or adsorb to organic particles (Ohana et al. 1987; Dejoux and Elouard 1990). Given that detrital pathways are critical flows of energy in streams (Cummins et al. 1983), the concentration of spores adherent to organic particles may be cause for concern, especially in light of Kreutzweiser et al.'s (1992) observation of a significant increase in larval mortality of the detritivorous stonefly *Taeniopteryx* at exposure to extreme concentrations of *Btk*.

In this study, we used a replicated field experiment to test effects of additions of a commercial *Btk* formulation on a stream benthic community in flow-through mesocosms. We measured benthic densities, adult emergence, drift rates, and leaf pack decomposition in response to experimental application of the *Btk* suspension. In a trough mesocosm, biological processes are integrated, providing invertebrates with natural food levels and environmental conditions as they are in the natural stream, and this allows for replication and high levels of statistical power which enables considerable extrapolation of findings to larger stream reaches (Perrin et al. 1992). Our specific objectives were to test for any detrimental effect of additions of a formulation of *Btk* on survival, and behavioural responses of stream invertebrates, contrasted with control troughs. Another specific hypothesis was that given the adsorption of *Bt* spores and their toxin to organic particles, leaf packs of deciduous leaves would decompose slower when *Btk* was added if detritivores were able to detect and avoid high spore concentrations or if their feeding activities were otherwise deterred.

Methods and Materials

Study Site and Mesocosm Assembly

Stream mesocosms were established on the bank of Muir Creek, a second-order stream which drains westward from second-growth forest stands north of Sooke, on the southwest coast of Vancouver Island, British Columbia, Canada. Water temperatures were monitored daily on a maximum–minimum thermometer and were between 14 and 22.5°C, with temperatures decreasing slightly over the course of the experiment.

Each of the 15 mesocosm troughs (1.52 m long × 0.2 m high × 0.2 m wide) contained a gravel substratum that supported a representative stream benthic community. Water was supplied via a 15.24-cm-diameter pipeline that was laid upstream for a distance of about 200 m. The mesocosm design was similar to that originally described in Mundie et al. (1991, see their fig. 1) and Perrin et al. (1992). The water was delivered to each trough through a standpipe assembly that was adjusted to maintain water flow of approximately 500 mL/s in each trough and a surface current velocity of about 5 cm/s. The upstream end of each trough consisted of a mixing chamber having an angled baffle that created turbulence. Downstream of the mixing chamber was a 1.2-m section within which 2-cm-diameter drain rock was placed to a depth of 5 cm. This section supported the benthic community. A baffle placed at the downstream end of the gravel section controlled the water level and was set at 5 cm over the gravel surface.

Experimental Design

The experiment involved two treatments contrasted with a control with no addition of *Btk*: one treatment was a *Btk* addition at the high end of the range that might be expected in a small, shallow stream after routine spraying (i.e., 50 BIU/ha) and the other treatment was a high-level dose

TABLE 1. Arithmetic mean concentrations of *Bt* (±1 SE) in water samples collected from channel outflows during treatment and 4 d following treatment expressed as colony-forming units (CFU)/mL.

Date	Control	Low dose	High dose
27 August (during treatment)	27.4 (5.04)	2.1×10^4 (5.3×10^3)	1.8×10^8 (9.67×10^7)
31 August	4 (2.26)	8.17×10^3 (7.96×10^3)	332 (102.84)

≥100 times the previous rate. Each treatment was replicated five times resulting in a total of 15 experimental units (flow-through troughs). *Btk* was added as Foray 48B®, a commercially available aqueous concentrate that has a potency of 12.7 BIU/L. The low-dose rate of Foray® addition was calculated based on an aerial application rate of 50 BIU/ha to the area of a trough (0.3 m^2) with current velocity of 5 cm/s and water depth of 5 cm.

In most field applications, there is a limited window of direct application and transport in a stream. An application duration of 2.5 h fits this time course, mimics a worst-case scenario, and is close to that tested in other related studies (Eidt 1985; Kreutzweiser et al. 1992), thus allowing comparability between studies. Hence, transport in the water is likely to be transient, with elevated concentrations persistent on the order of 1 or 2 h. While the concentration of the related bacterium *Bti* has been shown to decline exponentially as the water moves downstream due to settling, filtration by invertebrates, and adsorption (Merritt et al. 1989; Chalifour et al. 1990), we chose a constant dose as an extreme case and because dose responses would be easier to interpret.

Colonization of the troughs by aquatic invertebrates began with the commencement of water flows on 16 July 1992. Invertebrates entered the system through the water supply and benthic communities developed in each trough by colonization of the substrata gravels. The discharge water during application was collected in a basin and pumped upslope to the forest floor. A single discharge line was branched to five separate lines to avoid channelization and pooling in depressions of the forest floor.

The Foray® treatments occurred on 27 August 1992. Stream water was used to dilute the *Btk* solution and stock solutions were kept in ten 3-L glass carboys, one for each trough receiving treatment. The three treatments were randomly assigned to the 15 troughs. The *Btk* solution was delivered to the low-level treatment troughs through a Technicon precision metering pump, and the high-dose troughs received the Foray® via Mariott tubes inserted into the appropriate glass carboy. The pump maintained a constant delivery rate of solution to the low-dose troughs; however, the Mariott bottles and tubes had to be recalibrated during the course of the application due to the high viscosity but were close to target levels throughout the 2.5-h treatment period.

Concentrations of viable *Btk* spores in the outflow of each trough were measured in water samples collected during treatment. A second set of water samples was collected 4 d later for a second count of *Bt* that may have been retained, perhaps in association with organic particles. The water samples were analyzed using the membrane filtration technique with plating on *Bacillus cereus* selective agar (EVS Consultants, North Vancouver, B.C.). Representative colonies were confirmed as *Bt* and counted using phase contrast microscopy. This method does not distinguish between varieties of *Bt*, but given the high rates of application in our experiment, most colonies can be attributed to *Btk*.

Drift has been used as a measure of behavioural and mortality responses to a manipulation in many studies, ecotoxicological and otherwise. Drift rates of stream insects from the troughs were determined during four 24-h periods, with starting dates of 21, 27, and 31 August and 2 September. On the first two dates (21 and 27 August), before and on the day of treatment, the drift periods were separated into 3 and 21 h to isolate immediate effects during the 2.5-h period of addition from nonimmediate effects, e.g., the night following additions. Most large invertebrates drift primarily at night and a severe exposure may be needed to overwhelm this behavioural predisposition. Drift was sampled with nets made of 250-μm-mesh Nitex® attached to the outflow of each trough. Using the replicated design, we contrasted the net differences in drift rate from each trough from before *Btk* addition to after addition (i.e., drift after minus drift before) and analyzed the data by treatment in a one-way ANOVA. Using the net difference assures the maintenance of the appropriate degrees of freedom, accounts for day to day differences across the experiment, and tests whether drift numbers changed more due to treatment than other sources of variation.

Emergence of adult insects was used as a measure of the ability of individuals to complete development and metamorphosis under experimental conditions. Emergent adult aquatic insects from the troughs were caught and preserved in Plexiglas® traps which covered the entire surface of the troughs. Adults were collected for a 1-wk period before treatment (12–19 August) and 1 wk following treatment (27 August to 3 September). Adults were identified to the family level and the differences (number after − number before) in emergence rate between dates was analyzed by treatment in a one-way ANOVA.

One week after *Btk* treatment, all benthic organisms were harvested from each trough by agitating the gravel in each trough for a standard period of 7 min and collecting the animals in 250-μm nets at the outflow. Samples were immediately preserved with 10% Formalin. In the laboratory the samples were sieved, and all organisms retained on 250- and 500-μm sieves were sorted from debris, identified to the lowest reliable taxonomic level, and counted. The data were tested for treatment effects using a one-way ANOVA for those taxonomic groupings where there were high enough densities to meet statistical assumptions (generally >30 animals per sample), and also for the numbers of taxonomic units (taxonomic richness).

Since *Btk* spores were expected to adhere to the surfaces of organic particles (Menon and de Mestral 1985), there was a danger that detritivores might ingest a concentration of the microbe that is higher than that in the water column, or *Btk* may affect other processes of leaf decomposition by

TABLE 2. Summary of taxa identified from experimental troughs on Muir Creek, British Columbia. Means (±1 SE for those taxa where numbers were high enough and with enough nonzero values to have meaningful parametric error terms) are shown for each taxon by treatment. Differences tested by one-way ANOVA. Benthos samples were sorted down to 250 μm. Some taxa were represented by only 1–10 specimens across the entire 15 troughs. These include *Malenka* (Plecoptera: Nemouridae), *Calineuria* (Plecoptera: Perlidae), *Hydropsyche* (Trichoptera: Hydropsychidae), *Dicosmoecus* and *Ecclisomyia* (Trichoptera: Limnephilidae), *Rhyacophila* (Trichoptera: Rhyacophilidae), *Hydrochus* (Coleoptera: Hydrophilidae), *Hexatoma* (Diptera: Tipulidae), and Tubificidae (Oligochaeta).

Order or class	Family	Genus, subfamily, or tribe	Controls	Low *Btk* dose	High *Btk* dose	Probability of no difference
Ephemeroptera	Baetidae	*Baetis*	584.6 (122.30)	562.4 (139.60)	729.6 (87.60)	0.57
		Centroptilum	10.4	3.4	6.4	
	Ephemerellidae		33.8 (3.11)	40.0 (9.79)	53.0 (5.63)	0.17
	Heptageniidae	*Rhithrogena*	5.2	20.0	5.0	
		Heptagenia				
	Siphlonuridae	*Ameletus*	11.2 (5.68)	10.8 (3.31)	7.8 (1.85)	0.80
	Leptophlebiidae	*Paraleptophlebia*	65.8 (10.37)	83.0 (8.86)	94.0 (10.50)	0.17
Plecoptera	Chloroperlidae	*Suwallia*	39.6 (10.02)	43.8 (8.83)	42.6 (13.97)	0.96
	Nemouridae	*Zapada*	4.2	6.2	6.4	
	Perlodidae	*Isoperla*	6.4	4.4	4.2	
Coleoptera	Elmidae	*Zaitzevia*	64.2 (14.95)	60.6 (9.06)	55.0 (4.34)	
Trichoptera	Hydroptilidae	*Agraylea*	21.2	24.2	42.0	
		Oxyethira				
	Lepidostomatidae	*Lepidostoma*	5.0	7.6	5.0	
	Polycentropodidae	*Polycentropus*	81.4 (12.81)	68.6 (16.47)	92.6 (20.77)	0.62
Diptera	Chironomidae	*Tanytarsini*	155.0 (42.21)	124.2 (50.69)	181.4 (16.59)	0.87
		Chironomini	410.2 (46.24)	442.0 (63.1)	637.4 (41.90)	0.02
		Diamesinae	0.2	4.2	0	
		Corynoneura	98.0 (12.50)	104.6 (31.57)	157.6 (15.3)	0.14
		Orthocladiinae (excluding *Corynoneura*)	236.8 (34.58)	202.4 (36.7)	326.0 (26.98)	0.05
		Tanypodinae	300.4 (45.01)	229.0 (34.95)	329.8 (44.27)	0.25
		Pupae	86.6 (7.62)	75.8 (13.39)	115.6 (8.96)	0.05
	Simuliidae		9.4 (2.46)	7.0 (2.17)	11.8 (4.83)	0.60
	Tipulidae	*Dicranota*	0.4	1.2	0.6	
	Ceratopogonidae		21.4 (8.73)	12.0 (4.00)	17.6 (4.83)	0.57
	Athericidae	*Atherix*	0.8	1.6	0	
	Dixidae		4.8	8.6	7.0	
Oligochaeta	Naididae		590.6 (41.47)	537.4 (71.76)	732.6 (75.92)	0.13
Totals			3054 (226.3)	2886 (266.7)	3668 (189.0)	0.08
Richness			17 (0.95)	18.6 (0.60)	17.6 (0.51)	0.31

fungi and bacteria. To test these possibilities, we used two leaf packs per trough composed of 5.0 g (±1%) of dried alder leaves as an assay for any detrimental effect particularly associated with detrital processing and detritivores. The leaf packs were added to the troughs on 6 August to allow 3 wk of incubation before treatment. At the end of the experiment the leaf packs were removed, invertebrates were washed from the leaves, and the invertebrates from one leaf pack of each trough were identified and counted in the laboratory. Each of the two leaf packs per trough, including any smaller particles in the leaf pack, were dried, weighed, ashed, and reweighed to determine ash-free dry mass (AFDM). The mean value of the two leaf packs per trough was used as the variable and tested for treatment effects with a one-way ANOVA. Likewise the data for invertebrates were analyzed by ANOVA for those groups where densities were sufficient for meaningful tests.

Results

Bacterial Counts

Counts of cultured colonies of *Bt* cells collected from each trough during the addition of the *Btk* formulation, and 4 d later, were analyzed by ANOVA of $\log_{10}(x + 1)$ transformed data. During application of *Btk*, counts from the high-*Btk*-dose troughs were >6 orders of magnitude above those of the controls and >3 orders of magnitude greater than in the low-dose troughs (Table 1). All three treatments were significantly different from each other (ANOVA, $p < 0.0001$, Scheffé's test). Bacterial counts remained elevated 4 d later in troughs that received *Btk* treatments (ANOVA, $p < 0.006$), and water from the high-dose treatment had concentrations statistically indistinguishable (Scheffé's test) from those in the low-dose troughs.

TABLE 3. Mean numbers (±1 SE) of animals within taxonomic and functional groups, total numbers, and number of taxa found per leaf pack in each treatment. Samples sorted to 250 μm. Differences between treatments tested by one-way ANOVA.

Grouping	Control	Low dose	High dose	Probability of no effect
Total numbers	157.6 (16.6)	169.8 (6.2)	157.6 (11.2)	0.71
Richness	17.2 (1.2)	19.0 (0.8)	16.6 (1.0)	0.28
Chironomini	41.6 (4.2)	45.4 (5.3)	39.4 (4.8)	0.67
Tanypodinae	13.6 (0.48)	12.4 (1.5)	11.8 (1.5)	0.62
Corynoneura sp.	15.2 (4.4)	21.8 (4.1)	11.0 (2.2)	0.15
Orthocladiinae (excluding *Corynoneura*)	15.8 (3.4)	9.4 (2.7)	16.8 (3.3)	0.23
Shredders	15.2 (4.7)	19.8 (5.4)	9.0 (1.4)	0.23

Benthos

The mesocosm supported a diverse and abundant benthic community. More than 40 taxa were found representing mayflies, stoneflies, beetles, caddisflies, chironomids, other true flies, worms, snails, nematodes, mites, ostracods, and even Cladocera (Table 2). Occasionally, terrestrial taxa were found in low numbers but they were not included. Midge larvae (Chironomidae) were the most abundant taxonomic grouping accounting for 39% of all invertebrates in the control troughs (Table 2). Larvae of the mayfly genus *Baetis* were the second most abundant taxon in the troughs accounting for 20% of all invertebrates. Although naidid worms (all between 250 and 500 μm) are often overlooked in stream studies, they represented about the same density as the baetid mayflies in Muir Creek. An average of 3200 animals were found in each trough, or about 10 500 animals/m^2 in the size fraction >250 μm. About a third of these animals were in the size fraction >500 μm. This density is typical of that found in natural streams.

No significant treatment effect was found for total benthic densities and number of taxa (ANOVA, $p > 0.05$; Table 2). Total densities were 20% higher in the high-*Btk*-dose troughs than in the control troughs, and about 75% of this difference was due to increased chironomid numbers. Fifteen taxonomic groups occurred in sufficient numbers for statistical analysis as listed in Table 2. The Chironomini, Orthocladiinae (excluding *Corynoneura*), and chironomid pupae appeared to increase significantly at the high dose of *Btk* (individual ANOVA's, $p \leq 0.05$; Table 2), but with application of the Bonferroni correction to avoid random effects in multiple comparisons (Rice 1988), the critical probability level was reduced to 0.003 (0.05 divided by 15 taxonomic groups), resulting in no statistically significant treatment effect. For all chironomid taxa, naidid worms, all mayflies except *Ameletus* sp., and the caddisflies, greatest mean abundance occurred with the highest dose of *Btk*, but there was no consistent ranking between the control and low dose. There was also no consistent ranking among the other taxa analyzed.

Since black fly larvae, and filter feeders in general, may ingest high numbers of spores during their feeding activities, and since black flies are susceptible to *Bti*, we analyzed numbers of simuliids and total filter feeders (including Simuliidae, *Hydropsyche*, and *Polycentropus*) separately. There was no significant effect of the *Btk* treatment (nontransformed or $\log_{10}$ transformed data) on either simuliids (ANOVA, $p > 0.6$) or all filter feeders ($p > 0.5$). Densities of black fly larvae and combined densities of filter feeders were highest in the high-*Btk*-dose troughs, followed by the controls and the lowest densities in the low dose (Table 2).

Leaf Packs

AFDM's of the alder leaf packs were determined for the two leaf packs per trough and the mean value per trough analyzed, except for one trough (a low-dose trough) for which only one complete leaf pack was available. Leaf packs were incubated for 3 wk prior to treatment and then for a further week posttreatment. There were significant differences in the leaf pack mass due to treatment (ANOVA, $p < 0.02$), with the high dose (2.68 g AFDM ± 0.031, mean and 1 SE) greater than the low dose (2.60 ± 0.065) and both greater than the controls (2.41 ± 0.056). Approximately half the starting leaf mass of 5 g was lost over the 4-wk incubation period in all troughs. An a posteriori comparison of means showed that the significant difference was between the leaf packs from the high-*Btk* treatment and the controls (Scheffé's test, $p < 0.05$).

Invertebrates on leaf packs were counted from one randomly selected leaf pack from each trough. Total numbers and the abundance of four taxonomic groups (Chironomini, Tanypodinae, *Corynoneura* sp., and Orthocladiinae (without *Corynoneura* included)) occurred in sufficient numbers for analysis. All other taxa occurred incidentally (i.e., <5 animals per sample) and were not separately analyzed. The numbers of taxa were also analyzed. Since leaf packs are the food source for "shredding" invertebrates (detritivores feeding on coarse particles), the total numbers of shredders per leaf pack were also tested for treatment effects. This functional group was selected based on the potential for exposure to adsorbed *Btk* spores and toxin on detrital particles. None of the taxonomic or functional groupings showed significant differences between treatments (ANOVA, all with $p > 0.15$; Table 3). The total numbers of invertebrates per leaf pack were highest from the low-*Btk*-dose troughs, and the other two treatments had identical mean numbers per leaf pack (Table 3). Numbers of the midge tribe Chironomini and the midge genus *Corynoneura* sp. (subfamily Orthocladiinae) were highest in the low-dose troughs, while the remaining orthoclads were lowest in the low-dose treatment (Table 3). The number of shredders showed no significant differences ($p = 0.23$), and the numbers were highest in low-*Btk* troughs, followed by controls, but numbers in the leaf packs in the high-dose troughs were less than half those of the low dose. There was no significant difference in the numbers of taxa per leaf pack ($p = 0.28$).

TABLE 4. Numbers of adults emerging per trough during the 1-wk period after treatment and the net difference between numbers for 1-wk periods before and after treatment. Probability of no treatment effect on the net difference based on ANOVA.

	Control		Low dose		High dose		
Grouping	After treatment	Net difference	After treatment	Net difference	After treatment	Net difference	Probability of no effect
Baetidae	51.2	42	67.8	39	43.2	48.6	0.84
Chironomidae	24	−11.4	28.6	−15.4	27.6	−12.8	0.9
Hydroptilidae	6	−4.6	12.4	−5.4	17.4	−15.8	0.4
Simuliidae	7.6	−8.4	10.8	−7	8.2	−2.8	0.2
Nemouridae	5	0.4	2.8	0.4	3.0	0.4	1.0
Total	97.6	18.8	128	21.4	103.4	21.4	0.92

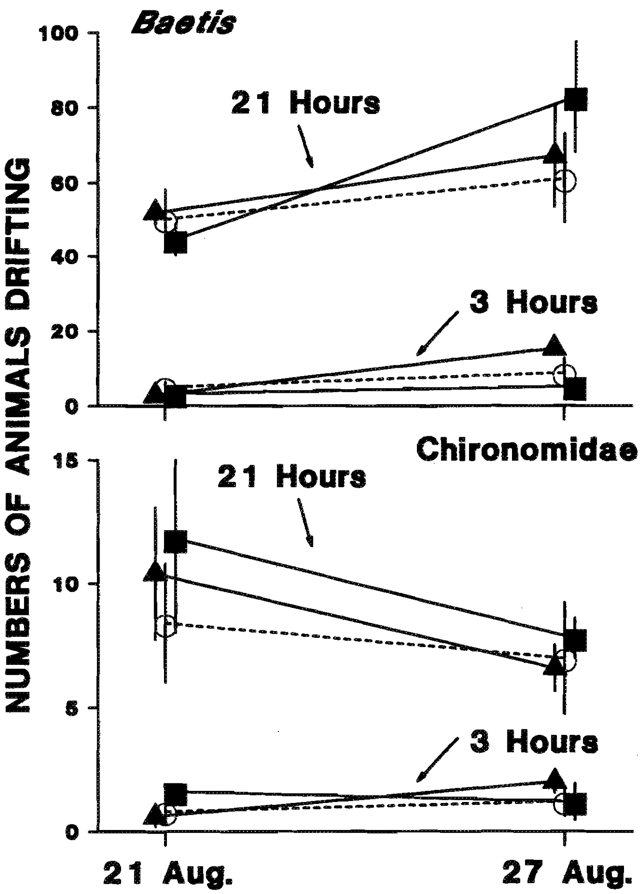

FIG. 1. Numbers of the mayfly *Baetis* sp. and larval Chironomidae collected in drift samples from the experimental troughs before (21 August) and during/after treatment of the appropriate troughs with *Btk* (27 August). Points represent means of the five troughs and have been slightly offset to separate the error bars (±1 SE). ■, controls; ○, low *Btk* dose; ▲, high *Btk* dose. The slopes of the lines remain appropriate for assessing differences before and after *Btk* additions. The lines are not meant to indicate a continuum of measures between the two points. Note the difference in scales on the y-axes.

Adult Emergence

Adults of the insect families Baetidae, Nemouridae, Chironomidae, Simuliidae, and Hydroptilidae emerged in sufficient numbers to allow statistical analysis. The difference in numbers emerged per trough from before treatment and after treatment was used to account for any systematic differences in troughs and maintain the appropriate degrees of freedom. There were no significant treatment effects for any of these taxa or for the total numbers of adults collected (ANOVA, all with $p > 0.2$; Table 4). There was also no consistent rank order of the numbers emerging by treatment.

Drift

Drift rate is defined as the number of animals leaving the troughs per unit time and can be regarded in this experiment as a measure of the behavioural response of stream animals to perturbation of their environment. The drift collections were made for a 3- and a 21-h period both before application of *Btk* and on the day of treatment. The 3-h sampling was organized to occur at the time of treatment. Only four groupings of data had sufficient numbers for analysis, and these were *Baetis*, larval Chironomidae, total number of drifting invertebrates, and the number of taxa represented. *Baetis* was the most abundant taxon in the drift samples followed by chironomid larvae, so most of the patterns detectable in total numbers drifting are largely based on responses by these two taxa.

There were no significant differences between troughs (based on their preassigned treatments) before application of *Btk* for either the 3- or the 21-h periods (Fig. 1) for any of the groupings of data (ANOVA, all with $p > 0.14$), thus confirming no trough effect on drift rates. To ensure trough effects were avoided over the time interval between measurements before and after treatment, statistical analyses used differences in numbers drifting before and after treatment.

During treatment, there was a significant increase ($p < 0.04$; Fig. 1) in 3-h drift rates of all invertebrates (mostly *Baetis* larvae), but not in the subsequent 21-h period ($p = 0.53$). Numbers of species drifting also increased with treatment ($p < 0.02$) but the net differences were as little as one taxon. An a posteriori test (Scheffé's test) showed that the 3-h drift from the high-dose troughs was significantly higher than the controls ($p < 0.05$) for *Baetis*, total invertebrates, and numbers of taxa. Drift rates from the low-dose troughs were not significantly higher than those of the controls (Scheffé's test). There was no significant effect of treatment on the drift rates of Chironomidae drifting during the 3-h application period as shown by lack of treatment interactions (indicated by differences in slopes) between dates in Fig. 1. There were no significant differences in the drift rates of any of the four groupings in the 21 h after treatment (ANOVA, all with $p > 0.50$), and drift rates from control troughs were slightly higher than from the treated troughs (Fig. 1).

Mortality due to toxic effects of the *Btk* formulation would be expected to show up to 1–4 d after exposure of the invertebrates. Drift samples (24-h duration) from 1 and 3 September showed no significant differences in drift rates for any of *Baetis*, Chironomidae, total invertebrates, or taxonomic richness. There were no consistent patterns in the order of drift rates between treatments (Fig. 2). For example, on 1 September, total numbers drifting were highest from the controls, but on 3 September, total numbers from the controls were lowest of the three treatments.

Another approach to examine treatment effects on drift rates is to examine per capita drift rate, i.e., the ratio of numbers drifting per unit time to benthic abundance. It may also be considered a rough estimate of "turnover time". Since *Baetis* was the most common taxon in drift, we used it to examine the influence of *Btk* additions on per capita drift. During the 3-h drift sample during treatment, per capita drift rates of *Baetis* were 1.2% for the controls (based on densities from the end of the experiment), 3.7% in troughs receiving the high dose, and 2.3% for the low dose. In the subsequent 21 h the rates were highest in the controls at 19.0%, followed by the high-dose troughs with 16.1% and the low-dose at 15.7%. The combined 24-h per capita drift rate on the day and night of treatment was higher for the controls, followed by the high dose and the low-dose treatments. Since *Baetis* accounts for most of the drift, this comparison shows that there was no change in turnover time or net emigration associated with the *Btk* addition, at least for the 24-h period.

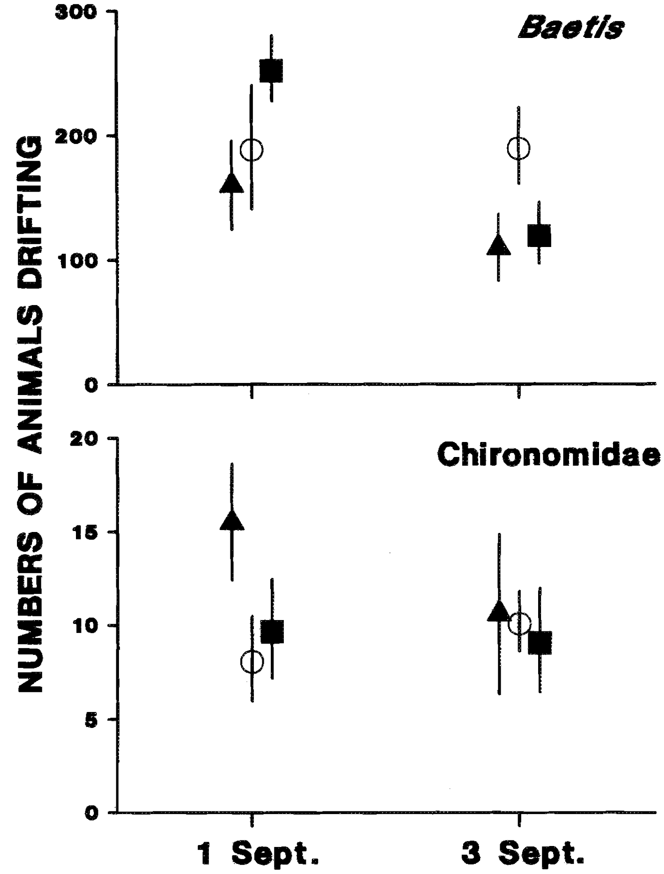

FIG. 2. Numbers of the mayfly *Baetis* sp. and larval Chironomidae collected in drift samples for 24-h periods 5 and 7 d after treatment of the appropriate troughs with *Btk*. Points represent the means of the five troughs per treatment and have been slightly offset to separate the error bars (±1 SE). ■, controls; ○, low *Btk* dose; ▲, high *Btk* dose.

Discussion

Most of the measures from our experiment showed no significant effect of the application of *Btk* on a benthic stream community even at 100 times the highest operational dose rate. Indices of community structure and processes including benthos abundance, numbers of animals associated with leaf packs, adult emergence, and drift rates did not change significantly in response to the *Btk* treatments. In fact, final benthic densities for most taxa were highest in the high-*Btk*-dose troughs. The mesocosms provided conditions of food and abiotic environment suitable for aquatic invertebrates, and 7 d posttreatment should have been sufficient to see any losses to increased mortality, since *Btk* usually has its toxic effect within 3–4 d (van Frankenhuyzen 1990).

One observation indicated that catastrophic drift behaviour may be associated with the high-*Btk* dosing. There was an increase in short-term (3-h) drift of *Baetis* with the onset of treatment. Assuming the final benthic densities of this mayfly were similar to the density at the time of *Btk* additions (1 wk earlier), the 3-h per capita drift rates were 0.0119 in the controls and up to 0.037 in the high-dose treatment. While drift rates tripled in the high-dose relative to control troughs, the nighttime per capita drift rates measured in the following 21 h were 0.16 in the low- and high-dose treatments and 0.19 in the controls. Thus, there was no residual effect after the actual application and, in fact, drift rate for the 24-h period that included the 2.5 h of *Btk* additions was actually higher in the controls than in the treatment troughs. Since *Baetis* larvae made up the largest proportion of the drift, the effect noted in the total numbers is attributed mainly to this mayfly. Since there was no treatment effect on *Baetis* in benthos (Table 2), these per capita drift data suggest that the elevated drift rate was a short-term response, perhaps related to increased turbidity that occurred with the addition of the high dose of *Btk* or due to ingredients in the *Btk* formulation (see below). There was no increase in drift rates in the days following the treatment as might be expected if there was delayed mortality due to toxic effects of ingestion of the insecticide.

One of our initial hypotheses was that leaf packs would decompose more slowly if *Btk* particles adsorbed onto the surface of the leaf tissue and this somehow deterred the feeding activity of shredders. Since bacterial counts were still higher in water from the treated troughs 4 d after treatment, it is clear that adsorption occurred and that Foray® was released to the water column from adsorption sites over time, either in association with sloughed particles or by desorption. While there was more leaf tissue remaining in the troughs receiving *Btk*, the data do not support our original hypothesis, since there was no significant treatment effect on the number of shredders on leaf packs. We cannot reject this hypothesis entirely, since shredder density on leaf packs was lower in high-dose troughs, but we suggest some further possibilities. First, there could have been some negative interaction between the bacterial insecticide or its carrier solution and the fungi and bacteria associated with the decaying leaf tissue. Since densities of "shredders" were an order of magnitude lower than in smaller coastal streams that have greater influence from detrital processes (Richardson 1992), most of the decomposition was likely due to microbes and these may have been adversely affected. A second possibility is that detritivorous invertebrates may have been feeding on the *Btk* particles or the starchy matrix adsorbed to the leaf surface and thereby ate less of the leaf tissue itself. Testing these hypotheses would require more detailed microbial experiments and feeding studies.

There have been other tests of the effects of *Btk* application on freshwater organisms. In an experiment in flow-through troughs, Kreutzweiser et al. (1992) found no significant effect of *Btk* application on drift or mortality rates of aquatic insects even at an application rate 100 times that expected under field conditions. However, in a laboratory toxicology experiment, there was a significant increase in the mortality rate of the detritivorous stonefly *Taeniopteryx nivalis* (Kreutzweiser et al. 1992), which is one of the reasons we considered effects of *Btk* application on shredders and leaf pack decomposition. In a field study, net-spinning hydropsychid (Trichoptera) larvae were collected with concentrated spores of *Btk* in their guts with no apparent toxic effects (Melin and Cozzi 1990). The only other study to have examined the effect of *Btk* on stream insects was a static toxicity bioassay in which black fly larvae, especially *S. vittatum*, showed a significant increase in mortality relative to controls at the highest dose of *Btk*, which was 200 times the expected environmental concentration (Eidt 1985). We found no negative effect of *Btk* application on black flies or filter feeders in terms of final densities and the numbers of emergent adult black flies.

Adverse effects of fish exposure to *Bti* or *Btk* have been shown to be due to formulation components rather than the bacterial toxin itself, since fish lack the gut biochemistry for any direct toxic effects. High concentrations of *Bt* formulations can cause gill clogging and suffocation (Snarski 1990; Wipfli 1992; R. Watts, Environment Canada, North Vancouver, B.C., personal communication). Preservatives in the formulation, such as xylene (Fortin et al. 1986), or high acidity (R. Watts, personal communication) is the likely culprit of toxicity towards fish at extremely high concentrations tested in laboratory assays. Nevertheless, commercial formulations of *Bti* had the lowest level of toxic effect of any of a series of pesticides tested on the mummichog (*Fundulus heteroclitus*) (Lee and Scott 1989).

One of the other ways that sublethal effects may propagate through ecosystems in through food-web effects. There were no obvious negative effects of *Btk* in our study, nor in that of Kreutzweiser et al. (1992) which would have suggested possible food-web effects. Studies of the black fly and mosquito larvicide *Bti* have shown potential for food-web effects. For instance, while Merritt et al. (1989) found no significant effects of *Bti* treatment on density or growth of most stream invertebrates (other than the target black flies), there was a significant increase in mortality of the tanytarsine midge *Rheotanytarsus*, another filter feeder, with a mortality rate of 27%. There were also food-web effects of *Bti* application on growth rates of some species of invertebrates in field mesocosms due to changes in abundance and ease of capture of moribund black fly larvae (Wipfli 1992). Such effects are unlikely to occur due to exposure to *Btk* based on the results of our experiment.

Our experiment found only minor evidence of any effects of experimental application of *Btk* to a benthic stream community, and those effects noted were not obviously detrimental. In fact, the final benthic densities, particularly of the chironomids, reached their highest levels in troughs having received a dose of the formulation more than 100-fold higher than could be expected from operational spraying over a small and shallow coastal stream. This study provides evidence that *Btk* is not harmful to benthic stream invertebrates under operational field application. The small, but statistically significant, reduction in decomposition of leaf packs demonstrates that there are ecosystem processes which are affected by *Btk*, but the relevance of these changes requires longer term and larger scale experiments.

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