A BROAD EVALUATION OF *B.T.I.* FOR BLACK FLY (DIPTERA: SIMULIIDAE) CONTROL IN A MICHIGAN RIVER: EFFICACY, CARRY AND NONTARGET EFFECTS ON INVERTEBRATES AND FISH

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ABSTRACT. Efficacy for black fly control, carry and nontarget effects of *B.t.i.* (Teknar® HP-D), applied in the Betsie River, Michigan, were studied in June 1988. Black fly mortality was high (~100%) for a 2,200 m stretch downstream from the application site, declined to 30% at 3,200 m, and was nil at 4,500 m. Drift of black flies greatly increased after application at a downstream site, but did not change at an upstream site. There were no detectable nontarget effects of *B.t.i.* application on: 1) invertebrate macro- or micro-drift; 2) numbers of invertebrates in benthic Surber samples; 3) mortality or feeding of drifting and nondrifting insects; 4) growth or mortality of caged *Stenomena* sp. larvae; 5) invertebrate functional group composition; 6) mortality or weight change of caged rock bass; or 7) fish numbers, species composition, length-weight (rock bass only) relationships or rock bass diet. Sampling of *Rheotanytarsus* sp. midges on natural substrates indicated low (27%) mortality owing to *B.t.i.* at only 100 m downstream from the application site, with negligible mortality at all other downstream and upstream sites. This information, combined with no pronounced changes in numbers of midges in macro-drift after application, indicated that midge populations were not adversely affected by *B.t.i.* in the study.

INTRODUCTION

Judged by the degree of annoyance and irritation they cause in North America, black flies rank with mosquitoes as major pests of people in recreation areas (Newson 1977, Kim and Merritt 1987). Annually, black flies account for large fiscal losses in Canada and the north central and northeastern United States. They greatly discourage tourism and outdoor recreation, and interfere with lumbering, mining and building activities during early spring and summer (Jamnback 1969, Fredeen 1977, Merritt and Newson 1978). In addition to humans, other animals also are affected by black fly attacks (Steelman 1976). Fredeen (1985) estimated that losses to beef and dairy producers in Sasketchewan in one year exceeded US $2.9 million owing to mass outbreaks of a single species. Besides economic effects, the bites of these insects can create a variety of pathological conditions in humans requiring hospitalization of sensitized individuals (Newson 1977).

In recreation areas, spraying to control adult black flies with insecticides has sometimes been effective, but the benefits are of short duration and this method presents risk of environmental contamination. Broad spectrum chemical insecticides applied to streams for larval control result in death of many nontarget organisms (Fredeen 1975, 1983; Mohsen and Mulla 1982). In addition, biomagnification of insecticides in the food chain has undesirable consequences (Woodwell et al. 1967). These adverse effects have prompted the development of more ecologically sound management strategies employing nonpersistent agents presumed to have little or no toxicity to nontarget organisms.

The most successful biological control agent developed to date for black flies is *B.t.i.* [*Bacillus thuringiensis* var. *israelensis* de Barjac (serotype H-14)], first isolated by Goldberg and Margalit (1977) from samples taken in the Negev Desert of Israel. It has proven to be very effective in a multinational black fly control program conducted by the World Health Organization in West Africa (Lacey et al. 1982), and at several locations in North America (Molloy and Jamnback 1981, Molloy 1989, Colbo and O'Brien 1984, Back et al. 1985, Gibbs et al. 1986, Pistrang and Burger 1984). Lacey and Undeen (1986, 1987), and most recently Molloy (1989) have reviewed uses of *B.t.i.* for black fly control.

To be effective, *B.t.i.* must be eaten by black fly larvae. The principal mortality agent of *B.t.i.* is a parasporal particle which contains a proteinaceous protoxin (Dubois and Lewis 1981, Aronson et al. 1986). The toxin becomes active after solubilization of protoxin in the presence of proteases and alkaline pH in the black fly larval midgut. The toxic polypeptides bind to surface receptors on the midgut epithelial cells and cause the cells to lyse and disintegrate, and the larva dies.

To date, there have been no published studies in the use or effect of *B.t.i.* against black flies in Michigan waterways. In June 1988, the Michigan Water Resources Commission and Department of Natural Resources issued a permit allowing *B.t.i.* to be experimentally applied to a section of the Betsie River, a brown trout stream located in the lower peninsula. This paper reports results of this study, the major objectives of which were to: 1) determine the extent of

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mortality of black flies and nontarget organisms caused by *B.t.i.*; 2) evaluate the effects of *B.t.i.* on macroinvertebrate abundance, short-term growth, and on micro- and macro-drift; 3) examine the downstream carry of *B.t.i.*; and 4) assess the effect of *B.t.i.* on fish abundance, growth and diet.

MATERIALS AND METHODS

Study site: The study was conducted from June 13 to 19, 1988, in Benzie County, Michigan, in the northwestern quarter of the lower peninsula of Michigan. Topography of the region is dominated by large, wooded sand dunes, while lowlands include many wetlands and small lakes. The study site was the Betsie River, a fourth-order stream which originates from Green Lake and flows westward for 50 km before emptying into Betsie Lake, near Lake Michigan.

The portion of the Betsie River used in the study was a 4.5 km stretch downstream from World's Bridge (8 km northeast of Thompsonville). The river was shallow (10–30 cm depth) and approximately 30 m in width at its widest point (Fig. 1). The stream bed consisted of small cobbles, sand, and occasional pools. Owing to drought (estimated recurrence interval of 100 years), the flow was low, and discharge was ca. 170,000 liters/min at the beginning of the study. Water temperature was high, with a diel range of 22 to 24°C. Black fly larvae occurred mainly on trailing vegetation and to a lesser extent on cobble. Larval black flies (collected in drift nets and from substrates) consisted mainly (>50%) of *Simulium jenningsi* Malloch. Other species included *Simulium vittatum* Zetterstadt cytospecies IIIL-1, *Simulium vittatum* cytospecies IS-7, *Simulium verecundum* Stone and Jamnback cytospecies AA-A/C, and *Simulium tuberosum* Lundstrom complex.

Application of B.t.i.: The *B.t.i.* used was a commercially available formulation, Teknar® HP-D (3,000 *Aedes aegypti* units per mg, equivalent to 1,200 ITU per mg). This is a liquid, "low-spore" formulation (ca. 10^4–10^5 spores per ml; D. H. Ross, Zoecon Corp., personal communication) and consists of spores, crystalline protoxin, cell fragments and water. The liquid was applied to the test site at 2000 h on June 15, 1988. The application rate was determined in accordance with the label requirements and the calculated stream discharge, such that the final concentration in the water would be 22.5 ppm for 1 min. The volume of Teknar® required was calculated to be 3.78 liters, which was measured into a small bucket and poured by hand over a 1-min period so that it was evenly dispersed over the width of the stream section.

Carry of B.t.i.: Direct counts of bacterial

Fig. 1. Betsie River study site, Benzie Co., Michigan.

spores in the river water were done to estimate the carry of the *B.t.i.* downstream from the release site. Water samples were taken at stations 100, 300, 600 and 1,800 m below the treatment site. For each station, samples were drawn 5 min before *B.t.i.* application and at intervals of 5, 10, 15, 30 and 60 min after application. Samples were taken by submerging and completely filling 500 ml sterile tissue culture flasks. Samples were put on ice immediately after collection and kept on ice until they were returned to the lab, where subsamples were drawn and preserved in 10% formalin (final concentration, v/v). Dilutions (1:5 or greater) of the samples were prepared using filter-sterilized, deionized water and then stained with DAPI fluorochromatic stain (Porter and Feig 1980, Walker et al. 1988) at a concentration of ~6 μg/ml for 25 min at 4°C in the dark. Slides were prepared from each sample according to the method of Hobbie et al. (1977), using Irgalan-black stained filters for a neutral, nonfluorescing background. Direct counts of total bacteria (including *B.t.i.* spores) were done for 4 replicates of each sample, from at least 15 fields to a total count of at least 200 bacteria for each filter, using a Leitz Laborlux 11 microscope with the appropriate epifluorescent light fittings and excitation and barrier filters. Counts were converted to numbers of bacteria per ml of river water sample, using a standard formula (APHA 1980).

Two attempts were made to obtain indirect counts of bacterial spore numbers using agar plating procedures of Gibbs et al. (1986). Subsamples (10 ml) drawn from the original samples were heat-shocked in a water bath at 60°C for 30 min to kill vegetative growth, serially diluted, and 1 ml of each dilution plated onto agar in petri plates, which were then incubated for 24 h before inspection for the presence of bacterial colonies. In the first trial, dilutions were in powers of 10 from 1:1 to 1:10,000, and plating was directly onto the top of solidified blood agar. In the second trial, dilutions were in powers of 5 from 1:1 to 1:625, and the samples were mixed into unsolidified tryptose agar during the plating procedure.

Macro-drift samples: Drift sampling has proved an effective method for evaluating the effects of *B.t.i.* on black flies and nontarget invertebrates (Gibbs et al. 1986, Molloy 1989), and was used in this study. Drift nets were placed in riffle areas above and below the site of *B.t.i.* release. Six nets were set in a transect across the width of the stream just above the release site (ca. 20 m below World's Bridge), and six more were set in a similar transect 100 m below the treatment site. The drift nets had a mesh size of 350 μm and were anchored in the stream with iron rods passed through the long axis of an aluminum frame (45 × 15 cm rectangular aperature) holding the net. All drift samples were preserved in 70% ethanol and returned to the lab for later analysis.

Drift was sampled from 2000 h to 0800 h (for 12 h) during each of 6 successive days or sampling periods, as natural drift of stream organisms is greatest during this time (Waters 1972). The first two sampling periods started 48 and 24 h before *B.t.i.* application. The beginning of the third period was concurrent with the application, while the remaining three periods began 24, 48 and 72 h after application. The drift net positions were the same for each of the 6 sampling periods. Stream depth and current flow in front of each net were measured just prior to removal of the sample to allow for corrections in calculating actual drift densities. Drift collections per net per day were then expressed uniformly as the number of individuals of each taxon per total volume of water that passed through each net during the sampling period (Waters 1972).

The sampling design (6 replications at each site with 6 sampling periods, 2 before the treatment and 4 after the treatment) provided information on pretreatment and posttreatment drift above and below the *B.t.i.* release point. Use of an experimental design incorporating both spatial (above-and-below) and temporal (before-and-after) sampling (cf., Green 1979) provided controls to the treatment effect in space and time.

Large numbers of chironomids were captured in drift nets; therefore, collections of these organisms were subsampled with a device modified from Waters (1969). An evaluation of the subsampler with drift samples revealed no significant differences in the number of invertebrates distributed among subunits after subsampling (χ^2 tests, $P > 0.05$). Total counts were made for other invertebrates, including black fly larvae.

Micro-drift samples: In order to determine the effects of the *B.t.i.* application on smaller-sized insects and to determine the suitability of using the coarser mesh-sized, larger volume nets for sampling drift, micro-drift sampling was conducted as follows. The nets used to collect micro-drift were of the "windsock" type, 0.8 meters long with a mesh size of 250 μm and a collection cup, with a removable screw cap, at the terminus of the net (Wilzbach et al. 1986). Four sets of five replicate drift samples were taken. Two sets of the five replicates were taken the night before *B.t.i.* release (one set above and one below the release point), and similar sets were taken immediately after *B.t.i.* treatment. The sets taken before release indicated the natural variability between the upper (above the release point) and lower (below the release point) sampling loca-

tions. All sampling commenced within 20 min of 2200 h and was of a 2 h duration. Comparison of these collections with those taken at the same locations after treatment with *B.t.i.* permit evaluation of the immediate effects of the pesticide.

The invertebrates from the micro-drift samples were sorted to taxa, enumerated by size classes (nearest mm) and converted to estimated biomasses with a special computer program which uses an array of 32 length-weight regression equations that are suited for the various groups of organisms. The coefficients and formulations for the regressions were from the literature or independently determined. Drifting invertebrates were also categorized according to functional feeding group (Merritt and Cummins 1984, Cummins and Wilzbach 1985) and habit (i.e., mode of attachment or locomotion; Merritt and Cummins 1984).

Drift/benthos mortality experiments: In order to compare mortality rates between drifting and nondrifting (benthic) invertebrates both before and after release of *B.t.i.*, a specifically designed sampler was used to partition these drifting and nondrifting population components. The plexiglas partitioner (Wilzbach and Cummins 1989) encloses a 0.1 m^2 area of stream bottom and has 250 μm mesh panels at the fronts and sides that permit circulation of stream water. The sampler can be seated several cm into a gravel stream bottom, with heavy cobbles on the lid to hold it in place. Water and invertebrate drift can exit the enclosure through two downstream ports. These ports can be fitted with 250 μm mesh micro-drift nets to collect the drift. The design of the partitioning sampler permits collection of drift and nondrifting benthos from a defined area of stream bottom and prevents colonization by drift from outside of the area.

The experiment was conducted the night before and the night after *B.t.i.* release at a point 100 m below the release point. Each night, the partitioner was seated in place for 10 h (from 1000 to 2000 h) with ports open, then the ports were closed with drift nets for 2 h (2000–2200 h). The ports were opened until the next morning (0900 h) at which time the drift nets were again attached and the bottom sediments gently disturbed to wash individuals from the benthos into them. These individuals represented benthos that had not drifted out of the enclosed area the previous night.

Drift and benthos collections were carefully washed into enamel trays, to which ice was added to maintain temperature, sorted under a microscope, and insects were transferred with fine mesh scoops to holding chambers for assessment of mortality. These were 50-cell plastic culture chambers (each cell 12 × 12 × 8 mm), with 250 μm mesh on the bottom to allow water circulation into each cell. Animals were placed in the floating culture chambers with one individual per cell. The taxon and length of each individual (nearest mm) and the fullness (full, 75%, 50%, 25% or empty) of the fore-, mid- and hindgut were recorded after visual inspection at 12× or greater magnification. The chambers were placed in a closed cooler with stream water for 12 h. Styrofoam blocks attached to the chambers allowed them to float so that each cell was 75% full of water. Water temperature was maintained at 18–22°C by adding ice as needed.

After being held without food in the dark for the 12 h period, the animals were censused for mortality under a dissecting microscope without removing the individuals from their cells. Any individual that exhibited damage from handling was excluded from the mortality census. The procedure yielded 12-h mortality estimates from partitioned samples of drift and benthos prior to and after release of *B.t.i.* In the latter case, the partitioner was in place when the *B.t.i.* was introduced.

Substrate samples for black fly and midge mortality estimates: The effect of *B.t.i.* on black flies and on the chironomid midge, *Rheotanytarsus* sp. (Chironominae, Tanytarsini), was determined by counts of living and dead larvae present on natural and artificial substrates following *B.t.i.* application. *Rheotanytarsus* was chosen as a test nontarget organism because: 1) it is a nematocerous dipteran, closely related to the Simuliidae, that was common at the study site, 2) it attached to substrates identical or similar to those used by black flies, and 3) it is a filter-feeding insect that removes fine particles from the passing water that are in the same size range used by black flies (Wallace and Merritt 1980). Thus, *Rheotanytarsus* would be the kind of non-target organism most likely to be affected by *B.t.i.* (Ali et al. 1981, Back et al. 1985, De Moor and Car 1986).

Two types of artificial substrates were placed in the stream (in April and May 1988) just above the treatment site, and at 100, 300, 600, 1,800 and 4,500 m below the treatment site. The first type of substrate consisted of 20-cm^2 clay tiles placed on cobble or sand in the stream bed. The second type consisted of a combination of 4 × 35 cm clear acetate strips and 5 × 60 cm colored vinyl flagging tape anchored in the stream with wire pegs. All substrates were placed in the stream at least 48–72 h prior to the pesticide application to allow time for colonization to occur. In addition to artificial substrates, naturally occurring, trailing leaves of the grass *Vallisneria* sp. were used as a substrate when artificial substrates were missing or had been vandalized.

Mortality counts were made 2 h after treat-

ment at the site above the release of *B.t.i.*, and at the 100, 300, 600, 1,800 and 4,500 m sites. Counts were also made at 12 h after the *B.t.i.* application at all 6 sites, and also for additional sites at 2,200 and 3,200 m, where there were no artificial substrates and counts for both organisms were from *Vallisneria.* Counts were accomplished by taking note of the total number of dead and live black fly or *Rheotanytarsus* larvae on 3–6 substrates at each site and recording the number and percent dead per substrate. Because these larvae respond to touch with wriggling movements, it was easy to discern live and dead individuals. Qualitative pretreatment counts showed no apparent mortality of black flies or *Rheotanytarsus* prior to *B.t.i.* release.

Substrate samples for benthos estimates: Samples of benthic invertebrates were taken with a Surber sampler (mesh size, 1 mm) at transects across the stream located 105 and 110 meters downstream from the release site. Five samples were taken before and 5 after *B.t.i.* application, and samples were evenly distributed across the length of the transects, after allowing for a 3-m buffer zone from each bank to minimize edge effects. Samples taken before release were from the 105 m transect at 0700 h, June 15; and samples taken after release were from the transect at 110 m on June 16. All samples were taken at 0700 h and were preserved in 70% ethanol and returned to the lab for sorting and identification.

Short-term invertebrate growth experiment: Live larvae of the scraper mayfly, *Stenonema* sp., were collected from the study site prior to the release of *B.t.i.* and held for 6 days (138 degree-days) in plastic field growth chambers (30 × 16 × 9 cm). These enclosures were fitted with 500 μm mesh panels on all sides and in the removable lid to allow circulation of stream water. The growth box chambers were positioned in the current 12 h before release of *B.t.i.*, with 3 above and 3 below the release site. Chambers were held in position with sections of reinforcement bar driven into the stream bottom. The boxes were provisioned with natural food from the location, which consisted of small cobbles of uniform size coated with periphyton. Care was taken to remove all macroinvertebrates from the cobbles before placing them in the boxes. Each box was stocked with 25 larvae and before stocking the boxes, a random subsample of 51 larvae was removed for determination of initial weights. These larvae were air-dried and then placed in individual Beam® capsules with perforated lids and placed in a dessicator. The capsules were oven-dried (50°C for 24 h) desiccated for 24 h, and then each larva was weighed to the nearest 0.1 μg. After the 6-day incubation period, the surviving larvae removed from the growth chambers were treated in the same fashion as the others for determination of final weight.

Fish studies: Species composition, relative abundance and length-weight relationships of resident fish were studied to examine effects of *B.t.i.* application. A boat equipped with an alternating current, electrofishing shocker with gasoline generator was used to collect fish along 100 meter stretches situated above (ca. 1 km) and below (300 m) the release point. Two passes were made for each collection along each stretch both before and after application of *B.t.i.*, giving a total of four collections (above-before, above-after, below-before, below-after). A crew of four people shocked and netted the fish, and all fish from both passes collected at a site were retained live for species identification and length (nearest mm) and weight (nearest 0.1 g) determinations. Fish were anesthetized with club soda (CO_2) prior to measuring and weighing.

One day prior to release of *B.t.i.*, rock bass (*Ambloplites rupestris*) representing a range of the sizes in the population were taken from the above- (10 fish) and the below-release (8 fish) stretches and placed in 1-m^3 cages constructed on 1-cm mesh wire. Prior to caging, gut contents of the fish were removed to evaluate diet composition and to insure that all fish had the same gut fullness initially. Gut contents were flushed from the digestive tract with water injected from a syringe, and preserved in alcohol. Cages were placed along the bank under cover of overhanging vegetation, and fish were held there for 3 days. At the end of the 3-day period, the cages were checked for mortality, and all individuals were measured and weighed again, and their gut contents were removed.

RESULTS

Carry of B.t.i.: Bacterial numbers in the river ranged from 2,027,834 to 4,424,365 per ml and were within the range observed in other rivers (e.g., Kondratieff and Simmons 1985). One-way ANOVA and Duncan's multiple range test (Steel and Torrie 1980) revealed distinct peaks in bacterial numbers, appearing above the natural, background bacterial numbers in the river, which we attributed to the presence of *B.t.i.* spores moving in the water column down river (Fig. 2). At the site 100 m downstream from the release point, there was a peak in direct counts at 5 min posttreatment; afterwards, direct counts dropped to preapplication levels. At the 300-m downstream site, there was a peak in direct counts at 10 min postapplication, and at the 600 m downstream site there were peaks at the 10 to 15 min sampling times. At the 1,800 m

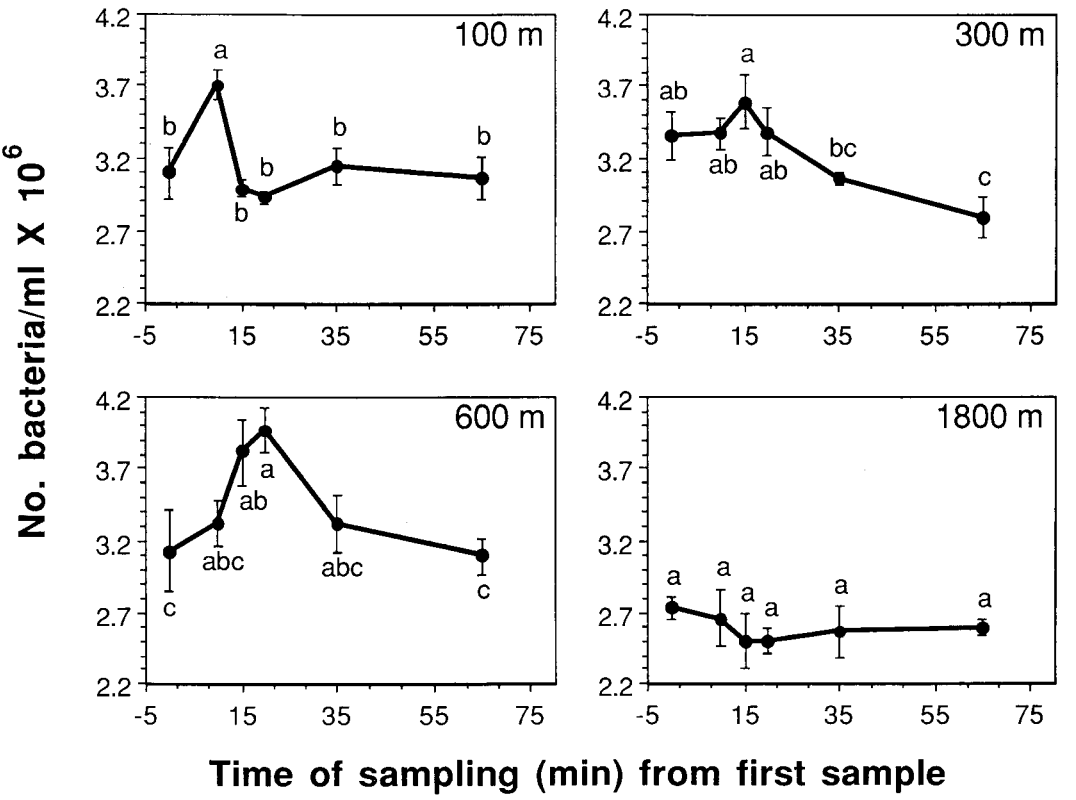

Fig. 2. Direct counts of bacteria (including viable *B.t.i.* spores), using DAPI stain and epifluorescence microscopy, in the Betsie River at four sites downstream from site of *B.t.i.* release. Data are means (± SEM) of four replicates per site. Means with the same letter are not significantly different (one-way ANOVA and Duncan's multiple range test, $P > 0.05$).

downstream site, there were no peaks in direct counts, which were equal among sampling times.

Attempts to culture spores on media for colony counts were unsuccessful.

Macro-drift sampling: Thirty-eight invertebrate taxa were identified from the macro-drift samples (Table 1). The drift was dominated by gammarid amphipods and larvae of hydropsychid caddisflies, black flies, baetid mayflies and chironomid midges. Chironomid pupal exuviae, indicating adult emergence, were abundant in the collections but were not counted. Interpretation of the effect of *B.t.i.* treatment on invertebrate drift must take into account both the results of ANOVA (Table 1) and a graphical depiction of the temporal and spatial pattern of drift (Figs. 3 and 4). Drift of black fly larvae generally decreased over time at the site above the treatment point (Fig. 3, A and B), suggesting the occurrence of natural population processes at the study site. At the site below the treatment point, black fly drift was similar to that above for the 2 days of pretreatment sampling, but greatly increased immediately following application of *B.t.i.* For the final 3 days of sampling, black fly larvae were rare in the below-treatment site drift samples. ANOVA showed a highly significant effect ($P < 0.001$) of both site location and sampling day on black fly drift. Drift of chironomid midge larvae showed slightly different patterns at the above-treatment and below-treatment sites ($P = 0.045$) and was generally higher at the above treatment site among the 6 sampling days (Fig. 3, C and D). There was significant variation of chironomid drift among sampling days nested within treatment sites ($P < 0.05$). In general, there was no change in chironomid midge drift at the down-stream site (on posttreatment sampling days 3–6) that could be attributed to *B.t.i.* application (Fig. 3D). Baetid mayfly larvae (Fig. 3, E and F) and gammarids (see Fig. 5, G and H) varied in drift samples among sampling days ($P < 0.001$ and $P < 0.01$, respectively), but did not differ in numbers of individuals in drift at above-treatment and below-treatment sites ($P > 0.05$). Hydropsychid caddisflies varied significantly in drift both between treatment sites ($P < 0.05$) and among

Table 1. Relative abundance (as total in 72 collections) of individuals of the invertebrate taxa (order and family) in macro-drift net collections in the Betsie River, and results of nested ANOVA on drifting numbers collected above and below the site of application of *B.t.i.*, and among days before and after application of *B.t.i.*

Taxon	Relative abundance	ANOVA *F*-tests	
		Sites	Days within sites
Amphipoda			
Gammaridae	10,000	0.00NS	2.35*
Decapoda			
Palaemonidae	24	ND	ND
Isopoda			
Asellidae	78	ND	ND
Odonata			
Aeshnidae	8	ND	ND
Libellulidae	1	ND	ND
Calopterygidae	1	ND	ND
Coenagrionidae	7	ND	ND
Ephemeroptera			
Baetidae	3,817	2.44NS	3.59***
Heptageniidae	668	8.87**	0.61NS
Ephemerellidae	82	ND	ND
Leptophlebiidae	13	ND	ND
Siphlonuridae	13	ND	ND
Tricorythidae	1	ND	ND
Non-Baetid, Non-Heptageniid	109	2.04NS	1.13NS
Plecoptera			
Perlidae	439	40.73***	2.98**
Hemiptera			
Pleidae	1	ND	ND
Saldidae	2	ND	ND
Megaloptera			
Corydalidae	3	ND	ND
Coleoptera			
Elmidae (larvae)	483	6.32*	0.59NS
Elmidae (adults)	44	ND	ND
Dytiscidae (larvae)	6	ND	ND
Dystiscidae (adults)	71	ND	ND
Gyrinidae (larvae)	13	ND	ND
Haliplidae (adults)	1	ND	ND
Hydrophilidae (larvae)	34	ND	ND
Hydrophilidae (adults)	7	ND	ND
Diptera			
Chironomidae	3,901	4.18*	2.23*
Simuliidae	6,970	29.40***	16.00***
Tipulidae	4	ND	ND
Athericidae	1	ND	ND
Trichoptera			
Hydropsychidae	7,791	5.69*	2.07*
Brachycentridae	2	ND	ND
Helicopsychidae	1	ND	ND
Hydroptilidae	13	ND	ND
Limnephilidae	9	ND	ND
Philopotamidae	42	ND	ND
Polycentropodidae	23	ND	ND
Psychomyiidae	13	ND	ND
Non-Hydropsychids	103	0.11NS	3.38**
Lepidoptera			
Pyralidae	2	ND	ND

Degrees of freedom were: sites, 1; days, 10; and error, 60.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
NS, not significant ($P > 0.05$); ND, ANOVA not done.

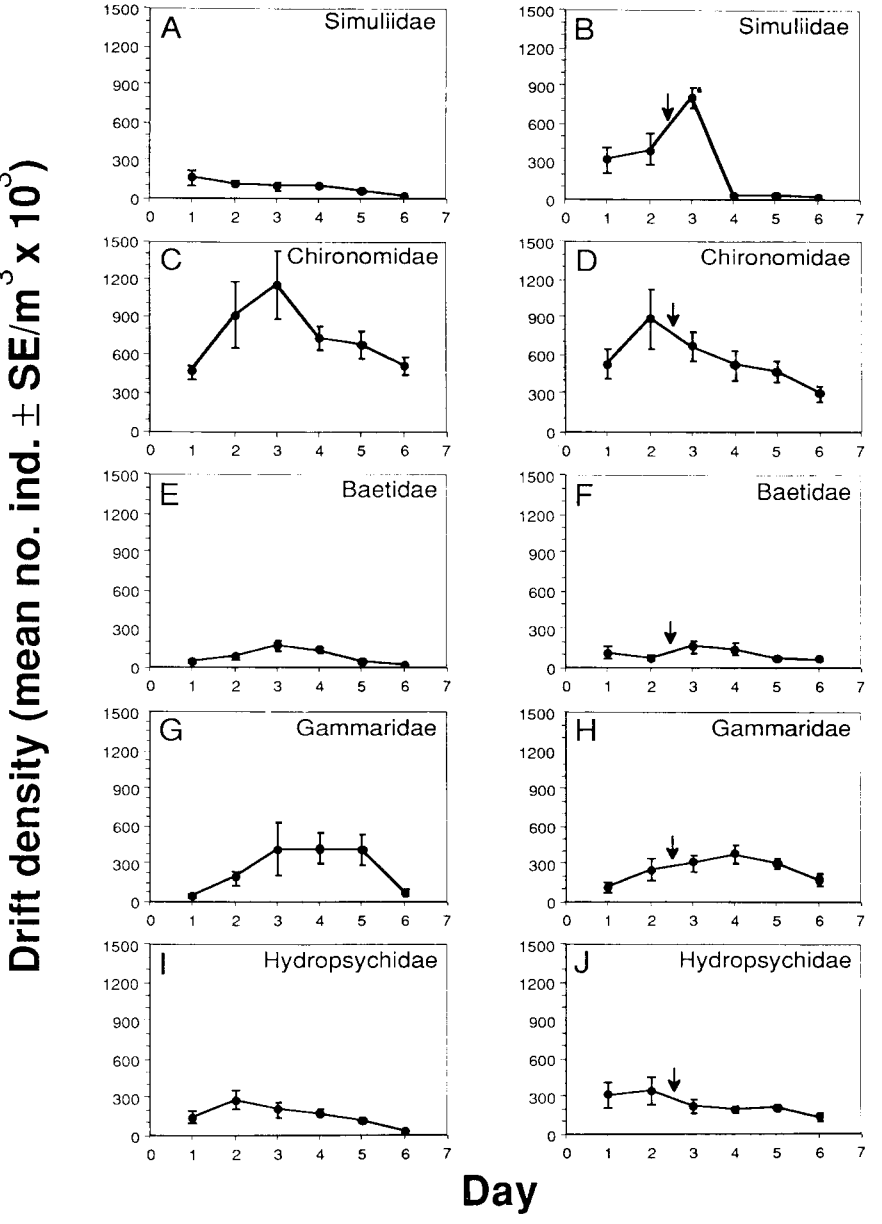

Fig. 3. Drift density [expressed as mean number of individuals (± SEM) per drift net per $m^3 \times 1{,}000$] of selected invertebrate taxa in macro-drift collections at above-(left column) and below-(right column) treatment sites; collections were made for 2 days before and 4 days after application, at night. Arrows indicate when *B.t.i.* was applied above the below-treatment site.

sampling days ($P < 0.01$). However, the patterns of drift at the above- and below-treatment sites appeared rather similar, except that drift was higher at the above-treatment site on the first sampling day (pretreatment) and somewhat lower on the last sampling day (posttreatment). Hydropsychid drift on days 3–5 (the first 3 days after treatment) was similar between above-and-below treatment sites.

Results of macro-drift samples for less abundant organisms are shown in Fig. 4. There was large variation in numbers of heptageniid mayflies in the drift, with significant variation between treatment sites ($P < 0.01$), but no significant variation among sampling days ($P > 0.05$). Early larval instar mayflies (mainly Heptageniidae, but also including Ephemerellidae, Leptophlebiidae, Siphlonuridae and Tricorythidae)

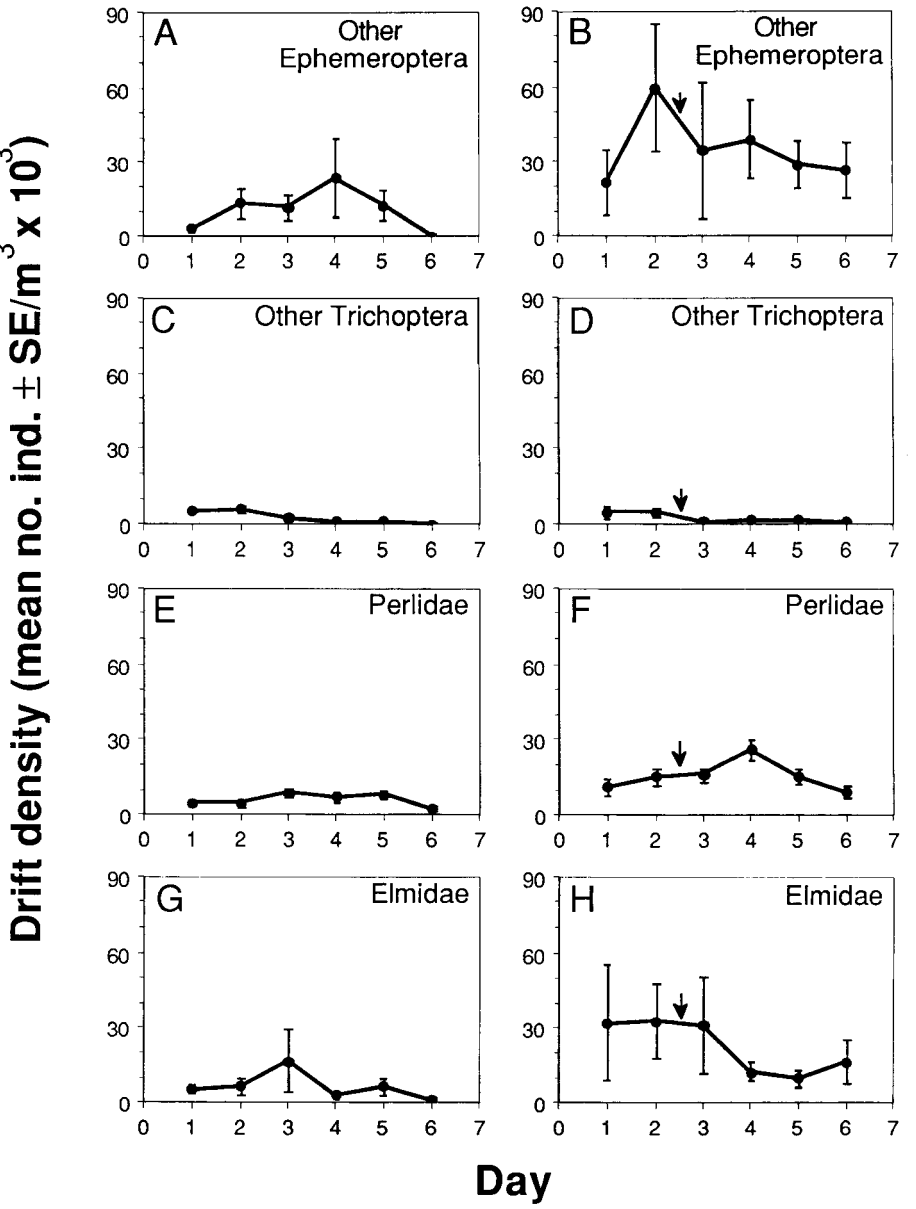

Fig. 4. Drift density (as in Fig. 3) of selected invertebrate taxa in macro-drift collections at above-(left column) and below-(right column) treatment sites. Collection times are as in Fig. 3.

were more abundant in the drift at the below-treatment site than at the above-treatment site both before and after *B.t.i.* application (Fig. 4, A and B). When numbers from these last four families were combined, there were no significant differences among sampling days or between treatment sites ($P > 0.05$). Caddisflies in the families Brachycentridae, Helicopsychidae, Hydroptilidae, Limnephilidae, Philopotamidae, Polycentropidae and Psychomyiidae were also uncommon in collections, and numbers of individuals from these families were combined for analysis as well (Table 1; Fig. 4, C and D). There was no significant difference between treatment sites for this group ($P > 0.05$), however, the numbers of these caddisflies did vary significantly among sampling days ($P < 0.01$). In general, collections for this group decreased in both above- and below-treatment sites over time. Perlid stoneflies (Table 1; Fig. 4, E and F) were significantly more abundant in below-treatment drift compared to above treatment drift ($P <$

0.001) and also varied significantly among treatment days ($P < 0.01$). Beetles were generally uncommon in the drift; however, there were sufficient numbers of elmid larvae for analysis (Table 1; Fig. 4, G and H). Elmid larvae were significantly more abundant in below-treatment drift compared to above-treatment drift ($P < 0.01$). Drift collections of elmid larvae did not vary significantly among sampling days nested within treatment sites ($P > 0.05$).

Micro-drift sampling: The micro-drift samples indicated that only an insignificant amount of drift was represented by size classes of animals in the size range of 1 mm or less (Fig. 5). Thus, the macro-drift samples adequately represented the total drift composition in the stream during the sampling period. This was true for the sites above and below the point of release of *B.t.i.* and for the same sites before and after the pesticide was applied.

In addition, no significant general effect of the *B.t.i.* application on size class composition of the drift could be demonstrated when comparing paired observations (n = 9) either at the above-treatment site before and after treatment (Wilcoxon signed-rank statistic = 17.5, $P > 0.05$) or at the below-treatment site before and after treatment (Wilcoxon signed-rank statistic = 16.5, $P > 0.05$). Comparisons of total drift numbers at the site below treatment (Mann-Whitney U = 8.0; $n_1,n_2 = 5,5$; $P > 0.05$) or total biomass (Mann-Whitney U = 8.0; $n_1,n_2 = 5,5$; $P > 0.05$) showed no significant differences between pre- and posttreatment samples. Further, no discernible shifts were observed in the taxonomic or functional group composition of the samples that could be associated with the application of *B.t.i.* (Fig. 6). The major source of variation was due to the sporadic presence in the samples of a few fish fry, included along with invertebrates in the predator category, which had high biomass relative to drifting invertebrates.

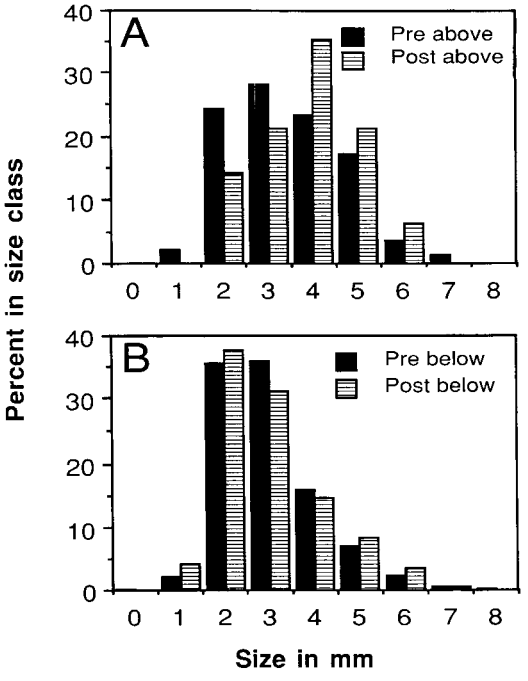

Fig. 5. Comparison of size class distributions of the micro-drift before and after the application of *B.t.i.* both above (A) and below (B) the application site.

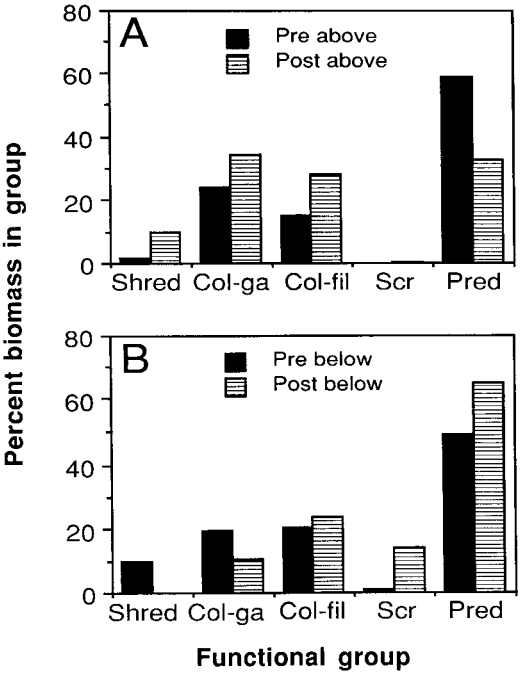

Fig. 6. Comparison of the relative percent biomass in each functional group in the micro-drift before and after treatment above (A) and below (B) the application site. Abbreviations: SHRED, shredders; COL-GA, gathering collectors; COL-FIL, filtering collectors; SCR, scrapers; PRED, predators including fish fry (see Merritt and Cummins 1984, Cummins and Wilzbach 1985).

Drift/benthos mortality experiments: Drift and benthos collections taken using the drift/benthos partitioner showed no significant effects of the *B.t.i.* release on mortality of the partitioned population components (Table 2), with the exception of an increase (from 1 to 19) in the number of dead black fly larvae encountered in the drift samples prior to the mortality tests. As reported in a previous study (Cummins and Wilzbach 1988), mortality of the animals held for 12 h was always higher among individuals from the drift than from the benthos collections, but in neither case was there a significant dif-

Table 2. Mortality and gut fullness of invertebrate taxa from the drift-benthos partitioner.

Time/sample	Number of taxa	Number of individuals	% mortality	Mean % gut full	Mean % foregut
Before/drift	13	29	20.7	69.3	69.2
After/drift	8	30	16.7	65.7	71.4
Before/benthos	9	33	6.1	58.8	51.5
After/benthos	9	47	1.0	53.1	85.1

ference between the collections taken before and after treatment. In addition, there was no effect on the feeding (gut fullness) of the invertebrates that could be attributed to the application of the pesticide. No significant difference was measured before and after *B.t.i.* release in either the percentage of individuals with full guts or those with greater than half-full foreguts (the latter being a reflection of the most recent feeding activity; see Table 2).

Mortality estimates from substrates: Counts of live and dead black flies and midges for mortality estimates came from both artificial and natural substrates because there was variation in colonization by these insects on the different substrates. Mean mortality estimates for these two groups at the different sites are shown in Fig. 7. For black flies (Fig. 7A), there was 0% mortality at the site upstream from the point of application, and 0% mortality at the 4,500 m downstream site; these two sites did not differ in percent mortality (one-way ANOVA and Duncan's multiple range test, $P > 0.05$). At sites ranging from 100 to 2,200 m downstream, there was nearly 100% mortality of black fly larvae, and these sites did not differ (one-way ANOVA and Duncan's multiple range test, $P > 0.05$). At the 3,200 m site, black fly mortality averaged 30%, and this site differed from all other sites (one-way ANOVA and Duncan's multiple range test, $P < 0.05$).

Rheotanytarsus midge mortality was much lower than black fly mortality at the downstream sites (Fig. 7B). There was some apparent unexplained midge mortality (possibly owing to handling), as the mortality estimate for the site upstream of the release point was about 10%; one-way ANOVA and Duncan's multiple range test showed that the mortality at this site was not significantly different ($P > 0.05$) from the 300, 1,800 and 2,100 m downstream sites. However, there was a significantly higher mortality estimate (37%, $P < 0.05$) at the 100 m downstream site than at the other sites.

Mortality estimates from benthos: Results of Surber samples are shown in Table 3. There were sufficient numbers of larvae from the following insect families to compare numbers collected between pre- and posttreatment samples: Baetidae, Ephemerellidae and Heptageniidae

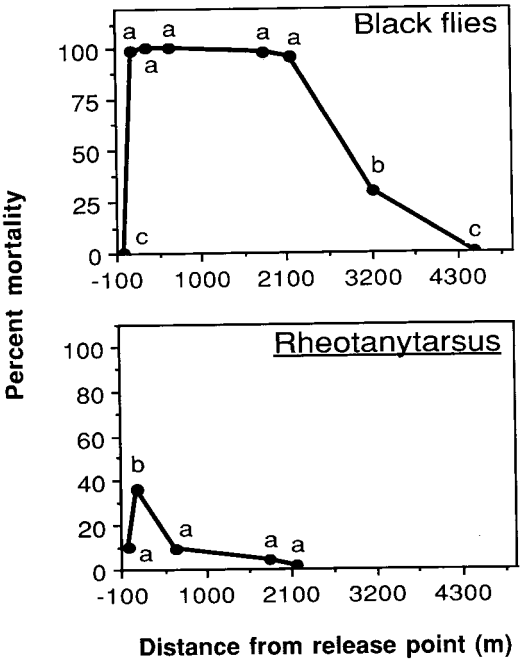

Fig. 7. Mean percent mortality of black fly and *Rheotanytarsus* larvae on artificial and natural substrates in the Betsie River, after release of *B.t.i.* Means with the same letter are not significantly different (one-way ANOVA and Duncan's multiple range test, $P > 0.05$).

(Ephemeroptera); Gomphidae (Odonata); Hydropsychidae, Leptoceridae and Philopotamidae (Trichoptera); Elmidae (Coleoptera); and Chironomidae (Diptera). *T*-tests of Surber samples paired by position in the transects showed no significant difference ($P > 0.05$; see Table 3) between pre- and posttreatment samples for these groups.

Short-term invertebrate growth experiment: The short-term in situ growth experiment with *Stenonema* sp. larvae showed no measurable effects of the *B.t.i.* treatment when the chambers placed above and below the release site were compared. Mortality, which was high in both the boxes above (mean = 64%) and below (mean = 78%) the point of *B.t.i.* release, was not significantly different (Mann-Whitney U = 75; $n_1,n_2 = 18$; $P > 0.05$). Growth rates (Table 4)

Table 3. Results of Surber samples taken before and after *B.t.i.* application below the site of application in the Betsie River.

Taxon	Mean (range) Before	After	*t*-test
Tricladida			
Planariidae	0.6 (0–2)	0.2 (0–1)	ND
Oligochaeta	0.1 (0–1)	0.0 (0)	ND
Gastropoda			
Pleuroceridae	1.4 (0–7)	1.6 (0–3)	ND
Pelecypoda			
Sphaerium	2.0 (0–9)	1.2 (0–5)	ND
Isopoda			
Asellidae	0.0 (0)	0.2 (0–1)	ND
Amphipoda			
Gammaridae	0.2 (0–1)	0.2 (0–1)	ND
Ephemeroptera			
Baetidae	6.2 (0–13)	10.4 (4–14)	1.69NS
Ephemerellidae	5.0 (2–10)	9.6 (2–24)	0.32NS
Heptageniidae	11.6 (1–20)	12.6 (1–29)	0.90NS
Siphlonuridae	0.0 (0)	0.2 (0–1)	ND
Odonata			
Gomphidae	1.6 (0–4)	3.6 (2–6)	0.06NS
Megaloptera			
Nigronia	0.0 (0)	1.0 (0–5)	ND
Trichoptera			
Hydropsychidae	148.0 (91–229)	129.6 (65–242)	0.69NS
Leptoceridae	4.8 (0–22)	6.0 (0–12)	0.85NS
Philopotamidae	2.8 (0–6)	12.2 (0–59)	0.43NS
Glossosomatidae	0.2 (0–1)	0.0 (0)	ND
Helicopsychidae	0.0 (0)	0.2 (0–1)	ND
Hydroptilidae	0.2 (0–1)	0.0 (0)	ND
Limnephilidae	1.8 (0–5)	1.0 (0–4)	ND
Coleoptera			
Elmidae	23.0 (10–50)	35.6 (11–92)	0.54NS
Diptera			
Chironomidae	89.8 (38–173)	72.6 (41–140)	0.67NS
Simuliidae	2.8 (0–8)	0.4 (0–2)	ND

ND, paired *t*-test not done; NS, *t*-test not significant.

Table 4. Weight gain and growth rate of *Stenonema* sp. larvae (Ephemeroptera: Heptageniidae) held for six days (138 degree-days) in enclosures above and below the point of *B.t.i.* release. Relative growth rate (RGR, Waldbauer 1968) is given as percent body weight increase per day and per degree-day.

Weight by site/time	n	Mean weight in mg (SE)	RGR Per day	Per degree-day
Initial	51	0.778	13.9	0.6
Final above	18	1.896	13.9	0.6
Final below	12	2.619	18.1	0.8

were also high in both sets of chambers, being slightly greater in those below the treatment site.

Fish studies: The electrofishing collections were dominated by rock bass and creek chub (*Semotilus atromaculatus*). These were the only species that were taken in significant numbers in every sample (Table 5), although 21 species of fish were collected. A few brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) were taken, but their distributions were highly restricted to areas at groundwater seeps because of the warm water temperatures. In general, fish were more abundant at the above-release stretch both before and after treatment, than at the below-release stretch before and after treatment. There was no significant difference in the relative numbers of fish caught below the *B.t.i.* release point when collections made before and after application were compared (Mann-Whitney U = 174; n_1,n_2 = 19,19; $P > 0.05$). Similarly, there was no significant difference in the relative numbers of fish caught above the *B.t.i.* release point when collections made before and after application were compared (Mann-Whitney U = 172; n_1,n_2 = 19,19; $P > 0.05$). Also, the variations between the upstream and downstream collections were indistinguishable from those observed between the samples taken in the control area above the release point before and after treatment.

Using rock bass as a test species, length-

Table 5. Fish diversity and abundance at sample sites in the Betsie River. Numbers are totals of two passes of electrofishing collections from 100 meter stretches above and below the *B.t.i.* application site. Collections were made at both sites in the day prior to, and the two days after, *B.t.i.* application.

Taxon	Above release		Below release	
	Before	After	Before	After
Petromyzonidae				
Lampetra lamottei	3	6	0	1
Salmonidae				
Salvelinus fontinalis	0	0	3	2
Salmo trutta	0	1	1	2
Umbridae				
Umbra limi	3	9	0	0
Cyprinidae				
Notropis cornutus	1	1	2	2
N. atherinoides	0	0	1	0
Semotilus atromaculatus	37	85	19	16
Clinostomus elongatus	11	0	0	0
Rhinichthys atratulus	12	1	2	4
R. cataractae	0	0	1	1
Catostomidae				
Catostomus commersoni	0	0	6	0
Ictaluridae				
Ictalurus natalis	0	0	0	1
I. nebulosus	1	1	1	3
Noturus sp.	2	2	0	0
Centrarchidae				
Ambloplites rupestris	29	16	9	11
Lepomis gibbosus	1	4	0	0
Micropterus salmoides	1	0	2	0
Percidae				
Perca flavescens	7	0	4	0
Etheostoma caeruleum	1	0	0	0
E. nigrum	1	1	0	0
Cottidae				
Cottus cognatus	0	1	2	0

weight relationships were compared using fish from both the free-ranging population and the groups held in cages. The length-weight relationships for all samples conformed best to an exponential fit (R_2 values, 0.97–0.98; see Fig. 8). There was no mortality of caged rock bass. There was no significant difference in length-weight relationships of free-ranging rock bass collected below the application site before and after application of *B.t.i.* (Fig. 8A; Mann-Whitney U = 42; n_1,n_2 = 9,11; $P > 0.05$). Nor was there a significant difference in length-weight relationships of caged rock bass held above or below the application site (Fig. 8B; Mann-Whitney U = 26; n_1,n_2 = 8,10; $P > 0.05$). A few individual fish could be specifically identified permitting comparisons of weight changes over the 3-day holding period. In all cases, the rock bass lost between 0.8–1.0 g regardless of whether they were located above or below the point of *B.t.i.* release.

The rock bass consumed a wide variety of prey items, including many crayfish and terrestrial prey, which comprised 37% and 28% of total prey biomass, respectively. Caddisfly and mayfly larvae comprised 25% and 18% of total prey items. Prey selectivity was not evident, except with respect to size of prey, as items less than 2 mm in length were rarely present. Mean prey size, which was estimated from head capsule width when items were not intact, was 5 to 6 mm. Only 2 larval black flies were identified from 38 fish guts; both were from fish caged below the release site after *B.t.i.* application. Dipterans in general comprised less than 1% of prey biomass. Diet composition of rock bass was highly variable among individuals, both within and between the above- and below-release stretches, and before and after *B.t.i.* application. Kendall's coefficient of concordance (W), which ranges from 0 to 1 (perfect concordance), was used to evaluate similarity in rankings of the four treatments (above-before, above-after, below-before, below-after), with respect to diet composition classified by taxonomic groupings and by functional groups of prey items. Expressed either by biomass or density of prey items, similarity among the treatments in all cases was low and not significant for functional group density ($W = 0.29$), functional group bi-

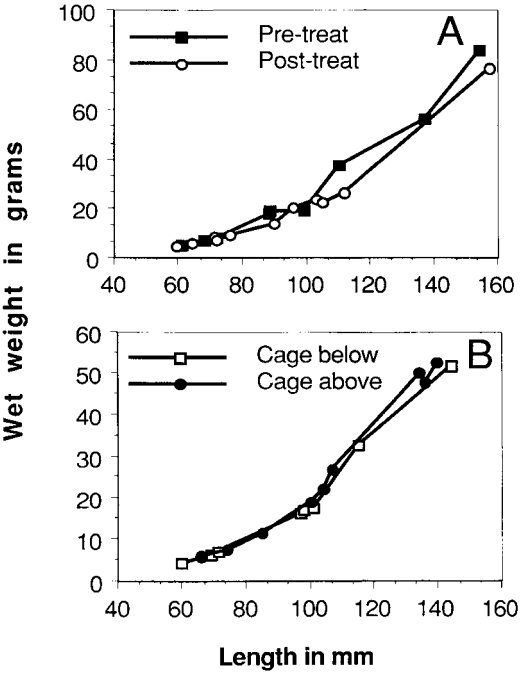

Fig. 8. Length-weight relationships of (A) free-ranging rock bass collected below the site of *B.t.i.* release both before and after treatment, and (B) caged rock bass held above and below the site of release.

omass ($W = 0.15$), taxon density ($W = 0.10$), and taxon biomass ($W = 0.40$), respectively ($P > 0.05$ for all).

DISCUSSION

Carry and effects of B.t.i. *on black flies:* In the Betsie River, direct counts of bacterial particles (including viable *B.t.i.* spores) using DAPI and epifluorescence microscopy revealed that the *B.t.i.* moved downstream as a slug after release (Fig. 2). This slug was detectable as far as 600 m below the release site, but by 1,800 m the *B.t.i.* spores had blended with background bacterial counts and could not be detected by our method. Black fly mortality was recorded as far as 3,200 m downstream from the release site, suggesting that direct counts of spores in river water samples may not accurately reflect *B.t.i.* carry or toxicity. This may be because modern liquid formulations contain predominantly crystalline toxin.

The effectiveness of *B.t.i.* against black fly larvae in the Betsie River was evident by the significant increase in drift density (Fig. 3B), and by mortality observed on natural and artificial substrates following treatment (Fig. 7A). Results from the drift/benthos mortality experiments and micro-drift studies showed that all black fly species were susceptible to *B.t.i.*, regardless of instar. However, most individual larvae were greater than 1 mm in size, as determined by the above studies.

The action of Teknar HP-D was fairly quick; larvae on substrates in the first 1,800 m were moribund or dead 2 h after treatment, but we did not observe larval detachment or drift until 12 h posttreatment. De Moor and Car (1986) observed that larvae often remained attached to substrates for several days after treatment, before being washed off by the current. Twenty-four hours after treatment and for the remaining 2 days, black fly drift densities were extremely low, far below pretreatment and above treatment levels, indicating a decline in the number of drifting larvae in the treated area. This occurrence is explained by studies showing that the distances traveled by drifting invertebrates is often less than 10 m (Townsend and Hildrew 1976). Thus, in this stream section it would probably have taken several days before drifting black fly larvae recolonized the treated areas from upstream sites.

Efficacy of *B.t.i.* declines as one moves further downstream from the application site (Molloy and Jamnback 1981). Maximum black fly mortality rates in our study (100%) occurred in the first 2,200 m downstream from the release site (Fig. 7A). However, a significant decline in the mortality rate (to ~30%) occurred at the 3,200 m site, and no mortality was recorded at 4,300 meters. Whether this decline was due to natural dilution of the toxin as it moved downstream, or to other factors, is unknown. However, the changes in river bed and channel morphology may have influenced carry. Immediately below 2,200 m the profile of the Betsie River changed from a cobble-gravel bottom, long riffle, shallow pool stream to a sand bottom, deep pool and meandering stream. A variety of factors are known to affect downstream carry of *B.t.i.* (see reviews by Lacey and Undeen 1986, Molloy 1989), two of them being stream profile and morphology (Undeen et al. 1984, Colbo and O'Brien 1984, Lacey and Undeen 1986). Both Molloy and Jamnback (1981) and Colbo and O'Brien (1984) found that "pooling" was a major impediment to good carry of *B.t.i.* in their stream systems, and there is some evidence from mortality studies on selected nontarget benthic insects that *B.t.i.* may adhere to substrates or be retained in depositional sediments (Undeen et al. 1984, Back et al. 1985, Molloy 1989). In these lower reaches of our study site, the possible "settling out" and/or "dilution" of *B.t.i.* caused by its movement through deep pools and contact with finer sediments could have decreased its carry.

When comparing other field trials that assessed the efficacy and carry of different *B.t.i.* formulations in temperate climates (Molloy 1989), we noted that the downstream distance in which larval black fly mortality occurred was very good in our study. Our comparisons were based on studies with rivers and streams having similar discharges and/or comparable application rates as those in the Betsie River (Car and De Moor 1984, Lacey and Undeen 1984, Back et al. 1985). We observed 100% mortality at 2,200 m and 30% at 3,200 m, while in other studies mortality never occurred or was not recorded more than 1,000 m below the application point. A number of factors could have been responsible for these differences. First, the elevated stream temperatures (22–24°C) during a severe drought year in Michigan undoubtedly influenced the level of activity of *B.t.i.* Comparative stream temperatures in other trials ranged from 9 to 20°C, with most below 15°C. Several investigators (Lacey et al. 1978, Molloy et al. 1981, Olejnicek et al. 1985, Lacoursiere and Charpentier 1988) have reported a positive correlation between temperature and *B.t.i.* activity against black flies, and the high mortality values that we obtained in this study (100%) were possibly associated with high water temperature conditions. Second, the formulations among studies were different. We used the liquid formulation Teknar HP-D, whereas wettable powder formulations or older liquid formulations

were used in some of the other studies. Field trials have indicated that WP formulations generally have less carry than liquid ones (Guillet et al. 1982, Lacey and Undeen 1984), based on the physics of particle size (Molloy et al. 1984). Other treatment parameters (i.e., concentration, application duration), although similar, were not standard for each study and may have influenced carry and mortality. Finally, factors such as stream discharge, profile (depth-to-width ratio) (Undeen et al. 1984), gradient and vegetation (Frommer et al. 1981) also may have acted solely or in combination to enhance the downstream carry and activity of *B.t.i.* in this study.

Effects of B.t.i. *on nontarget organisms:* We used several different methodologies in this study, including experiments not employed in previous studies that have assessed the effects of *B.t.i.* on target and nontarget organisms. Macro-drift, artificial or natural substrate colonization and Surber or Hess-type sampling have been the most common ways of assessing invertebrate mortality due to *B.t.i.* and other insecticides (Kingsbury 1975, Lacey and Mulla 1989). For invertebrates, we employed micro- and macro-drift studies, drift/benthos mortality experiments, short-term growth and feeding experiments, and examination of functional group composition. Few in situ fish studies have ever been conducted; therefore, we studied fish species composition, relative abundance, diet and length-weight relationships.

Our comprehensive approach to evaluating the effects of *B.t.i.* on nontarget organisms was developed after carefully reviewing published field studies and discovering that sampling variability, rather than the *B.t.i.*, may have been responsible for some of the reported population changes in nontarget organisms after treatment. This was particularly true for invertebrates not considered to be susceptible to *B.t.i.* (e.g., Ephemeroptera, Trichoptera). Molloy and Jamnback (1981) noted that large increases in nontarget organisms after treatment in their study may have been the result of small sample size and choice of sampling sites. Posttreatment increases in Trichoptera and Ephemeroptera in the Vaal River, Africa, were due to colonization of vacated habitats created when simuliid larvae drifted downstream (Carr and De Moor 1984). Several authors attributed decreases in benthic densities of nontarget organisms to emergence and pupation after treatment rather than to *B.t.i.* (Colbo and Undeen 1980, Burton 1984,[3] Gibbs et al. 1986). Although Pistrang and Burger (1984) recorded moderate increases of some drifting mayflies and caddisflies following *B.t.i.* treatment, there was no spatial control to the study and the emergence of some mayflies occurred during the study period. In the above studies, increases in drift of nontargets also may have been due, in part, to a temporary increase in particles introduced into the stream with the *B.t.i.* application (Lacey and Mulla 1989) or to effects of adjuvants and dispersants included in the product formulation (Pistrang and Burger 1984, Lacey and Mulla 1989). Thus, sampling variability can result from the choice and operation of sampling devices, physical features of the environment, field and laboratory sorting procedures, product ingredients and formulation, and biological features of the study organisms themselves (*cf.* Resh 1979, Merritt et al. 1984).

In addition to sampling variability, the spatial and temporal dynamics of a stream require special consideration in an environmental impact study such as this. A design must simultaneously include both spatial and temporal controls. As Green (1979) stated: "If the spatial control is missing and only before- and after-impact samples from an impacted area are available, one runs the risk that a significant change may be unrelated to the impact. . . . If the temporal control is missing, one may not detect that a difference between an area subject to the impact and an area not subject to it existed before the impact occurred." Although Green (1979) recommended an areas-by-times *factorial* analysis of data collected in an impact study, we advocate a *nested* analysis, where the temporal information is a level nested within the spatial level. A nested analysis provides for variation in natural temporal changes at different sampling sites and is especially applicable when each site is sampled several times before and after the impact occurs. We successfully applied a nested ANOVA to our macro-drift data, and indeed different patterns of drift at the above and below stream sites, before and after treatment with *B.t.i.*, were observed for certain taxa. For comparisons when samples were taken only immediately before and after treatment with *B.t.i.* at upstream and downstream sites, we opted to use simpler statistical comparisons (*t*-tests, Mann-Whitney U-tests) to examine differences within the "above-below and before-after" concept.

Within the 20 families of aquatic invertebrates investigated in this study, some variation in macro-drift was recorded above and below, and before and after treatment (Figs. 3 and 4). These observed increases and decreases in macro-drift patterns can be ascribed to sampling error, random factors and population processes

[3] Burton, D. K. 1984. Impact of *Bacillus thuringiensis* var. *israelensis* in dosages used for black fly (Simuliidae) control, against target and non-target organisms in the Torch River, Saskatchewan. M. S. Thesis, Department of Entomology, University of Manitoba, Winnipeg.

unrelated to *B.t.i.* application. We found no significant effect of *B.t.i.* on invertebrate microdrift composition, for either size class, total drift numbers, or total biomass when above and below sites were compared, before and after treatment. Further, no discernible shifts in taxonomic or functional group composition could be associated with the *B.t.i.* application. Short-term growth experiments with *Stenonema* showed no measurable effects of the *B.t.i.* treatment, and there was no effect on invertebrate feeding activity. The high mortality rates of *Stenonema* may have been largely a natural consequence associated with the growth of the larvae, as reported previously for *Leptophlebia* larvae (Cummins and Wilzbach 1988).

The numerous field trials evaluating the effects of *B.t.i.* on target and non-target invertebrates (see reviews by Molloy 1989, Lacey and Mulla 1989), indicate that only members of the dipteran suborder Nematocera have been shown to be significantly affected. We specifically examined chironomids belonging to the genus *Rheotanytarsus* because of published reports of their susceptibility to *B.t.i.* (Ali, 1981, Car and De Moor 1984, De Moor and Car 1986), and the fact that their microhabitat, food and mechanism of food acquisition were similar to that of black flies (Wallace and Merritt 1980, Coffman and Ferrington 1984). Our results showed that after considering field mortality, approximately 27% of the population of this midge was affected by *B.t.i.*, and that level of mortality was restricted to the first sampling station below the treatment site (Fig. 7B). *Rheotanytarsus* mortality was much lower than black fly mortality at sites farther downstream, and mortality at these sites was similar to or lower than mortality observed at the upstream control site.

No significant effect of *B.t.i.* on chironomid drift or midge densities from substrate sampling was detected in our study; however (except for *Rheotanytarsus*), we did not examine changes below the family level. If *B.t.i.* had caused acute toxicity and mortality in the resident chironomid populations in the Betsie River, we would have expected a similar pattern of macro-drift as was observed for black flies (compare Fig. 3B and Fig. 3D). Back et al. (1985) found no significant effect of *B.t.i.* on *Rheotanytarsus* but recorded reduced posttreatment densities of two other chironomid genera, *Eukiefferella* and *Polypedilum*. They also reported mortality in the Blephariceridae (Nematocera) at high dosages, whereas Gibbs et al. (1986) found no adverse effects of *B.t.i.* on this family at operational dosages in the field. Clearly, further research is needed, using comparable formulations and dosages, to properly assess the effects of *B.t.i.* on the Nematocera in the field.

There have been few field studies on the effects of *B.t.i.* on fish in streams. Laboratory studies report no effects of *B.t.i.* against mosquitofish, rain water killifish, sticklebacks (Garcia et al. 1980) or *Tilapia* sp. (Lebrun and Vlayen 1981) at labeled rates; however, with very high exposure rates (4,000 mg/liter), 50% of the *Tilapia* sp. died (LeBrun and Vlayen 1981). In a laboratory study, Fortin et al. (1986) reported no acute effects on brook trout fry either exposed to or fed *B.t.i.* at normal application rates. At a dosage of 3,000 mg/liter, the fry exhibited some behavioral changes but no mortality, while at dosages >4,500 mg/liter (i.e., levels 1,000 times greater than operational control dosages) there was observable mortality. Fortin et al. (1986) related this mortality experimentally to the presence of xylene and other monocyclic aromatic hydrocarbons in the particular formulation of *B.t.i.* used, rather than to *B.t.i.* toxins. Gibbs et al. (1986) found no changes in diets of slimy sculpin or brook trout in streams in Maine before and after treatment with *B.t.i.* and additionally found that black fly larvae comprised a small proportion of the diet of these fish. In the New River of West Virginia, Amrine (1982) similarly found that black fly larvae comprised a small proportion of shiner (*Notropis* sp.) diets. In a third order New York stream, Molloy (unpublished data) found no negative effects of *B.t.i.* application on brook trout, brown trout or slimy sculpin densities when sampled after 2.5 years of black fly elimination.

In our study, no effect of *B.t.i.* on numbers or species composition of fish was evident, because there was no change in numbers or composition of the fish community at the downstream stretch that occurred. The observed overall variation in collections is attributable to other, unknown factors related to the particular stretches sampled and to the prevailing hot, dry weather conditions. Detailed studies showed no shifts in length-weight relationships in wild-caught rock bass, nor any mortality or significant weight changes in caged rock bass, owing to *B.t.i.* treatment.

Diet composition of rock bass probably reflected prey availability. Because black flies comprised an insignificant portion of rock bass diets, it is not likely that black fly removal from a section of stream would affect diet composition or adversely affect fish growth. We conclude from these data that, under the conditions of our study, fish were not affected by *B.t.i.* release.

In summary, this short-term study found no significant effects of *B.t.i.* on major nontarget invertebrates and fish. The high selectivity, mortality and excellent carry obtained in this study showed that *B.t.i.* has good potential for use as a biological control agent against larval

black flies in Michigan streams. Future research should be directed at long-term studies in temperate climates, as are currently being conducted in the Adirondack Mountains of New York (D. Molloy, personal communication). In addition, studies evaluating the effects of black fly removal on stream predators, scavengers and the overall food web are advised and will be an avenue of research pursued by this laboratory in the future.

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