Monitoring the Black Fly Population Management Program in the New River below Bluestone Dam

Report Submitted to

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by

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Introduction

During the past few years there have been many complaints about black flies (Diptera: Simuliidae) in southern West Virginia. In the spring of 1986 the State of West Virginia announced plans to implement a pest management program in response to the requests of some citizens in the area. The West Virginia Department of Natural Resources (WVDNR) planned to apply the black fly larvicide Bacillus thuringiensis israelensis (Bti) to many miles of streams that were known to be breeding sites for Simulium jenningsi, the species responsible for the complaints.

One of the major breeding sites for S. jenningsi is the section of the New River from Bluestone Dam to Sandstone Falls. However, most of this section lies within the boundaries of the New River Gorge National River which is under the jurisdiction of the National Park Service (NPS). NPS policies prohibit the management of naturally occurring pests except under special circumstances. Therefore, the WVDNR planned to apply Bti in the New River only as far downstream as the Route 20 bridge, which is just above NPS boundaries. In order to assure appropriate management of the natural resources within a national river, the NPS contracted Virginia Polytechnic Institute and State University (V.P.I.& S.U.) to monitor the effects of the WVDNR pest management program on the biota of the New River.

The specific objectives of this study were:

1) to determine if black fly population densities were reduced within NPS boundaries;

2) to quantify the reduction in black fly population densities upstream from NPS boundaries;

3) to analyze the effects of Bti applications on nontarget organisms.

Literature Review

The control of black flies with Bti and other microbial pesticides has recently been reviewed by Lacey and Undeen (1986). The toxic component of the bacterium is the endotoxin located in the parasporal inclusions that are produced with the spore during sporulation. The toxin becomes activated in the high pH conditions of the midgut in black flies and mosquitoes. Within minutes after ingestion of Bti, histological changes begin to occur in the midgut.

The toxin is thought to produce a complete loss of osmotic regulation in the midgut cells. Eventually the cells swell, lyse, and are sloughed into the lumen of the gut. Death follows within 6 min at high concentrations or within 24 hrs at low concentrations.

Bti has been used widely in tropical regions as a successful means for suppressing populations of Simulium damnosum, the vector of onchocerciasis (river blindness). Blackflies do not vector debilitating diseases in North America, but Bti has begun to be used here for managing populations of several species which achieve pest status by virtue of their biting and general nuisance. Field evaluations have been reported in the literature from programs in Newfoundland (Colbo and O'Brien 1984), Quebec (Back et al. 1985), New York (Molloy and Jamnback 1981), New Hampshire (Pistrang and Burger (1984), Tennessee (Lacey and Undeen (1984), and South Carolina (Horosko and Noblet (1986). In addition, there is a large-scale program for controlling black flies in the Susquehanna River at Harrisburg, Pennsylvania, which has not yet been reported in the primary literature.

All of these studies indicate that applications of Bti can significantly reduce densities of black fly larvae. Concentrations of Bti and durations of application which have proven to be effective range from 10 to 15.9 ppm of stream discharge for 1 min, 0.2 to 2 ppm for 10 min, and 0.5 to 1.3 ppm for 15 min (summary by Gaugler and Finney 1982 and more recent papers by Colbo and O'Brien 1984, Lacey and Undeen 1984, Pistrang and Burger 1984, Lacey and Heitzman 1985, Horosko and Noblet 1986, Moor and Car 1986). Factors which affect downstream transport of Bti include discharge, turbulence, pools, and width/depth ratio; effectiveness is also influenced by larval feeding rates which are inhibited by excessive suspended particulate matter or low temperatures (Lacey and Undeen 1986). Populations of some species recover quickly after Bti applications, hence frequent larviciding is necessary, sometimes as frequently as every week (Horosko and Noblet 1986).

The effects of Bti on nontarget organisms have been examined in a few studies, but in general, far less attention has been devoted to measuring ecological effects than improving efficacy. Molloy and Jamnback (1981) treated a small stream at 0.5 ppm for 15 min and evaluated the effects on nontarget organisms by taking Surber samples at 1 day pre-treatment and 5 days post-treatment at downstream locations and an upstream control. They reported increased densities of Ephemeroptera, Trichoptera, Plecoptera, Chironomidae, and Elmidae, and concluded that there were no adverse effects on any nontarget populations. Their results were somewhat confusing because populations increased by

higher percentages at the upstream control site. Lacey et al. (1982) treated a large river in the Ivory Coast at 1.5 ppm for 10 min and collected nontarget organisms with artificial substrates before and 20 and 50 hrs after treatment. They found no evidence of adverse effects, although the numbers of organisms on the artificial substrates were quite low, probably due to the short colonization time (1 week). Pistrang and Burger (1984) treated a small stream at 10 ppm for 1 min and analyzed effects on nontarget organisms by taking drift samples and 5-min kick samples. They observed temporary increases in the drift of Ephemeroptera, Trichoptera, and possibly Plecoptera, but the only nontarget organisms for which they observed direct mortality were some Chironomidae. The results of this study are also difficult to interpret because of design problems. Sampling units were not large enough leading to few individuals being collected, and there were no replicates to facilitate statistical comparisons.

Chironomidae, which belong to the nematocerous Diptera along with the Simuliidae, are variably susceptible to Bti depending on feeding habits, physiology, and habitat (Lacey and Undeen 1986). Ali et al. (1981) conducted 48 hr laboratory bioassays with first and third instars of several species of chironomids. LC_{50} values for first instar larvae ranged from 0.034 to 0.18 ppm, while LC_{90} values ranged from 1.32 to 3.71 ppm. For third instar larvae LC_{50} values were 3.23 to 14.71 ppm, and LC_{90} values were 6.85 to 38.60 ppm. Back et al. (1985) deliberately treated a small lake-outlet stream at 5.86 ppm for 15 min, a dosage that was 5.48X greater than the maximum recommended application. They monitored the impacts on nontarget organisms using drift nets and artificial substrates. There was no evidence of increased drift in Ephemeroptera, Plecoptera, Trichoptera, or Chironomidae, but larvae of another family of nematocerous Diptera, the Blephariceridae, increased their drift by 50X. Densities of nontarget organisms on the artificial substrates were not reduced except for two genera of chironomids which were reduced by 74 and 61%. Drifting larvae of another genus showed symptoms of toxemia from Bti. The affected organisms were thought to have ingested Bti sedimented on the bottom of the stream or attached to periphyton growing on rocks. Moor and Car (1986) recorded a decrease in Chironomidae, as well as several genera of Ephemeroptera and Trichoptera, after a normal application of Bti (1.6 ppm for 10 min). However, their sampling design (5 replicate stones in current) was not sufficient to detect significant statistical differences. Moor and Car (1986) recommended careful screening of Bti against co-existing aquatic fauna in each area where it is to be used. They also pointed out that all tests on nontarget organisms have been of short duration, and recommended that studies on the long term effects of Bti be undertaken.

Methods

Samples were taken at two sites which were numbered the same as in a recent study (V.P.I.& S.U. 1985). Site 5 was immediately below Bluestone Dam at the Corps of Engineers Park. Site 6 was approximately 4 miles downstream from Bluestone Dam in an area known as Longbottom. Site 5 was not within NPS boundaries and was treated with Bti on 14 occasions from May 5 to September 29, 1986. Site 6 was approximately 1 mile within NPS boundaries and was not intentionally treated with Bti. Applications were made only as far downstream as the Route 20 bridge which is approximately 0.5 miles above the NPS boundary. Table 1 contains a record of the Bti treatments in the New River (Mike Zeto, WVDNR, personal communication). This study evaluated the effects of the Bti applications made on July 21 and September 5.

The sampling schedule followed the recommendations of Undeen and Lacey (1982) for evaluating the immediate effects of Bti on black flies and nontarget organisms. Their protocol recommends collecting pre-treatment samples within 24 hrs immediately prior to the application and post-treatment samples 48 hrs after the application. The rationale for this schedule is that by the time 48 hrs has passed, black fly larvae subjected to Bti will have died and been removed from their attachment sites, yet the total elapsed time of 72 hrs between samples makes it unlikely that natural population fluctuations (e.g. adult emergence) will have occurred. This protocol was followed exactly for the July 21 Bti application, with pre-treatment samples collected on July 20 and post-treatment samples collected on July 23. It was not possible to adhere to this schedule for the September 5 Bti application. Pre-treatment samples were collected on September 2 in anticipation of Bti application on September 3 (Mike Zeto, WVDNR, personal communication). A storm delayed the arrival of the helicopters used for spraying, and the treatment was not made until September 5. Post-treatment samples were then collected on September 8. Thus, for the September 5 Bti application, pre-treatment samples were collected 72 hrs before application and post-treatment samples were collected 72 hrs after application.

Sample locations within each site were chosen with a stratified random design. Benthic macroinvertebrates are notorious for having heterogenous spatial distributions that necessitate taking tremendous numbers of replicate samples in order to draw statistically valid conclusions (Elliott 1977, Resh 1979, Allan 1984). By stratifying the sampling to a particular type of habitat, the variability (and the number of replicates) can often be reduced to manageable levels (Resh 1979). An appropriate sampling stratum for the New River is within the growths of the attached macrophyte

Podostemum ceratophyllum on the rock outcrops (Voshell 1985). Analyses of previous data indicated that 20 replicates would provide what is considered to be a reasonable level of precision for benthic macroinvertebrate studies, a standard error within 20% of the mean (Elliott 1977). This level of precision would make it possible to detect changes in population densities of 50% or less for the major taxa on the outcrops.

Samples were collected with the Rock Outcrop Community Sampler (ROCS). This sampler was designed specifically for large river studies (Hiner and Voshell 1984) and has proven to be successful for other studies in the New River (V.P.I.& S.U. 1985, Voshell 1985). The ROCS is a square box that samples an area of 0.01 m^2 and is 80 cm high. It was placed randomly on a rock outcrop in an area where Podostemum was growing. A 6.5 cm wide strip of foam sealed the bottom, then long-handled tools were used to rub and scrape the rock surface. A light-weight diaphragm pump designed to handle slurries was used to transport the sample contents into a 100 um mesh nylon net. After the ROCS was pumped dry, approximately 2 liters of water was poured into the sampler and pumped out twice to make sure that all macroinvertebrates and vegetation were collected. The samples were preserved in a 5% solution of formalin and assigned codes that were unique for site, date, and replicate.

In the laboratory the preserved samples were washed through two sieves (355, 106 um), the smallest sieve having a mesh approximately the same as the 100 um mesh net on the ROCS. The material retained on each sieve was examined with a stereomicroscope at 4 - 10 X magnification. All macroinvertebrates were removed from the debris. Three taxa in the samples were enumerated: the target black fly Simulium jenningsi, a nontarget caddisfly Cheumatopsyche campyla, and a nontarget group of midges in the subfamilies Orthocladiinae/Chironominae. The nontarget taxa were chosen because they were known to be the dominant organisms in the benthic macroinvertebrate community (Voshell 1985). Results were expressed as numbers of organisms per square meter. The Podostemum in each sample was retained, dried at 60°C for 24 hrs, weighed to the nearest 0.1 g on a triple beam balance, and its abundance reported as g dry weight per square meter.

Several physical/chemical parameters were measured on each occasion in order to consider any possible complicating effects of water quality.

Temperature -- long-stem thermometer on each sampling date; continuous recording thermograph for the entire period.

Conductivity -- YSI Model 33 conductivity/salinity meter.

pH -- Fisher Accumet Model 640 meter with Fisher calomel reverse-sleeve reference electrode (#13-639-61) and Fisher universal glass electrode (#13-639-3).

Dissolved oxygen -- Azide modification of Winkler method (APHA et al. 1980).

Seston -- total suspended particulate organic matter as ash-free dry weight (Voshell 1983).

Discharge -- data obtained from Huntington District, U. S. Army Corps of Engineers. Bluestone Gauge located below Bluestone Dam but above confluence with Greenbrier River; Hinton Gauge located in town of Hinton below confluence with Greenbrier River.

Data were stored and analyzed using an IBM-PC computer with two software packages: SUPERCALC3 (Sorcim/IUS Micro Software, San Jose, CA) and STATISTIX (NH Analytical Software, St. Paul, MN). Separate t-tests were used to compare the pre- and post-treatment means for each of the two Bti applications monitored during 1986. In addition, separate t-tests were used to compare the pre- and post-treatment means from 1986 with the means from approximately the same dates in 1983 (Voshell 1985). The null hypothesis for all tests was stated as Ho: no significant difference between means. The null hypothesis was rejected (significant difference between means) at the 5% level of significance ($p < 0.05$). The main concern with using parametric statistics on benthic macroinvertebrate data is to make sure that the assumption of equal variances is not violated (Elliott 1977, Allan 1984). Prior to comparing the means with t-tests, separate F-tests were conducted on the same samples to compare their variances. For those comparisons in which the variances proved to be unequal ($p < 0.05$), the data were transformed by log $(x + 1)$, and the variances compared again. If the variances could not be made equal, then the means were compared (transformed data) using the precautionary methods recommended by Snedecor and Cochran (1980) for unequal variances.

Results

The temperature and discharge of the New River around the times of the two Bti applications that were monitored are presented in Fig. 1. Temperature was reasonably constant during both periods, with only slight, normal diel fluctuations occurring. Discharge decreased very slightly (300 - 400 cfs) during monitoring of the July 21 application, and increased substantially (6000 - 6100 cfs) during monitoring of the September 5 application. In order to facilitate comparisons between 1986 and 1983, temperature and discharge for each year were plotted in Fig. 2. The

shapes of the temperature curves were very similar in both years. Temperatures during July were about the same in both years, but the temperature at the first of September was about 6°C cooler in 1986. The annual cycle of discharge was consistent for both years with sharp peaks in winter and spring and relatively stable flows during the summer. The increased discharge that occurred during the September 5, 1986 monitoring was not similar to the flow during 1983. However, it can be seen from the hydrographs that the magnitude of the increase on that occasion was not unusual for the New River.

Other physical/chemical properties of the New River during the 1986 monitoring studies are presented in Table 2. There were no unexpected values for any parameters, and none of the measurements indicate any conditions that would be deleterious to aquatic life.

The counts of individual organisms in each of the separate replicate samples are recorded in Appendices 1 - 8 which are attached at the end of this document. These raw data are also stored permanently on diskette.

Descriptive statistics for the July and September monitoring are summarized in Tables 3 and 4, respectively. A noteworthy result is the comparativeley high precision of the sampling. The original goal of precision equal to 20% was met in almost all cases. The only exceptions were post-treatment samples of S. jenningsi where high variance would be expected from the effects of the pesticide. The results of this study have much higher levels of precision than commonly reported for benthic macroinvertebrates. This favorable outcome is likely the result of stratified sampling in a uniform habitat and taking numerous small replicate samples. The benefit of high precision is that one can place more confidence in the results and their interpretations.

Results of the separate t-tests for the July and September monitoring are presented in Table 5 and 6, respectively. After the July 21 Bti application, there were significantly lower densities of S. jenningsi at both Sites 5 and 6. The only other significant difference between pre- and post-treatment means was for chironomids at Site 6. In this case the post-treatment mean was significantly higher than the pre-treatment mean. Following the September 5 Bti application, the post-treatment means of S. jenningsi again were significantly lower than the pre-treatment means at both sites. The means of both Orthocladiinae/Chironominae and Cheumatopsyche also were significantly lower in post-treatment samples than pre-treatment samples from both Site 5 and Site 6 during the September monitoring.

Comparisons of means from 1986 monitoring studies with means from the closest dates during a 1983 production study (Voshell 1985) are presented in Tables 7 and 8. Comparisons were only possible for Site 5 because the 1983 study was conducted only at that site. Two dates from 1983 (one before and one after) were selected for comparison with each of the two dates in 1986. Table 7 contains the comparisons with the July 21, 1986 monitoring samples (July 4 and August 1, 1983). Means of S. jenningsi in both pre- and post-treatment July 1986 samples were significantly lower than 1983 samples. In most cases, means of chironomids and caddisflies were significantly higher in July 1986 than 1983. Means of Cheumatopsyche in post-treatment samples on July 23, 1986 did not test significantly different from means on July 4, 1983 and August 1, 1983. In no case was the mean from either July 4 or August 1, 1983 significantly higher than 1986.

Results of comparisons of September 1986 samples (Table 8) also demonstrated that densities of S. jenningsi were significantly lower than on August 29 and September 26, 1983. However, comparisons of means of chironomids and caddisflies in September 1986 with means in 1983 gave opposite results from the July 1986/1983 comparisons. Means of chironomids and caddisflies in both the pre- and post-treatment samples in September 1986 were significantly lower than means in samples taken on August 29 and September 26, 1983.

Discussion

It is not surprising that S. jenningsi densities were significantly reduced at Sites 5 and 6 by applications of Bti on July 21 and September 5, 1986. The dosages applied to the New River (22 - 30 ppm for 1 min) are among the highest reported in the primary literature (see Literature Review). These heavy dosages would be expected to carry from the Route 20 bridge downstream into the NPS boundaries, as documented by the statistically significant reductions at Site 6.

Analyses of the monitoring results for the July 21 application (Table 5) would seem to indicate that there were no adverse impacts to the dominant nontarget organisms (caddisflies and chironomids). There were no significant reductions of these taxa between pre- and post-treatment (chironomids actually increased at Site 6), and means were higher in 1986 than similar dates in 1983 (Table 7). However, the monitoring results for the September 5 application (Table 6) support the opposite conclusion that there were adverse impacts to the dominant nontarget organisms. In September there were statistically significant reductions of caddisflies and chironomids between pre- and post-treatment, and means were

significantly lower in 1986 than comparable dates in 1983 (Table 8). There are several possible explanations for these seemingly anomalous results.

A moderate increase in discharge occurred between the pre- and post-treatment sampling (Fig. 1). The higher flows could have dislodged some organisms, thereby lowering densities in the post-treatment samples. When the annual hydrographs are examined (Fig. 2), the increase in discharge between September 2 and 8, 1986 does not seem to be such an unusual event that it would reduce densities so drastically. Also, the mean densities estimated from pre-treatment samples on September 2 were not affected by the increased flow, and those means were still significantly lower than comparable means in 1983. Interpretation of the 1986/1983 comparison is complicated by the fact that water temperature was 6°C lower on September 2, 1986 than August 29, 1983. The lower temperature in 1986 was caused by an exceptional cold front in August when flows were lower than normal. The decreasing water temperature could have triggered an emergence of caddisflies and chironomids, thereby causing densities to be lower on September 2, 1986 than August 29, 1983. However, the caddisflies and chironomids in the New River do not enter diapause and disappear from the environment as the black flies do (Voshell 1985). The caddisflies and chironomids merely cease to recruit new individuals into their populations during the winter, and exhibit gradually declining densities due to natural mortality until recruitment begins again in the spring. The degree of difference between the comparable samples in 1986 and 1983 appears to be greater than what would be expected solely from natural mortality.

Another possible interpretation of these results is that the consistent application of high doses of Bti throughout the summer eventually produced adverse effects on the dominant nontarget organisms. Chironomids, especially the first instars, are susceptible to Bti, and the dosages applied to the New River might well have been high enough to be toxic (Ali et al. 1981). The chironomids in the New River would be likely to ingest the material from sediments in the mats of Podostemum (Back et al. 1985). Reduced densities may not have been detected in July because 48 hrs may not be long enough under field conditions for the effect to be manifested (Moor and Car 1986). Hare and Nasci (1986) found delayed mortality in mosquito larvae 72 hrs after they were treated and removed to habitats without Bti. In September there was a longer time interval between pre- and post-treatment samples, and higher flows would lead to better carry and mixing. Toxic effects to chironomids during most of the summer would probably be quickly compensated for by recruitment of newly hatched individuals. However, at the cooler temperatures in September 1986, there may have been little recruitment.

The reduced densities of Cheumatopsyche in September 1986 were probably not the result of mortality from the Bti applications because there is no indication of toxicity to caddisflies in the literature. Bti applications are known to induce drift by caddisflies (Back et al. 1985, Pistrang and Burger 1985). Bluestone Dam prevents recolonization from upstream, therefore, drift losses can only be compensated for by recruitment of newly hatched individuals. Recruitment from eggs hatching should have been common during the July Bti application but might not have been occurring during the September Bti application. The same reasoning about drift could also be applied to the chironomids in the New River below Bluestone Dam.

It should be clear from the preceding discussion that it is not possible to interpret the results of the 1986 monitoring studies with any reasonable degree of certainty. However, this was the most detailed field evaluation of the effects of Bti on nontarget organisms yet conducted, and the results themselves are statistically valid and scientifically defensible. Densities of nontarget organisms did not decrease during the July application, and they did decrease during the September application. All previously reported results of studies on nontarget organisms must be viewed with a great deal of skepticism, because the studies were poorly designed to answer the questions being asked.

In actuality, short term studies such as this one and all previously reported studies will never adequately assess the ecological risk from Bti or any other pesticide. Ultimately ecological risk must be assessed at the ecosystem level (USEPA 1986). Ecosystems have both structural and functional properties, and their processes operate over longer temporal units. Short term impacts may be ameliorated by the inherent resiliency of an ecosystem, or, conversely, a particular impact may magnified by the interactive properties of an ecosystem. Ecological risk assessments that are adequate for comparing the costs to benefits of pesticides will only be obtained by conducting ecosystem level studies of appropriate duration.

Conclusions

1) Stratified sampling (rock outcrops) with numerous small sampling units ($0.01m^2$) provides precise benthic macroinvertebrate data that are amenable to statistical analysis.

2) Applications of Bti made on July 21 and September 5, 1986 significantly reduced the densities of Simulium jenningsi larvae in the New River from Bluestone Dam downstream at least 1 mile into the boundaries of the New River Gorge National River.

3) The densities of the dominant nontarget organisms (Cheumatopsyche campyla and Orthocladiinae/Chironominae) were not changed significantly during the July 21 Bti application.

4) The densities of the dominant nontarget organisms were significantly reduced during the September 5 Bti application.

5) It was not possible to explain the results obtained from the 1986 monitoring studies with any degree of certainty. Likely explanations for the opposing results for nontarget organisms in July and September include flow, temperature, life cycles, mortality caused by Bti, drift induced by Bti, and all possible combinations of these factors.

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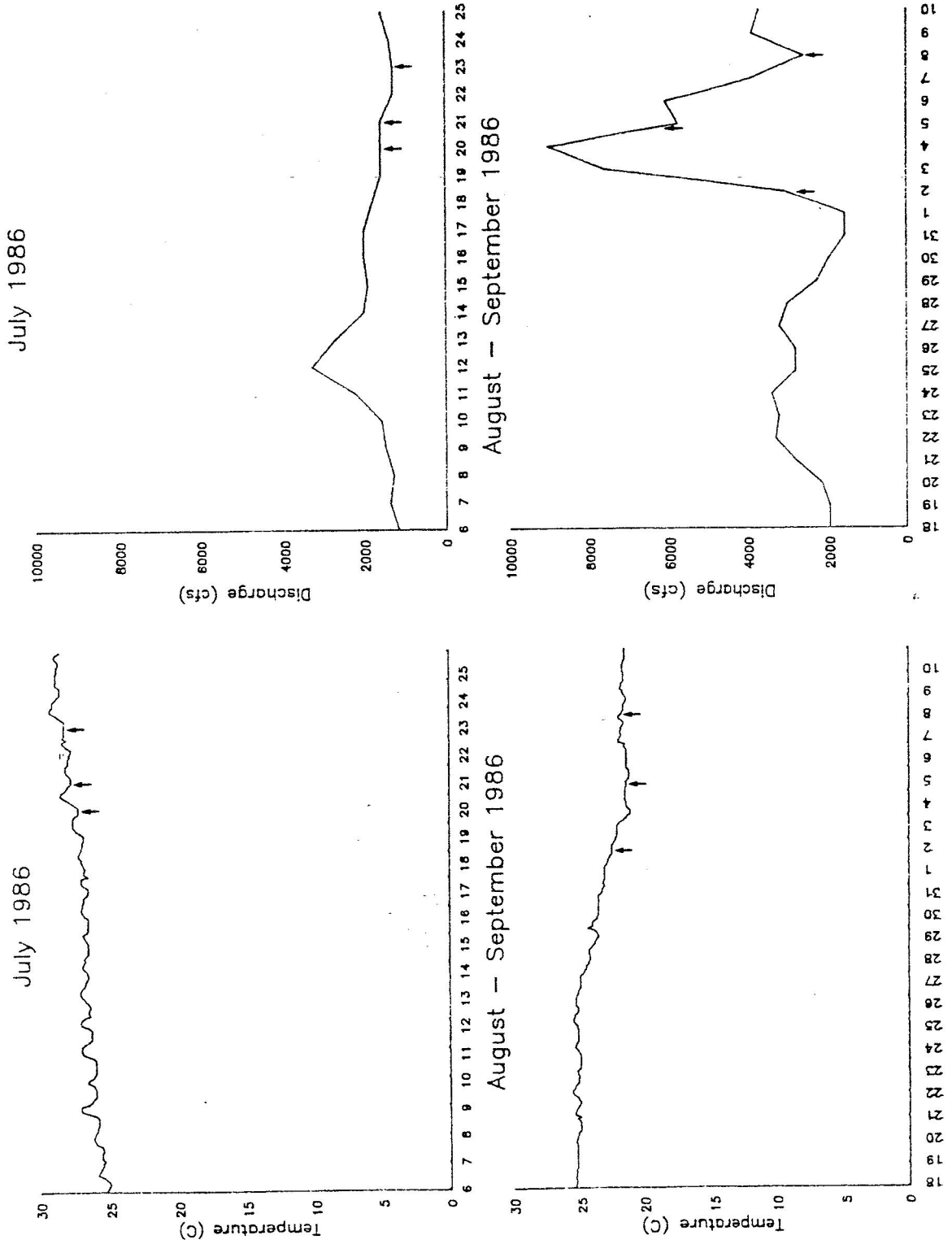


Fig. 1. Temperature and discharge at New River Site 5 during and two weeks preceding Bti applications that were monitored. Arrows indicate dates of pre- and post-treatment samples and Bti application.

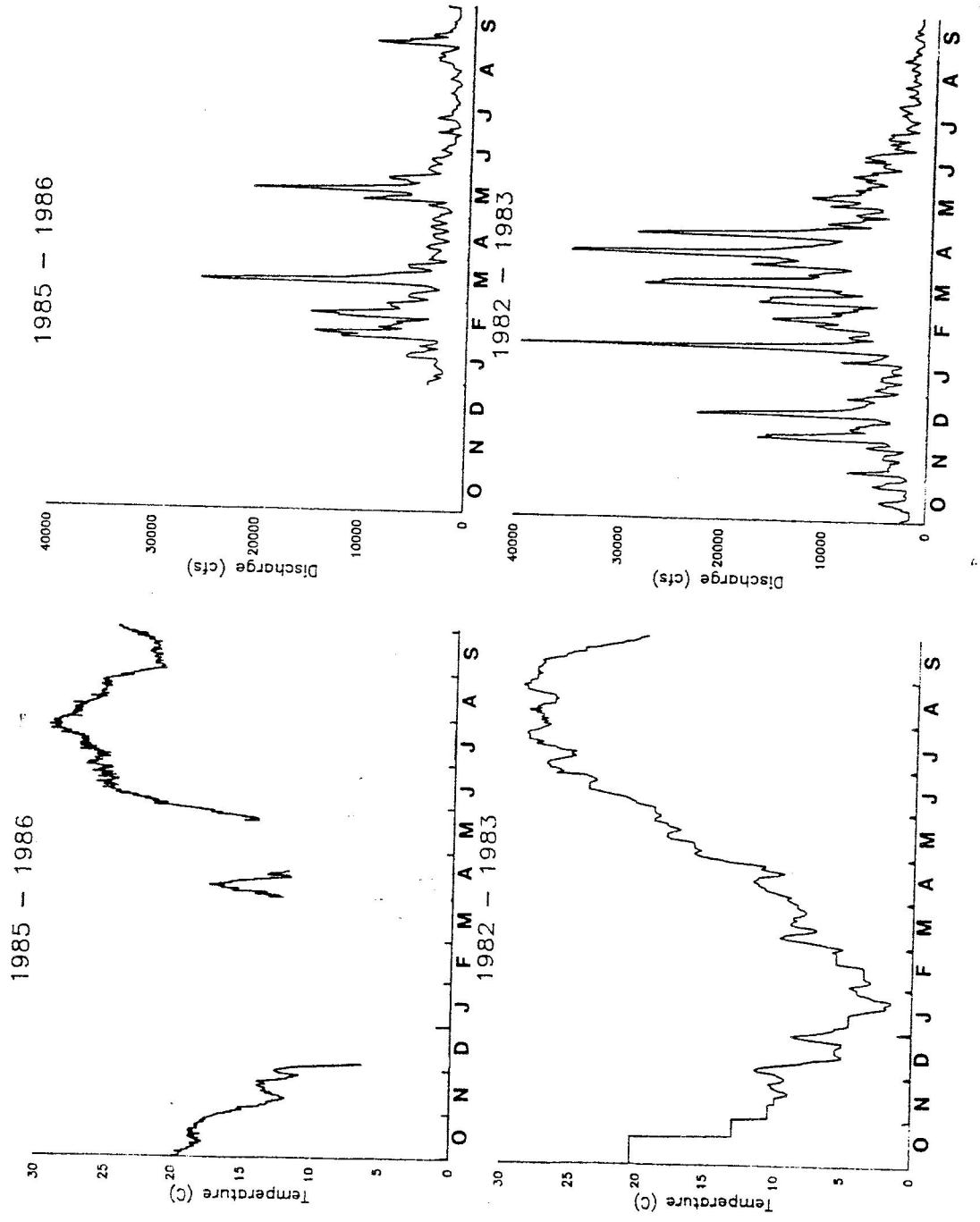


Fig. 2. Temperature and discharge at New River Site 5 during year with Bti applications (May - Sept 1986) and year with no applications.

Table 1. Records of Bti applications to the New River during 1986 (Mike Zeto, WVDNR, personal communication). Asterisk (*) denotes applications that were monitored.

Date	Bti Concentration (ppm for 1 min.)	
	Bluestone Dam to Rt. 3 Bridge	Rt. 3 Bridge to Rt. 20 Bridge
5 May	22	22
19 May	22	22
31 May	22	22
14 June	27	22
27 June	27	22
9 July	27	22
21 July*	27	22
30 July	27	22
8 Aug	27	22
16 Aug	30	30
25 Aug	30	30
5 Sept*	30	30
17 Sept	30	30
29 Sept	30	30

Table 2. Physical/chemical properties of the New River during Bti applications that were monitored.

Site/ Date	Temp (°C)	Cond. (umhos)	pH	D.O. (mg/l)	Seston (mgAFDW/l)	Discharge (cfs)
Site 5						
20 July	28.5	198	7.89	7.8	1.03	1600
21 July						1600
23 July	28.5	157	8.27	7.2	1.49	1300
Site 6						
20 July	27.0	180	8.01	8.0	.81	1900
21 July						1900
23 July	28.0	124	8.55	9.8	.56	1500
Site 5						
2 Sept	22.5	205	7.92	8.2	1.43	3100
5 Sept						5800
8 Sept	22.0	140	7.84	8.8	1.19	2600
Site 6						
2 Sept	21.0	250	8.71	9.9	1.27	3400
5 Sept						6500
8 Sept	20.0	155	7.85	8.1	1.10	3300

Table 3. Monitoring results (as density = number of individuals/m^2) for July 21, 1986 Bti application. SJENN = Simulium jenningsi; ORCHI = Orthocladiinae/Chironominae; CHEUM = Cheumatopsyche campyla; PRE = pre-treatment samples; POST = post-treatment samples; S.D. = standard deviation; S.E. = standard error; Prec. = Precision (S.E./Mean).

SITE	TAXA	TREAT.	MEAN	S.D.	S.E.	PREC.
FIVE	SJENN	PRE	3,710	2,382	533	.14
		POST	285	225	50	.18
	ORCHI	PRE	114,500	39,860	8,914	.08
		POST	110,600	63,600	14,220	.13
	CHEUM	PRE	55,930	17,220	3,851	.07
		POST	44,110	23,190	5,185	.12
SIX	SJENN	PRE	950	503	113	.12
		POST	640	317	71	.11
	ORCHI	PRE	7,780	3,786	847	.11
		POST	11,760	4,851	1,085	.09
	CHEUM	PRE	8,390	7,468	1,670	.20
		POST	4,930	2,667	596	.12

Table 4. Monitoring results (as density = number of individuals/m^2) for September 5, 1986 Bti application. Abbreviations same as Table 3.

SITE	TAXA	TREAT.	MEAN	S.D.	S.E.	PREC.
FIVE	SJENN	PRE	280	194	43	.15
		POST	5	22	5	1.00
	ORCHI	PRE	16,420	5,962	1,333	.08
		POST	8,555	3,570	798	.09
	CHEUM	PRE	20,850	12,000	2,683	.13
		POST	7,245	3,432	768	.11
SIX	SJENN	PRE	685	515	115	.17
		POST	10	31	7	.69
	ORCHI	PRE	4,245	2,038	456	.11
		POST	1,730	1,718	342	.20
	CHEUM	PRE	18,490	12,290	2,748	.15
		POST	5,015	3,822	854	.17

Table 5. Results of t-tests comparing pre-treatment (PRE) and post-treatment (POST) means for July 21, 1986 Bti application. SJENN = Simulium jenningsi; ORCHI = Orthocladiinae/ Chironominae; CHEUM = Cheumatopsyche campyla; EQ = equal variances, no transformation necessary; TR = equal variances after transformation by log (X + 1); UV = variances could not be equalized, t-tests conducted on transformed data by precautionary measures of Snedecor and Cochran (1980). Ho: no significance difference between means. Ho rejected at $p < 0.05$.

SITE	TAXA	TREAT.	EQUALITY OF VAR.?	MEAN	Ho Rejected?
FIVE	SJENN	PRE		3,710	
		POST	EQ	285	YES
	ORCHI	PRE		114,500	
		POST	EQ	110,600	NO
	CHEUM	PRE		55,930	
		POST	TR	44,110	NO
SIX	SJENN	PRE		950	
		POST	EQ	640	YES
	ORCHI	PRE		7,780	
		POST	TR	11,760	YES
	CHEUM	PRE		8,390	
		POST	EQ	4,930	NO

Table 6. Results of t-tests comparing pre-treatment and post-treatment means for September 5, 1986 Bti application. Abbreviations and explanations same as Table 5.

SITE	TAXA	TREAT.	EQUALITY OF VAR.?	MEAN	Ho Rejected?
FIVE	SJENN	PRE		280	
		POST	EQ	5	YES
	ORCHI	PRE		16,420	
		POST	EQ	8,555	YES
	CHEUM	PRE		20,850	
		POST	EQ	7,245	YES
SIX	SJENN	PRE		685	
		POST	EQ	10	YES
	ORCHI	PRE		4,245	
		POST	UV	1,730	YES
	CHEUM	PRE		18,490	
		POST	EQ	5,015	YES

Table 7. Results of t-tests comparing means from monitoring of July 21, 1986 Bti application with means from closest calendar dates during 1983 production study (Voshell 1985) at Site 5 only. Abbreviations and explanations same as Table 5.

Taxa	Date	Treat	Equality of var?	Mean	Ho Rejected?
SJENN	July 20, 86	PRE		3,710	
	July 4, 83	NONE	EQ	62,270	YES
ORCHI	July 20, 86	PRE		114,500	
	July 4, 83	NONE	EQ	47,930	YES
CHEUM	July 20, 86	PRE		55,930	
	July 4, 83	NONE	TR	42,860	YES
SJENN	July 23, 86	POST		285	
	July 4, 83	NONE	EQ	62,270	YES
ORCHI	July 23, 86	POST		110,600	
	July 4, 83	NONE	EQ	47,930	YES
CHEUM	July 23, 86	POST		44,110	
	July 4, 83	NONE	UV	42,860	NO
SJENN	July 20, 86	PRE		3,710	
	Aug 1, 83	NONE	EQ	13,000	YES
ORCHI	July 20, 86	PRE		114,500	
	Aug 1, 83	NONE	EQ	26,990	YES
CHEUM	July 20, 86	PRE		55,930	
	Aug 1, 83	NONE	EQ	34,000	YES
SJENN	July 23, 86	POST		285	
	Aug 1, 83	NONE	EQ	13,000	YES
ORCHI	July 23, 86	POST		110,600	
	Aug 1, 83	NONE	EQ	26,990	YES
CHEUM	July 23, 86	POST		44,110	
	Aug 1, 83	NONE	UV	34,000	NO

Table 8. Results of t-tests comparing means from monitoring of September 5, 1986 Bti application with means from closest calendar dates during 1983 production study (Voshell 1985) at Site 5 only. Abbreviations and explanations same as Table 5.

Taxa	Date	Treat	Equality of var?	Mean	Ho Rejected?
SJENN	Sept 2, 86	PRE		280	
	Aug 29, 83	NONE	EQ	3,965	YES
ORCHI	Sept 2, 86	PRE		16,420	
	Aug 29, 83	NONE	EQ	66,570	YES
CHEUM	Sept 2, 86	PRE		20,850	
	Aug 29, 83	NONE	TR	41,250	YES
SJENN	Sept 8, 86	POST		5	
	Aug 29, 83	NONE	EQ	3,965	YES
ORCHI	Sept 8, 86	POST		8,555	
	Aug 29, 83	NONE	EQ	66,570	YES
CHEUM	Sept 8, 86	POST		7,245	
	Aug 29, 83	NONE	EQ	41,250	YES
SJENN	Sept 2, 86	PRE		280	
	Sept 26, 83	NONE	EQ	3,115	YES
ORCHI	Sept 2, 86	PRE		16,420	
	Sept 26, 83	NONE	EQ	93,420	YES
CHEUM	Sept 2, 86	PRE		20,850	
	Sept 26, 83	NONE	EQ	46,880	YES
SJENN	Sept 8, 86	POST		5	
	Sept 26, 83	NONE	EQ	3,115	YES
ORCHI	Sept 8, 86	POST		8,555	
	Sept 26, 83	NONE	EQ	93,420	YES
CHEUM	Sept 8, 86	POST		7,245	
	Sept 26, 83	NONE	EQ	46,880	YES

APPENDIX 1. SITE 5, PRE-TREATMENT, JULY 20, 1986

Individuals per sample (0.01 sq. meter)

OCPRE = ORTHOCLADIINAE/CHIRONOMINAE PRE-TREATMENT
TANYPRE = TANYPODINAE PRE-TREATMENT
JENNPRE = S. JENNINGSI PRE-TREATMENT
CHEUPRE = CHEUMATOPSYCHE PRE-TREATMENT
PODPRE = PODOSTEMUM PRE-TREATMENT (grams dry weight)

CASE	OCPRE	TANYPRE	JENNPRE	CHEUPRE	PODPRE
1	1031.0	3.0	23.0	549.0	0.4
2	636.0	0.0	20.0	341.0	0.5
3	693.0	1.0	18.0	331.0	0.2
4	1846.0	1.0	33.0	771.0	0.6
5	1150.0	4.0	121.0	551.0	0.7
6	1533.0	0.0	29.0	752.0	0.4
7	1002.0	0.0	81.0	539.0	0.3
8	2196.0	2.0	39.0	948.0	0.7
9	1432.0	1.0	24.0	601.0	0.5
10	992.0	3.0	31.0	615.0	0.3
11	1302.0	1.0	32.0	654.0	0.4
12	1206.0	2.0	46.0	554.0	0.4
13	1119.0	3.0	28.0	614.0	0.6
14	1345.0	3.0	41.0	801.0	0.6
15	998.0	5.0	27.0	493.0	0.5
16	942.0	4.0	34.0	436.0	0.3
17	495.0	0.0	23.0	235.0	0.4
18	827.0	2.0	28.0	421.0	0.1
19	1134.0	1.0	35.0	464.0	0.5
20	1024.0	0.0	29.0	516.0	0.1

APPENDIX 2. SITE 5, POST-TREATMENT, JULY 23, 1986

Individuals per sample (0.01 sq. meter)

OCPOST = ORTHOCLADIINAE/CHIRONOMINAE POST-TREATMENT
TANYPOST = TANYPODINAE POST-TREATMENT
JENNPOST = S. JENNINGSI POST-TREATMENT
CHEUPOST = CHEUMATOPSYCHE POST-TREATMENT
PODPOST = PODOSTEMUM POST-TREATMENT (grams dry weight)

CASE	OCPOST	TANYPOST	JENNPOST	CHEUPOST	PODPOST
1	757.0	0.0	3.0	121.0	1.0
2	923.0	0.0	3.0	259.0	0.9
3	864.0	0.0	5.0	296.0	0.8
4	443.0	0.0	0.0	312.0	0.6
5	325.0	0.0	1.0	197.0	0.6
6	379.0	0.0	0.0	238.0	0.5
7	1378.0	0.0	4.0	467.0	0.4
8	1270.0	0.0	4.0	535.0	1.1
9	2485.0	1.0	4.0	902.0	0.3
10	1403.0	0.0	3.0	617.0	0.5
11	2271.0	0.0	3.0	799.0	0.7
12	1831.0	0.0	3.0	786.0	0.4
13	459.0	0.0	0.0	433.0	0.8
14	868.0	0.0	2.0	469.0	0.8
15	947.0	1.0	0.0	365.0	0.3
16	329.0	0.0	3.0	268.0	0.3
17	967.0	1.0	3.0	466.0	0.7
18	857.0	0.0	3.0	381.0	0.5
19	1491.0	1.0	3.0	135.0	0.6
20	1871.0	0.0	10.0	777.0	0.6

APPENDIX 3. SITE 6, PRE-TREATMENT, JULY 20, 1986

Individuals per sample (0.01 sq. meter)

OCPRE = ORTHOCLADIINAE/CHIRONOMINAE PRE-TREATMENT
TANYPRE = TANYPODINAE PRE-TREATMENT
JENNPRE = S. JENNINGSI PRE-TREATMENT
CHEUPRE = CHEUMATOPSYCHE PRE-TREATMENT
PODPRE = PODOSTEMUM PRE-TREATMENT (grams dry weight)

CASE	OCPRE	TANYPRE	JENNPRE	CHEUPRE	PODPRE
1	51.0	0.0	14.0	0.0	1.0
2	38.0	4.0	13.0	60.0	0.8
3	83.0	11.0	7.0	31.0	0.7
4	69.0	2.0	18.0	68.0	2.1
5	91.0	1.0	24.0	72.0	2.5
6	32.0	0.0	8.0	2.0	2.4
7	97.0	3.0	7.0	212.0	1.6
8	124.0	7.0	12.0	121.0	1.6
9	34.0	7.0	10.0	3.0	0.7
10	78.0	16.0	6.0	197.0	1.2
11	49.0	5.0	6.0	4.0	0.9
12	39.0	7.0	6.0	29.0	1.5
13	151.0	9.0	11.0	64.0	1.1
14	84.0	14.0	4.0	179.0	2.0
15	123.0	22.0	4.0	72.0	1.7
16	151.0	15.0	10.0	186.0	0.5
17	73.0	14.0	12.0	68.0	2.2
18	65.0	7.0	5.0	61.0	2.0
19	96.0	18.0	6.0	219.0	1.5
20	28.0	6.0	7.0	30.0	2.2

APPENDIX 4. SITE 6, POST-TREATMENT, JULY 23, 1986

Individuals per sample (0.01 sq. meter)

OCPOST = ORTHOCLADIINAE/CHIRONOMINAE POST-TREATMENT
TANYPOST = TANYPODINAE POST-TREATMENT
JENNPOST = S. JENNINGSI POST-TREATMENT
CHEUPOST = CHEUMATOPSYCHE POST-TREATMENT
PODPOST = PODOSTEMUM POST-TREATMENT (grams dry weight)

CASE	OCPOST	TANYPOST	JENNPOST	CHEUPOST	PODPOST
1	116.0	7.0	2.0	20.0	2.2
2	67.0	4.0	7.0	23.0	1.9
3	104.0	4.0	5.0	19.0	1.7
4	55.0	9.0	8.0	25.0	1.5
5	102.0	11.0	12.0	8.0	1.6
6	31.0	7.0	1.0	91.0	0.8
7	131.0	7.0	9.0	60.0	1.5
8	115.0	9.0	4.0	41.0	1.6
9	115.0	12.0	11.0	97.0	2.1
10	180.0	6.0	3.0	56.0	2.1
11	160.0	11.0	5.0	99.0	1.3
12	66.0	3.0	1.0	42.0	1.2
13	105.0	6.0	7.0	33.0	1.2
14	124.0	10.0	7.0	68.0	0.9
15	77.0	7.0	6.0	49.0	1.0
16	218.0	11.0	9.0	20.0	2.0
17	114.0	14.0	9.0	69.0	2.4
18	213.0	10.0	5.0	55.0	2.9
19	115.0	16.0	7.0	53.0	1.6
20	144.0	7.0	10.0	58.0	1.3

APPENDIX 5. SITE 5, PRE-TREATMENT, SEPTEMBER 2, 1986

Individuals per sample (0.1 sq meter)

OCPRE = ORTHOCLADIINAE/CHIRONOMINAE PRE-TREATMENT
TANYPRE = TANYPODINAE PRE-TREATMENT
JENNPRE = S. JENNINGSI PRE-TREATMENT
CHEUPRE = CHEUMATOPSYCHE PRE-TREATMENT
PODPRE = PODOSTEMUM PRE-TREATMENT (grams dry weight)

CASE	OCPRE	TANYPRE	JENNPRE	CHEUPRE	PODPRE
1	174.0	0.0	4.0	334.0	0.2
2	115.0	0.0	4.0	113.0	0.3
3	258.0	0.0	3.0	308.0	0.3
4	225.0	0.0	2.0	265.0	0.2
5	95.0	0.0	1.0	140.0	0.7
6	268.0	0.0	1.0	447.0	0.7
7	123.0	0.0	3.0	239.0	0.6
8	241.0	2.0	0.0	297.0	0.4
9	253.0	1.0	1.0	380.0	0.7
10	150.0	2.0	4.0	161.0	0.6
11	111.0	0.0	1.0	83.0	0.3
12	107.0	1.0	3.0	35.0	0.2
13	195.0	0.0	3.0	115.0	0.1
14	141.0	0.0	3.0	178.0	0.5
15	117.0	0.0	9.0	100.0	0.2
16	136.0	1.0	2.0	150.0	0.1
17	85.0	0.0	2.0	90.0	0.1
18	120.0	0.0	5.0	166.0	0.2
19	213.0	1.0	3.0	418.0	0.5
20	157.0	0.0	2.0	152.0	0.4

APPENDIX 6. SITE 5, POST-TREATMENT, SEPTEMBER 8, 1986

Individuals per sample (0.1 sq meter)

OCPOST = ORTHOCLADIINAE/CHIRONOMINAE POST-TREATMENT
TANYPOST = TANYPODINAE POST-TREATMENT
JENNPOST = S. JENNINGSI POST-TREATMENT
CHEUPOST = CHEUMATOPSYCHE POST-TREATMENT
PODPOST = PODOSTEMUM POST-TREATMENT (grams dry weight)

CASE	OCPOST	TANYPOST	JENNPOST	CHEUPOST	PODPOST
1	58.0	0.0	0.0	60.0	0.4
2	84.0	0.0	0.0	53.0	0.3
3	40.0	0.0	0.0	55.0	0.4
4	70.0	0.0	0.0	110.0	0.3
5	96.0	0.0	0.0	53.0	0.4
6	60.0	0.0	0.0	71.0	0.4
7	81.0	0.0	0.0	79.0	0.4
8	4.0	0.0	0.0	22.0	0.2
9	94.0	0.0	0.0	73.0	0.3
10	57.0	0.0	0.0	22.0	0.2
11	71.0	0.0	0.0	47.0	0.4
12	71.0	0.0	0.0	52.0	0.4
13	80.0	0.0	0.0	37.0	0.3
14	151.0	0.0	0.0	109.0	0.4
15	101.0	0.0	0.0	118.0	0.2
16	125.0	0.0	1.0	84.0	0.2
17	113.0	0.0	0.0	105.0	0.3
18	90.0	0.0	0.0	72.0	0.7
19	109.0	0.0	0.0	67.0	0.4
20	156.0	0.0	0.0	160.0	0.4

APPENDIX 7, SITE 6, PRE-TREATMENT, SEPTEMBER 2, 1986

Individuals per sample (0.01 sq. meter)

OCPRE = ORTHOCLADIINAE/CHIRONOMINAE PRE-TREATMENT
TANYPRE = TANYPODINAE PRE-TREATMENT
JENNPRE = S. JENNINGSI PRE-TREATMENT
CHEUPRE = CHEUMATOPSYCHE PRE-TREATMENT
PODPRE = PODOSTEMUM PRE-TREATMENT (grams dry weight)

CASE	OCPRE	TANYPRE	JENNPRE	CHEUPRE	PODPRE
1	30.0	9.0	13.0	68.0	0.7
2	17.0	4.0	5.0	47.0	0.4
3	41.0	9.0	7.0	69.0	0.6
4	34.0	3.0	3.0	24.0	1.8
5	56.0	13.0	1.0	277.0	1.3
6	32.0	11.0	10.0	16.0	1.0
7	9.0	2.0	4.0	35.0	1.4
8	67.0	2.0	21.0	324.0	1.1
9	36.0	16.0	3.0	215.0	1.9
10	28.0	10.0	4.0	198.0	1.4
11	80.0	25.0	3.0	183.0	1.5
12	74.0	18.0	9.0	404.0	1.8
13	54.0	10.0	10.0	201.0	1.9
14	53.0	9.0	9.0	287.0	1.8
15	8.0	8.0	2.0	50.0	1.9
16	54.0	8.0	6.0	389.0	2.1
17	29.0	5.0	6.0	212.0	1.0
18	45.0	5.0	15.0	272.0	1.9
19	66.0	9.0	0.0	271.0	1.9
20	36.0	11.0	6.0	156.0	1.1

APPENDIX 8, SITE 6, POST-TREATMENT, SEPTEMBER 8, 1986

Individuals per sample (0.01 sq. meter)

OCPOST = ORTHOCLADIINAE/CHIRONOMINAE POST-TREATMENT
TANYPOST = TANYPODINAE POST-TREATMENT
JENNPOST = S. JENNINGSI POST-TREATMENT
CHEUPOST = CHEUMATOPSYCHE POST-TREATMENT
PODPOST = PODOSTEMUM POST-TREATMENT (grams dry weight)

CASE	OCPOST	TANYPOST	JENNPOST	CHEUPOST	PODPOST
1	12.0	8.0	0.0	22.0	0.3
2	25.0	5.0	0.0	113.0	0.7
3	10.0	7.0	0.0	13.0	1.0
4	7.0	0.0	0.0	5.0	0.7
5	7.0	2.0	0.0	21.0	0.4
6	9.0	4.0	0.0	26.0	2.0
7	11.0	7.0	0.0	72.0	1.2
8	13.0	6.0	0.0	103.0	1.5
9	42.0	11.0	0.0	74.0	1.4
10	32.0	6.0	0.0	105.0	0.9
11	19.0	5.0	0.0	134.0	1.5
12	3.0	4.0	0.0	14.0	0.9
13	4.0	10.0	0.0	46.0	0.9
14	4.0	12.0	0.0	7.0	1.2
15	75.0	16.0	0.0	54.0	2.4
16	21.0	7.0	0.0	23.0	1.5
17	28.0	5.0	1.0	39.0	1.1
18	5.0	6.0	0.0	50.0	1.8
19	12.0	4.0	0.0	46.0	1.1
20	7.0	0.0	1.0	36.0	0.7