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THE EFFECTS OF LAMPREY LARVICIDE ON THE BOTTOM FAUNA AND PERIPHYTON OF THE CHOCOLAY RIVER, MARQUETTE COUNTY, MICHIGAN [1]

By Robert C. Haas

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INTRODUCTION

The sea lamprey (Petromyzon marinus) gained entry to the upper Great Lakes in 1829 upon completion of the Welland Canal. By the 1930's the sea lamprey had established itself in Lakes Huron and Michigan and a short time later in Lake Superior as well. This parasite thrived and by 1950 the commercial harvest of lake trout (Salvelinus namaycush) was down 95% from that of the 1930's (Eschmeyer, 1957). The disastrous decline in harvest of the highly valuable lake trout prompted cooperative research and lamprey control programs between Canada and the United States as early as 1946 and a treaty for joint action was signed in 1954. The international collaboration resulted in life history studies (Applegate, 1950; Applegate and Moffett, 1955; Hile, 1957; and others) which led the way to a lamprey control program based upon the vulnerability of adults in spawning streams. Various mechanical and electrical weirs were used to block lamprey migrations both up and downstream; but high costs, time required to reach desired results, and flood conditions made weirs impracticable (Applegate, Smith and Nielsen, 1952; Erkkila, Smith and McLain, 1956). It was found that larvae of the sea lamprey, called ammocoetes, live in the spawning streams for

about 5 years before maturation to the parasitic, lake dwelling adult. Hence, it was possible to treat the spawning streams with a toxic chemical, kill the larvae and eliminate several generations before they became parasitic.

Applegate et al. (1958) found a differential toxicity between fishes and larval lampreys for ten halogenated mononitrophenols. All of these compounds are more toxic to lampreys than most other aquatic organisms; however, one of them, 3-trifluormethyl-4-nitrophenol (TFM), met the requirements more closely and was selected for field use (Applegate, et al., 1961). Laboratory studies with TFM (Applegate et al., 1957; Applegate et al., 1958; and Applegate et al., 1961) established that this chemical is acutely toxic to larval lampreys at low concentrations (2-3 ppm) and that at these concentrations, it is non-toxic to other fishes.

Stream treatments with the lampricide are timed to remove the lampreys before metamorphosis. Most of these streams contain valuable resident fish populations and spawning habitat for Great Lakes fishes. Although numerous trials demonstrated that concentrations of TFM used in stream treatments (2-4 ppm) had little or no direct effect upon the resident fish, the possibility existed that this chemical might eliminate some of the stream invertebrates and algae and thus remove a part of the fishes' food supply.

In some instances, representative stream invertebrates were included in the laboratory analyses and early reports showed

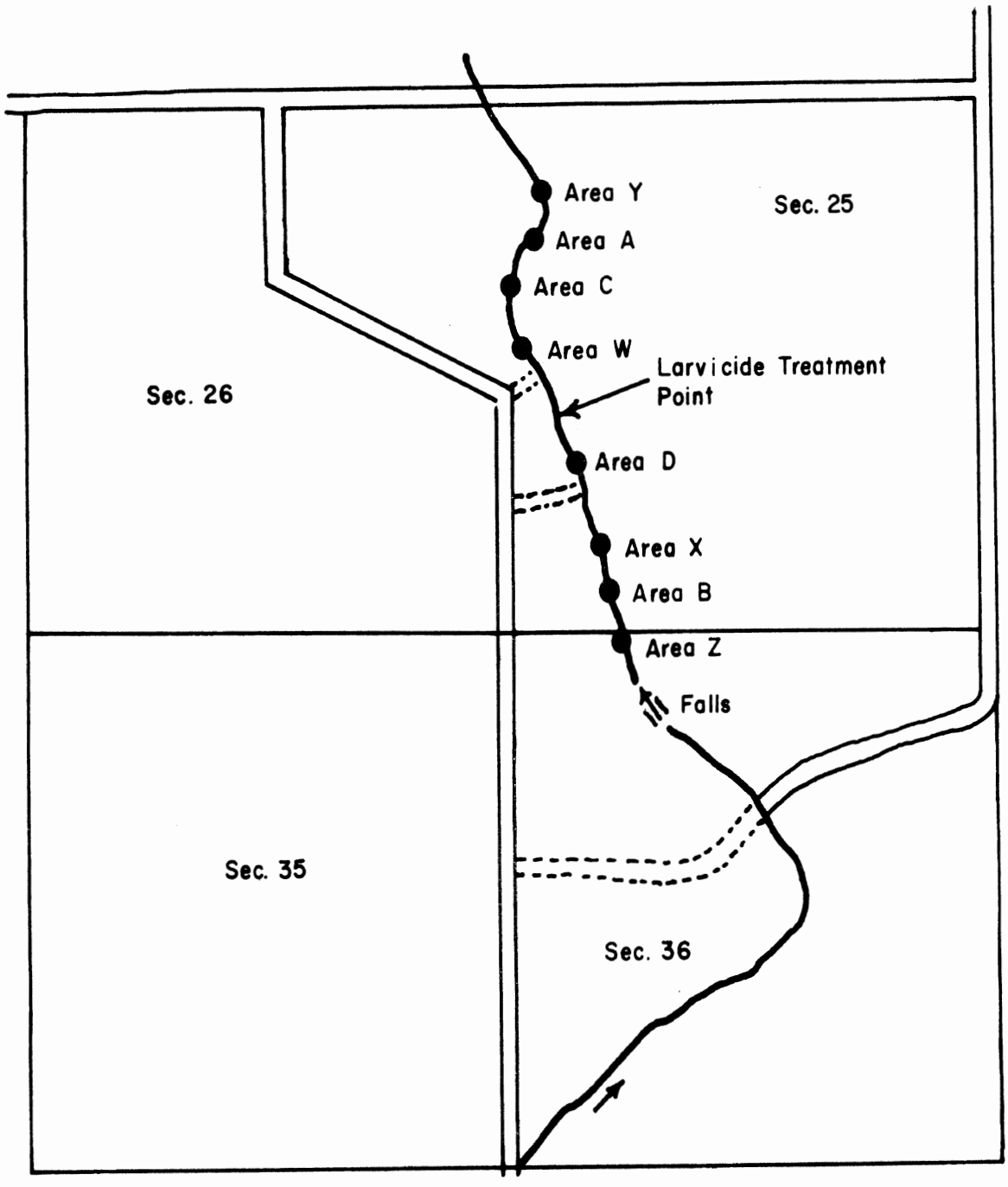
that crayfish and insects were not affected at the concentrations used to eliminate lampreys (Applegate et al., 1961). Field observations of actual stream treatments likewise did not indicate gross stream invertebrate mortalities. However, before the present study, an intensive investigation of the effects of TFM treatment upon stream invertebrates and algal communities under natural conditions had not been made.

In the fall of 1965, the East Branch of the Chocolay River in Michigan's Upper Peninsula was treated with TFM to determine the chemical's effects upon stream bottom fauna and periphyton. The purpose of this paper is to report and evaluate the short-term effects of the larvicide on these communities in the East Branch of the Chocolay River.

Study Area

The East Branch of the Chocolay River is located in Marquette County, Michigan. The Chocolay system drains approximately 94,000 acres of land and flows into Lake Superior at Harvey, Michigan. The East Branch is 8 miles long. It has old beaver dams and ponds at the headwaters. The river is surrounded by moraines covered by mixed northern hardwoods and conifers. Bottom soils of the East Branch consist mainly of sand and gravel with occasional rocks and boulders. Water color varies from light to dark brown due to organic compounds added by the beaver ponds at the headwaters.

Figure 1.--Study section of the East Branch of the Chocolay River, Marquette County, Michigan. The experimental and control riffle bottom fauna areas are designated A and B and the experimental and control pool bottom fauna areas C and D. The experimental and control riffle periphyton areas are designated Y and Z and the experimental and control pool periphyton areas W and X.


Area Y
Sec. 25
Area A
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Larvicide Treatment Point
Sec. 26
Area D
Area X
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Sec. 36
● Location of Bottom Fauna and Periphyton Sampling Areas

Figure 1

Portions of the stream flow through rolling farm country where bank erosion is quite noticeable. A biological and physical inventory of the drainage system by Galbraith (1954) indicated that temperatures in the East Branch might occasionally exceed the level generally considered lethal for trout. These high temperatures were attributed to lack of cover along the stream banks.

The area studied (Fig. 1) consisted of 1 1/4 miles of stream in Sections 25 and 36 (T 46N, R 24W) of Branch Township, Marquette County. This portion of the stream is about half shaded by bank vegetation with a typical habitat of pools alternated with swift running riffle areas. The width of the study area varies from 6 to 13 feet. The average current velocity was about 2 ft/sec in the riffle areas and 1 ft/sec in the pool areas during the study period. The substrates of the riffle areas consisted of rubble and gravel and the pool areas were gravel and sand. Water temperatures during the TFM treatment period varied from a maximum of 59 F on September 18 to a minimum of 37 F on October 15.

Fish species resident in the East Branch of the Chocolay River (Galbraith, 1954) were rainbow trout (Salmo gairdneri), brook trout (Salvelinus fontinalis), blacknose dace (Rhinichthys atratulus), redbelly dace (Chrosomus eos), mottled sculpin (Cottus bairdi), brook stickleback (Eucalia inconstans), and central mudminnow (Umbra limi). Migratory rainbow trout, brown trout (Salmo trutta) and sea lampreys are barred from the study area by natural waterfalls downstream.

Sampling Areas

The location of the sampling areas for bottom fauna and periphyton are shown in Figure 1. Four bottom fauna sampling areas and four periphyton sampling areas were selected; two riffle areas and two pool areas for both periphyton and bottom fauna. One area of each type (riffle and pool) served as an experimental area and the other as a control area. Control areas were located upstream from the point of treatment with TFM and the experimental areas were downstream. The corresponding experimental and control areas were selected to be as similar in physical and biological characteristics as possible. Two of the bottom fauna areas are shown in Figures 2 and 3.

Areas A and B were the experimental and control riffle bottom fauna areas, C and D were the pool bottom fauna areas. Likewise, Y and Z were the experimental and control riffle periphyton areas while W and X were the pool periphyton areas.

Riffle bottom fauna sampling area A (Fig. 2) was characterized by water depths of 3-11 inches with a substrate of rubble and gravel overlying sand. The percentages of materials in the substrate ranged from 50% rubble and 50% gravel to 90% rubble and 10% gravel. The average current velocity for the treatment period at A was 1.4 ft/sec.

Riffle bottom fauna sampling area B was very similar in physical properties to station A. Area B was characterized

by water depths of 6 to 11 inches and also had a substrate of rubble and gravel overlying sand. Composition of the substrate ran from 40% rubble and 60% gravel to 90% rubble and 10% gravel. Average current velocity for the treatment period was 1.4 ft/sec.

Pool bottom fauna sampling area C (Fig. 3) had water depths of 8 to 28 inches and a substrate of rubble overlying gravel and sand. Rocks covered the sand and gravel in varying degrees from 50% to 100%. Average current velocity for the treatment period at area C was 0.5 ft/sec.

Pool bottom fauna sampling area D was characterized by water depths of 10 to 24 inches with a substrate of rubble overlying sand and gravel, covering the latter two from 80% to 100%. Average current velocity for the treatment period at area D was 0.7 ft/sec.

The lampricide was introduced into the stream approximately midway between the experimental and control sampling area (Fig. 1). This site of introduction is shown in Figure 4.

METHODS

Current velocity was measured with a Gurley current meter. Each velocity measurement was determined from three separate readings of the meter for 40-second periods. Current measurements were made within 6 inches of the stream bottom at all locations.

Near the treatment site a staff gauge was placed in the stream bed so that the lower end was always below the minimum water level. This gauge, graduated at intervals of 1 foot and tenths of feet, was used to measure the height of the stream water.

A subsurface maximum-minimum thermometer was employed to register upper and lower stream temperatures throughout the study period. This thermometer was located in the middle of the stream near the treatment site.

Chemical analyses of the stream water was conducted several days prior to the first treatment with TFM. Procedures for these determinations were generally those set forth in the ninth edition of "Standard Methods for the Examination of Water and Sewage."

Bioassays and Application of Larvicide

Applegate et al. (1961) found that the amount of TFM and the time required to treat a given stream cannot be determined by chemical analyses of the water, therefore, pre-treatment bioassays have to be conducted for each stream treatment. Bioassays were made from a mobile laboratory using Chocolay River water. The methods used were essentially those described by Applegate et al. (1957). Test animals were placed in containers and subjected to various levels of TFM to determine the minimum lethal dose for lamprey larvae (concentration killing 100% of the test lamprey larvae in 24 hours), and the maximum allowable dose for fish (concentration killing 25% of the test fish in 24 hours). Larval brook lampreys (Ichthyomyzon fossor) and rainbow trout were utilized as test animals. The bioassays were adequate to determine the minimum and maximum allowable dosages of the lampricide. Test cages that contained specimens of the brook lamprey and rainbow trout were also placed in the stream well below the treatment point. Their purpose was to determine whether or not the concentration of TFM was sufficient to kill lamprey larvae but not trout within a 24-hour period.

The efficient application of TFM requires a highly accurate and controllable pumping system. The system as described by Applegate et al. (1961) was arranged to feed a concentrated stock

solution of TFM into a pipe containing a stream of water drawn from the river by a centrifugal pump. Stream deflectors were positioned at the treatment site to mix the TFM with the river water (Fig. 4). Metering of the diluted lampricide through a perforated pipe aided in mixing the chemical with the water.

Successful treatment with lampricide requires a precise method of analyzing the treated stream for TFM so that the needed concentration can be maintained. Accurate measurements of the amounts of TFM were made by colorimetric analysis based on the natural yellow color of the nitrophenols. This method was described in detail by Smith, Applegate and Johnson (1960). These analyses were made at three stations below the lampricide feeder unit. These stations were located about 200 yards downstream from the treatment site and arranged perpendicular to the flow with one on each side of the stream and one directly in the middle.

Bottom Fauna Sampling

The four bottom fauna areas were sampled during September and October of 1965, to evaluate the changes in abundance due to the effects of TFM treatment. The riffle and pool areas were separated upstream and down from the point of treatment by approximately 200 yards and the same areas were used throughout the sampling period.

Figure 5.--Grid method of selecting one-square-foot random bottom fauna stations at stream sampling areas.

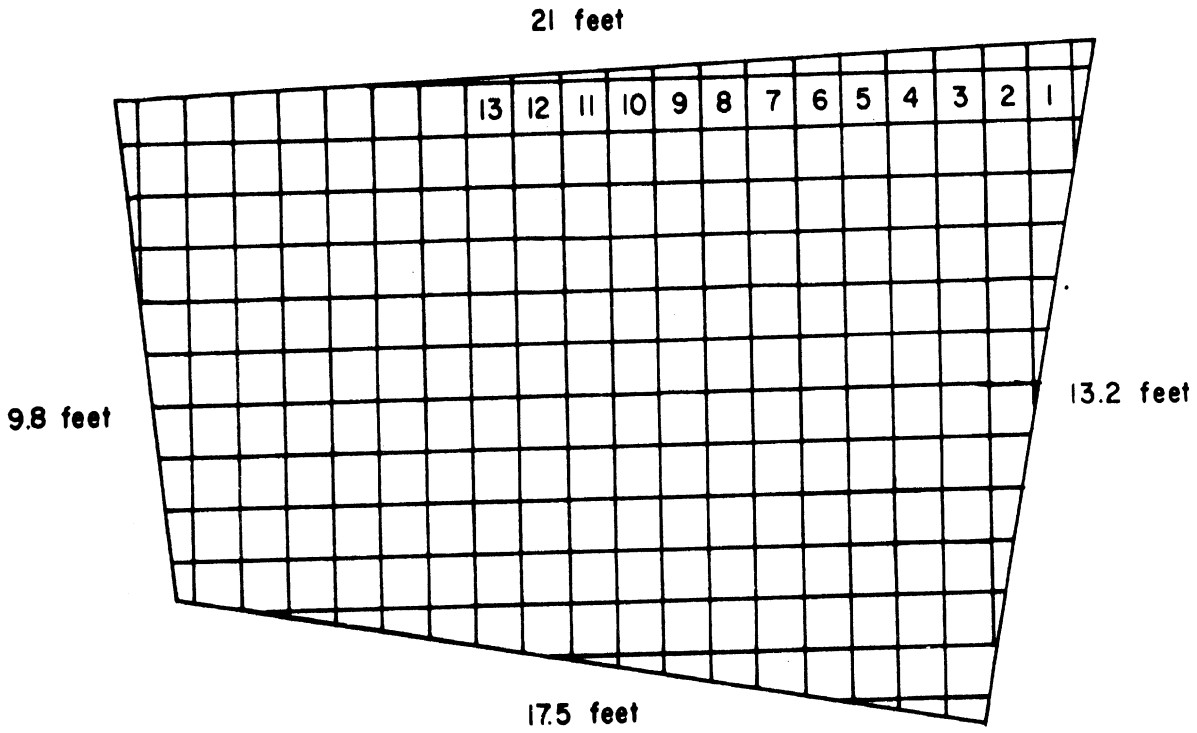


Figure 5

The area boundaries were first marked with permanent stakes driven into the stream bottom. These boundaries were at least 1 foot from the stream shore. Then each area was divided into numbered square-foot sections by a grid as exemplified in Figure 5. To locate each sampling station, a string was stretched around the perimeter of each area and a tape was used to measure the coordinates for each sample within the stream area.

Ten random square-foot samples were taken from each area during each sampling period. Numbers for sampling stations were selected from a table of random numbers. The square-foot sampler was used for all collections of bottom fauna. According to Welch (1948), it is especially suitable for collecting macroscopic organisms in stony and gravelly stream bottoms which possess enough current to hold the net open. Stations that fell partly or wholly on large rocks or logs were rejected, as were those stations that had been sampled previously.

The corresponding experimental and control areas were sampled on the same date. The bottom fauna samples were placed in shallow enamel pans and were picked immediately while the animals were alive. The rubble and gravel was returned to the sampling stations after being picked clean. The benthic organisms were then preserved in 95% alcohol.

Periphyton Sampling

A study of the effects of TFM on periphyton growth was made during the summer and fall of 1964 and 1965. Twelve plexiglass plates 2 inches by 5 inches were installed at each periphyton area. The plates were numbered from 1 to 12 with plate No. 1 on the west side of the stream and plate No. 12 on the east. None of the plates were placed in slack water and efforts were made to assure uniformity between experimental and control areas by selecting areas with comparable stream velocity and light intensity. The 12 plates for each area were set in a series across the stream. They were positioned 7 inches below the surface of the water and parallel to the current direction to eliminate collection of sediment particles.

The plexiglass substrates were left in the stream for a period of 14 days to insure the accumulation of a weighable amount of periphyton. The sampling period extended from July through November and included both treatment periods with TFM.

When the substrate plates were picked up, they were kept out of the sun and the macroscopic organisms, mainly blackflies, were carefully picked from the plates. The plates were then packed in individual freezer bags and kept on ice until returned to the laboratory. Fresh plates were installed at each station immediately after the samples were collected.

The periphyton plates were immediately placed in a refrigerator when returned to the laboratory. The growth was scraped from the top and bottom of each plate, using a microscope slide and a rubber policeman. The substrate was rinsed with filtered water and the sample collected in individual 2-ounce bottles. Before resetting in the stream, the plates were rinsed in 0.01 N HCl followed by a rinse in distilled water.

Millipore filter papers, with a disc diameter of 0.47 mm, were used to concentrate and weigh the periphyton samples. The experimental error in weighing periphyton papers was determined by the following procedure: Papers were weighed on a balance, filtered with distilled water, dried in a dessicator for 48 hours, and then reweighed. The average error from five weighing procedures was found to be 0.0006 g.

Each millipore filter paper was weighed on the balance and stored in the dessicator until used. The periphyton sample was then filtered through one of the weighed papers using a vacuum filter. The filter paper and periphyton sample were placed in a dessicator and dried for 48 hours. When the drying period was over, the papers were again weighed and the difference gave the dry weight of the periphyton sample.

When a weighable amount of periphyton was not present on a single substrate, four plates were pooled into one sample. In these instances, the corresponding plates in both experimental and control areas were pooled.

RESULTS AND DISCUSSION

It has been shown in several studies that the action of the larvicide is dependent upon physical and chemical conditions of the treated water. According to Applegate et al. (1961) the toxicity of TFM is strongly influenced by alkalinity and pH, but only slightly by temperature and oxygen. The chemical is most effective in killing lamprey larvae in soft, acid waters. Considerably higher concentrations are required as pH, conductivity and alkalinity increase. However, the differential toxicity of TFM to lampreys and other fishes appears to be maintained regardless of the chemical conditions and concentrations of TFM encountered.

Some of the physical and chemical properties of the East Branch of the Chocolay River on September 20, 1965, prior to the first treatment with TFM are given in Table 1. These conditions of pH, conductivity and alkalinity fall near the middle of the range for streams in the state of Michigan. There was a total alkalinity of 68.0 ppm calcium carbonate and a pH of 7.75. According to Applegate et al. (1961) for streams with approximately this alkalinity and pH, a minimum lethal dose of TFM would be 2 ppm and the maximum allowable dose would be 8-9 ppm. Prediction of

Table 1.--Some physical and chemical properties of the water of the East Branch of the Chocolay River on September 20, 1965, at the TFM treatment site

Aluminum	0.13 ppm
Calcium	44.00 ppm Calcium carbonate
Chloride	2.00 ppm
Copper	0.04 ppm
Alkalinity	
Phenolphathalein	0.00 ppm Calcium carbonate
Methyl purple	42.00 ppm Calcium carbonate
Total	68.00 ppm Calcium carbonate
Iron	0.30 ppm
Nitrogen	
Nitrate	1.00 ppm
Nitrite	0.00 ppm
pH	7.75
Conductivity	116 μohm
Water temperature	51 F

the precise toxicity for lampreys and fish in the East Branch could only be obtained through bioassay techniques.

Water stage, temperature and current velocity data for the study section of the East Branch during the sampling period in 1965 are given in Table 2. The stage recordings and current velocity measurements indicate relatively stable water levels with only moderate fluctuations from the end of July to the middle of October. The conditions observed during the treatment study indicate that fluctuations of water temperature and velocity were probably not abnormal. However, the data collected on October 21 show a sharp increase in flow over the previous two months' average. This change came several days after the second treatment with TFM and after the bottom fauna sampling was completed.

The maximum and minimum temperature data show wide daily variation. This was probably due to the lack of cover upstream from the study section. According to Applegate et al. (1961), the use of TFM as a lampricide is not impaired by low water temperatures; in fact, the differential toxicities are improved slightly. As the temperature is lowered from 55 F to 35 F, trout mortalities are reduced slightly, whereas lamprey mortalities remain essentially the same.

The water temperatures of the East Branch during both treatment operations are given in Table 4. On September 24, 1965, the temperature remained constant at 47 F. During the second

Table 2.--Temperature, water stage and current velocity for the study section of the East Branch of the Chocolay River in 1965

Date	Temperature (°F)		Water stage (feet)	Current velocity at bottom fauna stations (ft/sec)			
	Minimum	Maximum		A	B	C	D
July 28	37	74	1.3	0.7	0.9	0.2	0.2
July 31	56	64	1.0	0.8	0.8	0.2	0.1
Aug. 13	50	70	0.9	0.9	0.7	0.1	0.2
Aug. 31	50	66	1.6	1.6	1.4	0.4	0.9
Sept. 18 [a]	44	59	1.8	1.8	1.6	0.7	1.1
Oct. 2	37	54	1.9	1.9	1.5	0.8	1.2
Oct. 14 [b]	37	50	1.2	1.1	0.9	0.3	0.4
Oct. 21	38	54	2.2	2.3	3.4	1.4	1.6

[a] First treatment with TFM on September 24, 1965.

[b] Second treatment with TFM on October 15, 1965.

treatment the stream water averaged 43 F. It is felt that the temperatures encountered during this study period would not have altered the action of TFM to any extent.

Bioassays

The stream treatments with TFM were made on September 24 and October 15, 1965. Since the amount of TFM needed for lamprey control could not be determined from chemical analyses of the water, each treatment was preceded by a bioassay. Physical and chemical properties of the water, plus the biological activity of TFM as determined through these pre-treatment bioassays, are given in Table 3. The first bioassay, performed on September 21, gave a minimum lethal dose of 1.0 ppm TFM and a maximum allowable dose of 4.0 ppm.

The first treatment was made at the concentration of 1.0 ppm TFM, which was the minimum lethal dose. The brook lampreys that were held in the stream were not killed within the ensuing 24-hour period. Apparently the 1 ppm concentration was not suitable and plans were immediately made for a second treatment at a higher concentration.

The second treatment on October 15 was made using a concentration of 4.0 ppm or 1 ppm below the maximum allowable dose. The test lampreys in the stream were killed quickly at this concentration. Water temperatures and the variation in concentration

Table 3.--Some physical and chemical properties of the East Branch of the Chocolay River, and the biological activity of the larvicide in this water as determined from pre-treatment bioassays

Date, 1965	Test temperature (°F)	Minimum lethal dose TFM (ppm)[1]	Maximum allowable dose TFM (ppm)[2]	Conductivity at 20 C (μmho)	Alkalinity		
					phth (ppm)	MO (ppm)	pH
Sept. 21	55	1.0	4.0	117	0	43	7.75
Oct. 14	52	2.0	5.0	122	0	48	-

[1] Concentration of TFM killing 100% of the test lamprey larvae within 24 hours.

[2] Concentration of TFM killing approximately 25% of the test rainbow trout within 24 hours.

of TFM used in both treatments are given in Table 4. The desired concentration of 1.0 ppm was maintained very closely during the first treatment; however, for the second treatment, the actual concentration fluctuated considerably from the desired 4.0 ppm.

Bottom Fauna

Bottom fauna areas were sampled on a schedule designed to detect any immediate effects of TFM on the benthic community. The experimental and control bottom fauna areas were sampled and compared to discern changes in abundance. Significant mortalities, due to the lampricide, should been detected by this analysis. Long-term or sub-lethal effects of TFM upon the invertebrates would not have been detected. Torblaa (1968) found that aquatic invertebrate abundances were not significantly different one year after treatment with lampricide, suggesting that there is very little, if any, long-term effect upon the stream invertebrate communities. Smith (1967) conducted laboratory bioassays to determine the effects of TFM on aquatic invertebrates. He found that it is potentially toxic to some of the invertebrates but that mortalities would probably be low at concentrations used for most stream treatments.

The sampling and treatment schedule for the riffle and pool areas respectively are given in Figures 6 and 7. Benthic samples from one area on one day (10-square foot samples) are

Table 4.--TFM concentrations with variations and temperature in the East Branch of the Chocolay River during treatments

Time	Temperature (° F)	TFM (ppm)	Variation from desired concentration (ppm)
First treatment September 24, 1965			
0900	47	1.0	0.0
1000	47	1.2	+0.2
1100	47	1.2	+0.2
1200	47	1.1	+0.1
1300	47	1.0	0.0
1400	47	1.2	+0.2
Second treatment October 15, 1965			
0855	43	4.1	+0.1
0910	43	4.8	+0.8
0950	43	3.3	-0.7
1100	43	6.1	+2.1
1145	43	7.2	+3.2
1230	43	6.2	+2.2
1300	44	3.8	-0.2
1330	44	3.9	-0.1
1400	44	5.0	+1.0

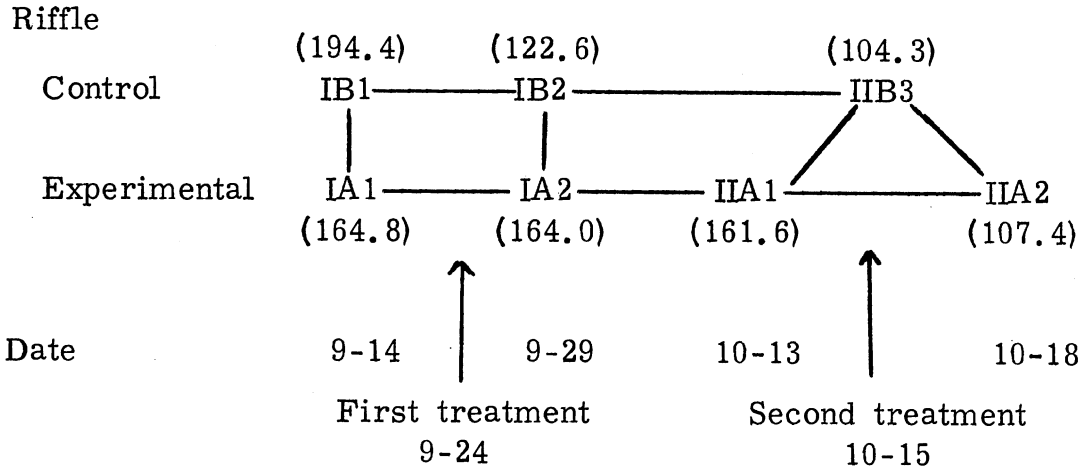
characterized by three symbols. The first, a roman numeral, indicates which treatment period. The second, a capital letter, tells which area was sampled; and third, an arabic numeral, tells whether the time of sampling was before or after the treatment.

Before and after samples were collected for both treatments except for control areas (B and D) during the second treatment. Because of a shortage of labor, these two areas were only sampled once; at the time of treatment. It was hoped that this would be sufficient for making meaningful comparisons.

Seven sets of samples were taken for both the riffle and pool areas. For these seven sample groups, nine statistical comparisons were made for the riffle areas and nine for the pool areas.

The bottom fauna samples were sorted and identified to genera for most of the benthic invertebrates utilized in the analyses. The taxonomic descriptions and keys used for identification were those provided by Burks (1953), Frison (1935), Leonard and Leonard (1962), Needham and Needham (1962), and Ward and Whipple (1918). A qualitative list of all macrobenthic organisms collected during this study is given in Table 5. The bulk of the organisms in the samples come from the orders Diptera and Ephemeroptera with the orders Plecoptera and Trichoptera in much lesser abundance. Six orders, 15 families and 18 genera were represented in the bottom fauna samples. All instars of each taxon of the benthic organisms were lumped together in the counts.

Figure 6.--Temporal arrangement of riffle bottom fauna sampling schedule with mean number of benthic organisms per square foot in parentheses.

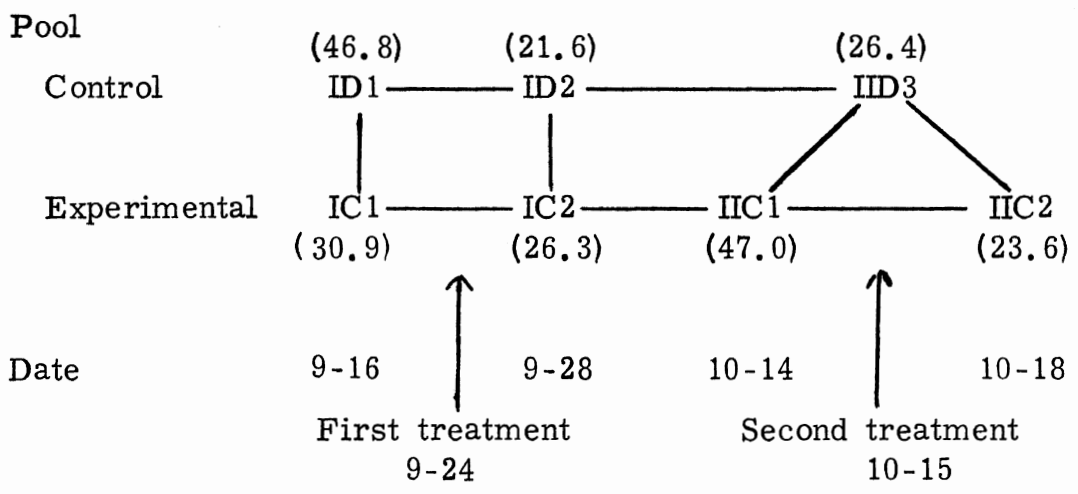


Area B - Control riffle above treated section
Area A - Experimental riffle in treated section
I - Means associated with first treatment
II - Means associated with second treatment
1 - Means before treatment
2 - Means after treatment
3 - Means during treatment

Lines connecting areas represent statistical comparisons by three-way analysis of variance

Figure 6

Figure 7.--Temporal arrangement of pool bottom fauna sampling schedule with mean number of benthic organisms per square foot in parentheses.



Area D - Control pool above treated section
Area C - Experimental pool in treated section
I - Means associated with first treatment
II - Means associated with second treatment
1 - Means before treatment
2 - Means after treatment
3 - Means during treatment

Lines connecting areas indicate comparison by three-way analysis of variance

Figure 7

Table 5.--List of macrobenthos taxa in the East Branch of the Chocolay River in the fall of 1965

Order	Family	Genera
Ephemeroptera	Heptageniidae	Epeorus
		Stenonema
	Baetidae	Paraleptophlebia
		Baetis
	Ephemerellidae	Ephemerella
Plecoptera	Perlidae	Acroneuria
	Perlodidae	?
	Taeniopterygidae	Taeniopteryx
Trichoptera	Rhyacophilidae	Glossosoma
		Rhyacophila
	Hydropsychidae	Hydropsyche
Diptera	Rhagionidae	Atherix
	Tipulidae	Antocha
	Simuliidae	?
	Ceratopogonidae	Palpomyia
	Tendipedidae	?
Coleoptera	Elmidae	Ancyronyx
Hydracarina	?	?

Some of the taxa were not abundant enough for meaningful comparisons and evaluations, so only 13 of the 18 taxa were utilized in this study for testing the effects of TFM. The average number of individuals per square foot before treatment for each of the taxa of riffle and pool bottom fauna, selected for evaluation by virtue of their relatively high abundance and wide variety, are given in Table 6. They were thought to effectively encompass the range of types of stream invertebrates which might be adversely affected by the chemical treatments.

All mayfly genera present in the samples were included in the analysis because many studies have shown that, in general, the Ephemeroptera are intolerant to most types of "pollutants" and chemicals. Applegate et al. (1961) and others suggested a low tolerance of this order to TFM. Moyle and Luckman (1964) showed that mayflies as a group are killed immediately by insecticides and that populations remain depleted for several years. Surber (1953) indicated that the mayflies were the least tolerant and first to disappear under polluted conditions. The Ephemeroptera, because of their low tolerance to environmental contamination, should be among the first taxa to exhibit any deleterious effects of the lampricide.

Twelve taxa of benthic organisms were selected from the riffle fauna for evaluating the lampricide treatments and eight taxa were selected from the pool fauna. The riffle fauna was more

Table 6.--Genera and relative abundance of macrobenthos selected for statistical comparison from before treatment samples

Area	Taxa	Average number per square foot
Pool	Stenonema	10.0
	Paraleptophlebia	1.0
	Ephemerella	1.5
	Perlodidae	1.7
	Taeniopteryx	0.4
	Hydropsyche	1.5
	Antocha	4.5
	Tendipedidae	15.3
Riffle	Epeorus	22.9
	Paraleptophlebia	11.1
	Baetis	9.6
	Ephemerella	6.4
	Perlodidae	3.7
	Taeniopteryx	3.4
	Glossosoma	6.0
	Hydropsyche	73.4
	Atherix	5.9
	Antocha	9.7
	Tendipedidae	22.1
	Hydracarina	5.5

diverse and supported considerably larger numbers of individuals of those taxa found in both the riffle and pool areas. Seven out of the eight taxa selected from the pool areas were also utilized in the riffle analysis.

The mayfly, Epeorus, the net spinning caddis fly, Hydropsyche, and the midges, Tendipedidae, were the most abundant members of the benthic community found at the riffle stations. The mayfly, Stenonema, and the midges were the most abundant members of the benthos at the pool stations. The remainder of the thirteen taxa were found to be in relatively low abundance.

Riffle and pool stations were analyzed separately to eliminate a large source of variation, even though the benthic organisms used in the pool areas were also used for the riffle areas. Differential effects of TFM were anticipated due to differences in water velocity, depth, and substrate type. These physical differences and the differences in distribution and abundance of the taxa between riffle and pool areas necessitated the separate evaluation.

The samples collected in experimental areas before treatments were utilized as indices of abundance for comparisons with post-treatment samples. Pre- and post-treatment samples from control areas were analyzed for random changes in abundance or those beyond the treatment effect. For general comparisons between taxa, the change in abundance over the specified time interval was

computed as the percentage of the former level of abundance. Values below 100% indicate a drop in abundance and those over 100% an increase in abundance. These calculations were made to eliminate some of the variation between benthos abundances and to put the data on more comparable terms. The changes for each taxon of benthic organisms used in the analysis of the riffle areas are given in Table 7. Relative abundance before the first treatment is given as the mean number of individuals per square foot (Table 6).

For the first treatment period (IA1-IA2), eight taxa in the experimental area showed a drop in abundance while four taxa increased. In the control area, ten taxa decreased during the same time interval. Only two, Hydropsyche and Antocha, decreased in the experimental area and not in the control. For the most part, decreases in abundance were greater in the control area than in the treated, experimental area. Since the decreases in benthic abundance were greater in the control area, there was probably no change in the experimental area due to the TFM.

Changes in abundance for each taxon over the 14-day period from after the first treatment to before the second treatment are included in Table 7. There appears to have been a slight increase in numbers in the experimental area although they may well be within sampling variation.

For the second treatment period (IIA1-IIA2) ten taxa showed a decrease in abundance and only one increased, while

Table 7.--Changes in abundance for each genera of bottom fauna from riffles over the specified time interval calculated as the percent of the former abundance

Taxa	Experimental area			Control area	
	IA1-IA2	IA2-IIA1	IIA1-IIA2	IB1-IB2	1B2-IIB3
Epeorus	107	72	102	86	79
Paraleptophlebia	94	138	71	31	152
Baetis	84	96	71	34	194
Ephemerella	163	86	48	84	89
Perlodidae	67	103	100	50	106
Taeniopteryx	84	185	31	158	195
Glossosoma	55	221	56	117	106
Hydropsyche	115	70	56	54	74
Atherix	113	87	72	74	100
Antocha	68	127	66	94	49
Tendipedidae	77	161	61	63	112
Hydracarina	74	109	69	67	31

five taxa in the control area decreased in abundance. Since the benthos in the experimental area decreased considerably in abundance, more than the benthos in the control area, the TFM probably did cause some mortality or movement out of the area. However the experimental and control sampling times were different. The control samples were taken on the day of treatment, thus less value can be placed on their comparison with the experimental.

Each taxon was checked for its reaction through the four separate treatment periods. Seven riffle taxa declined in abundance after successive treatments with larvicide. Two of these, Hydracarina, and the dipteran, Antocha, also declined in the control area during both sampling periods. Two other genera, Ephemerella and Hydropsyche, declined during both control periods, but only during the second treatment in the experimental riffle. All taxa declined in abundance during the second riffle treatment period except for Epeorus and Perlodidae which both remained at the pre-treatment level.

Smith (1967) found that, under lab conditions, a concentration of 4 ppm TFM would cause short-term mortalities of less than 25% for each of the groups of invertebrates represented by the 12 taxa. In this study some taxa decreased in abundance more than 25% but most of these showed considerable variations under the control and untreated conditions.

The pool community had a lower density and was more variable in abundance and had lower species diversity, than the riffles. The changes in abundance over the specified time interval, as the percent of the initial abundance, for each of the eight taxa utilized in the pool comparisons are given in Table 8.

After the first experimental treatment period (IC1-IC2) there were decreases in the abundances of five taxa and increases in three. However, all eight taxa decreased in the untreated area during the same time period. Hence, no harmful effects of the lampricide could be demonstrated in the pool areas during the first treatment.

The 16-day period between treatments was characterized by increases in abundance for all taxa in the experimental area. The control area showed six taxa increased and two decreased between treatments. It appears that the taxa in the experimental pool area were not very different from the control pool at completion of the first treatment.

The second treatment period (IIC1-IIC2) resulted in declines in abundance for all taxa in the experimental area. The control pool area showed six taxa increased and two decreased. Here again it appears, in the experimental area, that the treatment has resulted in substantial drops in the quantity of benthic invertebrates. However, when the control samples are compared, the wide fluctuations in abundance make a judgment nearly impossible.

Table 8.--Changes in abundance for each genera of bottom fauna from pools over the specified time interval calculated as the percent of the former abundance

Taxa	Experimental area			Control area	
	IC1-IC2	IC2-IIC1	IIC1-IIC2	ID1-ID2	ID2-IID3
Stenonema	109	108	56	44	144
Paraleptophlebia	62	620	61	25	433
Ephemerella	36	183	64	7	300
Perlodidae	80	120	92	67	67
Taeniopteryx	567	112	21	50	50
Hydropsyche	440	132	62	29	229
Antocha	67	117	48	29	111
Tendipedidae	60	295	42	53	133

Torblaa (1968) found that most groups of invertebrates declined in treated streams one week after treatment with TFM. He also showed that most groups had very rapid recoveries, usually after one week. Likewise he found a wide variability in numbers of organisms.

Statistical comparisons between the experimental and control areas were made by a three-way analysis of variance with ten replications. This analysis tested the differences between the means for the three main effects (sources of variation): area or treatment, stations or samples, and taxa. Three interactions were also tested to see if any combination of effects was significant.

Significant differences, at the 95% level, were expected between the different taxa of bottom fauna because they are normally different in abundance and distribution. Some variation was also expected between samples (stations) within each area but not to a significant degree since sampling procedures were carefully controlled to reduce sampling error. Significant differences were expected when testing the treatment effect in the experimental areas, and non-significant differences in the corresponding control areas for that same effect. Such a result would show that the lampricide had caused mortalities or movements of the benthic communities within the treated stream. The control area comparisons would also reveal any extraneous or natural changes in invertebrate abundances or natural differences between the experimental and the control areas.

The F-values for the six sources of variation for nine analyses of variance comparing riffle areas and nine analyses comparing pool areas, respectively, are given in Tables 9 and 10. The relative importance of these nine comparisons can be better understood by looking at the mean number of benthic invertebrates per square foot as shown in Figures 6 and 7. The statistical comparisons over the time periods were made to analyze the treatment effects. Those comparing the control and experimental areas were made first to establish that these areas were statistically identical and to follow the eventual changes in the benthic communities more closely.

The 95% level was established as the level of significance and any F-value equal to or exceeding it was considered significant.

Any general mortality or change in abundance should cause a significant F-value for the area term. This treatment source of variation is probably the most meaningful because it compares the total number of invertebrates between the two areas. The mean numbers of benthic organisms per square foot were 194.4 in the control riffle and 164.8 in the experimental riffle, so the control riffle had 29.6 more individuals per square foot than the experimental riffle area. However, the non-significant F for the area term comparing the experimental and control areas before treatment (IA1-IB1) establishes a statistical equality in benthic

Table 9.--F-values for sources of variation from three-way analysis of variance comparing riffle bottom fauna samples [1]

Source	df	IA1-IB1	IB1-IB2	IA1-IA2	IA2-IB2	IB2-IIB3	IA2-IIA1	IIA1-IIB3	IIA1-IIA2	IIA2-IIB3
Area	1	0.76	3.32	0.002	2.36	0.57	0.02	11.21*	11.65*	0.05
Stations	9	1.62	0.91	1.54	0.82	0.90	5.37*	2.73*	3.25*	1.71
Taxa	11	15.86*	13.86*	22.22*	13.64*	13.59*	28.56*	17.80*	15.17*	17.63*
Area x station	9	1.71	1.30	3.01*	1.35	1.22	2.35*	2.93*	1.91	1.44
Area x taxa	11	3.19*	1.30	0.03	0.73	0.46	2.07	1.59	1.10	3.86*
Station x taxa	99	0.97	0.70	0.90	0.54	0.68	1.95	1.17	0.83	0.70
Error	99									

[1] Significant F-values at the 0.95 level or above are indicated by *.

Table 10.--F-values for sources of variation from three-way analysis of variance comparing pool bottom fauna samples [1]

Source	df	IC1-ID1 [2]	ID1-ID2 [2]	IC1-IC2	IC2-ID2	ID2-IID3	IC2-IIC1	IIC1-IID3	IIC1-IIC2	IIC2-IID3
Area	1	4.27*	20.24*	1.42	3.99*	4.25*	17.01*	20.67*	23.25*	0.49
Stations	9	1.42	1.13	2.67*	1.18	1.37	0.62	2.03	0.99	2.23*
Taxa	7	31.90*	28.88*	32.86*	28.56*	41.15*	35.14*	55.19*	38.90*	29.11*
Area x station	9	0.96	1.49	1.48	1.95	2.72*	1.75	1.36	1.40	1.23
Area x taxa	7	1.41	3.25*	2.53*	2.03	1.08	8.44*	4.17*	65.95*	0.95
Station x taxa	63	0.93	0.94	0.97	0.73	1.36	0.71	1.42	0.86	1.10
Error	63									

[1] Significant F-values at the 0.95 level or above are indicated by *.

[2] Degrees of freedom are different for IC1-ID1 and ID1-ID2 because of a missing station.

abundances between the two areas. Sampling error is probably high enough to mask any difference in abundance between control and experimental area.

Two of the other eight F-values for the treatment term in the riffle area were significant and indicated that the benthic abundances were different in those areas. One occurred during the second treatment in the experimental area and the other resulted from a comparison between the experimental riffle area before the second treatment and the control riffle during the second treatment.

The significant term for the experimental area during the second treatment (IIA1-IIA2) is important because it shows that a change in abundances had taken place, while a non-significant F in the control area (IB2-IIB3) would establish the treatment with lampricide as the cause. A problem is encountered because there is a difference in the time of sampling the control area (B) and experimental area (A) during and after the second treatment. Since the control area was sampled on the day of the second treatment, the result in (IB2-IIB3) is not as easily compared. The non-significant F for the (IIA2-IIB3) area term is also confusing because it implies that the experimental benthic community had not changed from that of the control. This situation can probably be explained by the mean benthic numbers given in Figure 6. The control area, which had not changed significantly between sampling periods, had continually been

decreasing in mean number of individuals per square foot up to the last sampling period. A three-way analysis of variance comparing the control riffle before the first treatment (IB1) and during the second treatment (IIB3) was significantly different at the .995 level ($F = 9.71_{1, 99}$) validating the drastic and regular decline in the control benthic numbers.

The mean number of organisms per square foot in the experimental riffle area remained nearly constant until the second treatment. The decrease in the experimental riffle of 54.2 individuals per square foot after the second treatment was highly significant and when compared with the extremely small changes in that area for the previous sampling periods, gives strong indication that the second treatment with TFM (4.0 ppm) caused a significant decline in the riffle benthic abundances.

The significant F-values for the station term show that enough variation was present to make the stations different. This was not necessarily the reason for a significant area term because (IA2-IIA1) had a significant station term and a non-significant area term.

Even though the larvicide probably caused a decrease in the benthos, the conditions were satisfactory for rapid recolonization. None of the taxa were eliminated entirely and substantial populations probably were present upstream from the study area which could provide enough drift to compensate for a small effect. Waters (1964)

found that the mayfly, Baetis, returned to 100% of its former density 4 days after removal. He also has shown that drift is a mechanism capable of returning disturbed populations of many stream invertebrates to normal or capacity levels in a relatively short time.

The wide variation in the pool benthos makes analysis of the information very difficult. The significant F-value for the area term comparing the experimental and control pool areas before treatment establishes that these invertebrate communities were statistically different (Table 10). Also nearly all of the treatment terms were significant for both the control and experimental areas. The mean number of organisms per square foot dropped in both the experimental and the control pool area during the first treatment period. Both areas then increased to the second treatment period. There was a very substantial decrease of 23.4 benthic organisms per square foot in the experimental pool area during the second treatment. This decrease indicates that the second treatment at 4 ppm TFM did significantly lower the benthic abundance in the experimental pool area.

The control pool was also highly variable and the non-significant area term between the experimental after the second treatment (IIC2) versus the control during the second treatment (IID3) is probably a circumstance of that variation. The control pool area certainly did not provide a good measure for the

effects of the TFM in the experimental pool area, and little value can be placed upon these comparisons in judging the effects of the lampricide upon the benthic fauna.

Periphyton Results

Periphyton growth was of interest in this study because it provides the basis of the stream's productivity and because little work has been done on its reactions to environmental changes. Periphyton growth was examined during the years 1964 and 1965.

This community of organisms is made up of those that are attached or move upon submerged substrates. Reid (1961) says that the periphyton, typically, is an assortment of unicellular and filamentous algae with various attached protozoans, bryozoans and rotifers. Sladeckova and Sladecek (1962) define the true periphyton as those organisms which are attached, thus immobile, and which show various adaptations for sessile life. Sladeckova (1962a) found in a new reservoir that this group contained bacteria, algae, fungi, and rotifers. Clifford (1959) found that the periphyton community on artificial substrates in a Michigan stream was composed almost entirely of diatoms.

Stream velocity is thought to be an important factor in periphyton growth. It was measured at each area on the days of sampling as shown in Table 11. The importance of current velocity was made evident by Whitford (1960) when he demonstrated

Table 11.--Current velocities at periphyton areas in feet per second. (Each value is an average of four measurements.)

Date	Area Y	Area Z	Area X	Area W
1964				
Aug. 13	1.1	1.2	0.8	0.9
Aug. 31	1.4	1.7	0.6	0.8
Sept. 14	1.8	1.9	1.1	1.1
Oct. 12	0.3	0.1	0.1	0.3
Oct. 26	2.6	3.8	0.7	1.6
Nov. 9	3.9	3.8	0.9	1.8
1965				
June 23	1.2	1.1	-	0.6
July 7	0.8	1.1	0.1	0.4
July 21	0.8	0.7	0.2	0.3
Aug. 4	0.6	1.3	0.2	0.4
Aug. 18	0.9	0.8	-	-
Sept. 1	1.7	1.8	0.5	1.7

that many species of attached algae grew best in a current and that some died when placed in still water. Kevern and Ball (1965) also demonstrated higher periphyton productions with higher current velocities.

Periphyton standing crop in the Chocolay River was measured by allowing growth of the communities on the submerged plexiglass plates for a 14-day period. Sladeckova (1962b) used this method because the quantitative removal of the periphyton is easily accomplished.

The periphyton was collected, dried and weighed to measure the standing crop. The weight of organisms on a suitable, uniform surface is a more accurate and direct measure of the productive capacity of waters than other techniques (Cooke, 1956).

Figures 8 and 9 contain graphs of periphyton dry weights over the 2-year sampling period. The graph in Figure 8 shows the two pool sampling areas (W and X). Standing crop in these two areas remained very consistent over the study period. The control pool produced higher weights than the experimental pool area for most of that time. It is quite interesting to note that standing crop in both years showed three distinct peaks on approximately the same dates. It is also of note that area (X) had consistently slower current velocities than did area (W), and yet, still exhibited higher standing crops. It is possible that the increases in periphyton standing crop with increased current velocity occurred but were

not measured because of a high rate of sloughing and scouring which removed substantial amounts of periphyton from the plates. Whitford (1960) indicated that current velocities must exceed 0.5 feet per second to produce the steep diffusion gradient that is beneficial to growth. Kevern and Ball (1965) showed increased production with much lower increases in current velocity.

The graph of the periphyton weights in the pool areas does not show any drastic effects from the treatments with lampricide. Growth in both areas rose to a peak after the second treatment and then dropped off sharply in mid-November, as it had done in 1964.

The graph in Figure 9 shows the periphyton standing crops in riffle areas (Y and Z). The weights for these areas were considerably lower than those found in the pool areas. Again the explanation might be that the current velocities were high and might have sloughed off enough periphyton to allow greater weights in the pool area, not taking into consideration other unmeasured factors such as light and oxygen tensions. McIntire (1966) stated that in laboratory tests run at high oxygen tensions, rates of growth were higher for the slow current periphyton communities than for the fast current communities.

The experimental riffle area (Y) and the control riffle area (Z) produced nearly equal weights of periphyton at each time of sampling and they fluctuated together. Here, as with the pool

Figure 8.--Graph of periphyton dry weights over the entire study period for the pool areas.

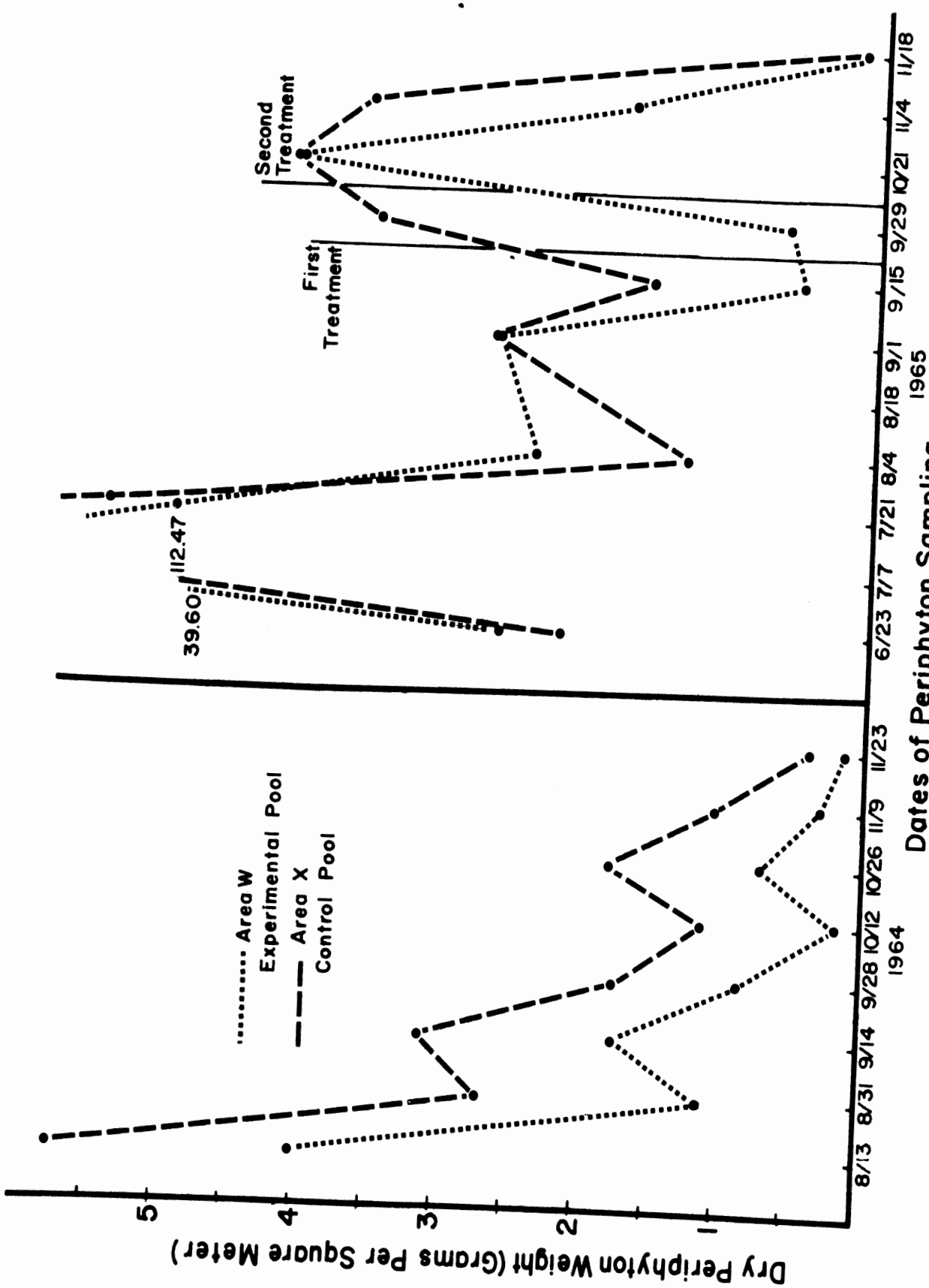

Dry Periphyton Weight (Grams Per Square Meter)
5
4
3
2
1
8/13 8/31 9/14 9/28 10/12 10/26 11/9 11/23
1964
6/23 7/7 7/21 8/4 8/18 9/1 9/15 9/29 10/21 11/4 11/18
1965
Dates of Periphyton Sampling
Area W
Experimental Pool
Area X
Control Pool
First Treatment
Second Treatment
39.60
112.47

Figure 8

Figure 9.--Graph of periphyton dry weights over the entire study period for the riffle areas.

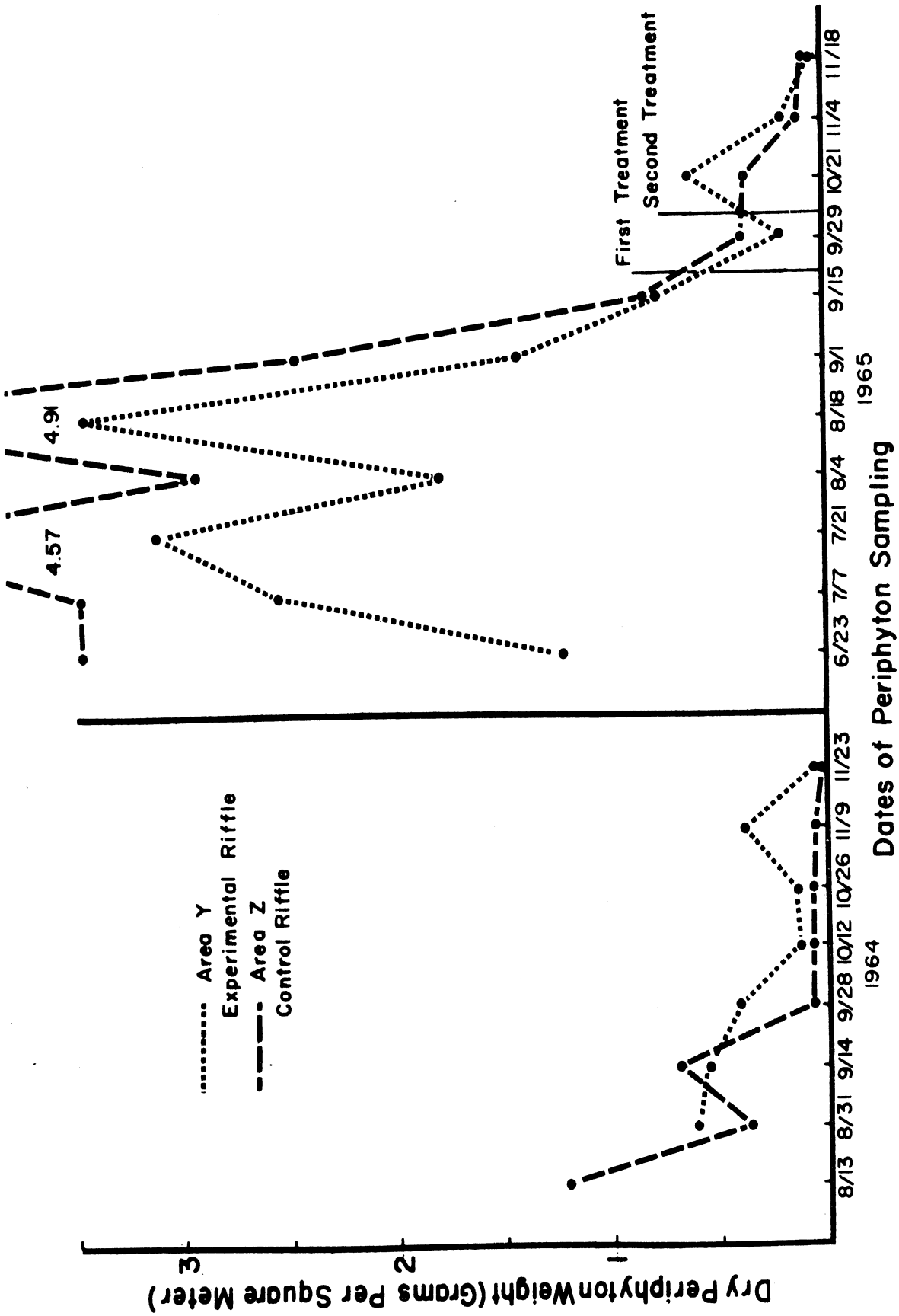

Dry Periphyton Weight (Grams Per Square Meter)
3
2
1
Area Y
Experimental Riffle
Area Z
Control Riffle
4.91
4.57
First Treatment
Second Treatment
8/13 8/31 9/14 9/28 10/12 10/26 11/9 11/23
1964
6/23 7/7 7/21 8/4 8/18 9/1 9/15 9/29 10/21 11/4 11/18
1965
Dates of Periphyton Sampling

Figure 9

areas, the treatments did not appear to affect the periphyton growth which was actually higher in the treated area after the second application of TFM.

Statistical comparisons between the periphyton weights in the experimental and control areas from 1964 and 1965 were made by a four-way analysis of variance with two- and three-way interactions. The F-values for the sources of variation are given in Table 12. There was a significant F, at the 95% level, for all four sources of variation. The weights were different between the two years, they were different on the various dates sampled, and the weights in pools and riffles were different. The weights in the experimental areas were also different from those in the control areas. This variation in standing crops is probably too large to make meaningful statistical comparisons between the areas.

There is little basis in this study for measuring the effects of the larvicide upon periphyton standing crop. The dry weights from both 1964 and 1965 show a drastic decline in October and November which is probably due to shortened photoperiod and cold water temperatures. These fluctuations at the time of treatment tend to mask any effects which might be due to the TFM.

Mean periphyton weights for the riffle and pool areas before and after each of the treatments with larvicide are given in Table 13. A two-way analysis of variance with 1 and 5 degrees of freedom with orthogonal contrasts was used to test whether the

Table 12.--F-values for sources of variation from four-way analysis of variance comparing periphyton sampling areas [1]

Source	df	SS	MS	F
Total	53	.0046111		
Years	1	.00012025	.00012025	8.95**
Dates	6	.00182944	.00030490	22.69***
Water [2]	1	.00084846	.00084846	63.13***
Area [3]	1	.00018793	.00018793	13.98***
Year x dates	6	.00041359	.00006893	5.13**
Year x water	1	.00007731	.00007731	5.75*
Year x area	1	.00000706	.00000706	0.53
Date x water	6	.00032349	.00005391	4.01*
Date x area	6	.00000000	.00000000	0.00
Water x area	1	.00014331	.00014331	10.66**
Year x dates x water	6	.00047606	.00007934	5.90**
Year x water x area	1	.00000844	.00000844	0.63
Date x water x area	6	.00009138	.00001523	1.13
Error	10	.00013439	.00001344	-

[1] Significant F-values at the 0.95 level are indicated by *, at the 0.975 level by **, and at the 0.995 level by ***.

[2] Comparison between riffle and pool areas.

[3] Comparison between experimental and control areas.

Table 13.--Mean periphyton weights in grams per square meter from two sampling periods immediately before and after each treatment with TFM

	First treatment period		Second treatment period	
	Experimental	Control	Experimental	Control
RIFFLE AREAS				
Before treatment				
First mean	1.44	2.48	0.82	0.85
Second mean	0.82	0.85	0.19	0.39
After treatment				
Third mean	0.19	0.39	0.62	0.37
Fourth mean	0.62	0.37	0.19	0.11
POOL AREAS				
Before treatment				
First mean	2.68	2.71	0.53	1.60
Second mean	0.53	1.60	0.65	3.57
After treatment				
Third mean	0.65	3.57	4.12	4.17
Fourth mean	4.12	4.17	1.77	3.64

mean standing crops were significantly different. Eight analyses were made comparing the experimental and the control areas during the two treatment periods. Two means before each treatment were compared with two means after the treatment for each of the four study areas. Eight more analyses were run comparing each of the four experimental areas with each of the control areas to test whether they were significantly different. None of the resulting 16 F-values were significant at the 0.95 level.

The direction of the changes of the mean standing crops during the two treatments also does not indicate any adverse growth changes due to the lampricide. Only the experimental riffle area during the first treatment showed a decreased mean weight and the corresponding control area also decreased. All other periphyton areas showed no change or increased standing crops during the treatment periods. So there is no evidence in this study that the periphyton standing crops were affected by either of the larvicide treatments.

SUMMARY

1. The Chocolay River, Marquette County, Michigan, was selected to study the effects of lamprey larvicide on the bottom fauna and periphyton. In 1965, two stream treatments with TFM (3-trifluormethyl-4-nitrophenol) were made. The concentration of larvicide was 1 ppm during the first treatment and 4 ppm during the second. Bottom fauna and periphyton samples were collected before and after each treatment to analyze the effects of the larvicide.

2. In order to evaluate the effect of the larvicide, twelve taxa of bottom fauna in riffle areas and eight taxa in pool areas were utilized. Percent change in abundance of each taxa did not reveal a larvicide effect from the 1 ppm treatment. However, all taxa, except two, decreased in numbers in the experimental riffle area from the 4 ppm treatment. The eight taxa in the experimental pool area also decreased in numbers during the 4 ppm treatment. These results indicated that the second larvicide treatment did cause a decline in bottom fauna.

3. Numbers of benthic organisms in the experimental and control areas were statistically compared. In the experimental riffle areas, the number of benthic organisms was significantly lowered after the second treatment. No other significant differences for the

bottom fauna in the riffles were found except between the experimental and control number before the second treatment. This difference was probably a function of the high variation in the control.

Pool bottom fauna areas exhibited high variation unrelated to TFM and most statistical comparisons showed the experimental and control to be significantly different in numbers of benthos during most sampling periods. These differences make a judgment on larvicide effects impossible for the experimental pool benthos.

The only positive result of statistical analyses strongly indicated that the 4 ppm larvicide treatment caused a decline in riffle bottom fauna abundance, but not the pool abundance.

4. During 1964 and 1965, periphyton standing crop was measured at two experimental and two control areas to test effects of the larvicide. A statistical analysis comparing periphyton weights revealed that standing crops were significantly different between years, areas, and within areas on different dates. Periphyton weights immediately before and after each treatment were compared to further test the effects of the larvicide treatments. No significant differences were found suggesting that the larvicide did not affect the standing crop of periphyton.

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