MONTANA FISH AND GAME DEPARTMENT
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SOME EFFECTS OF DDT ON COLD WATER FISH AND FISH-FOOD ORGANISMS

COMPLETION REPORT FOR JOBS II AND III, PROJECT NOS. F-21-R-4

A FEDERAL AID TO FISH RESTORATION PROJECT

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by

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ABSTRACT

Bio-assays were conducted on six species of cold water fish to determine mortality rates under different concentrations of DDT and varying physical, chemical and biological conditions. A total of 6000 fish was tested. The mortality rate of rainbow trout varied little at DDT concentrations between 0.5 and 10 ppm. Mortality rates increased as the size of the fish decreased. Hatchery rainbow trout had a higher mortality rate than wild rainbow trout. Mortality rates increased as the temperature increased but decreased when turbidity and alkalinity increased. Mortality rates were increased by feeding trout aquatic insects treated with DDT. A comparison of six species of fish tested in 1 ppm DDT showed the longnose sucker had a mortality rate of 94 percent while the salmonids had less than 10 percent. Delayed mortality occurred in all species of fish treated in DDT throughout a six month observation period.

Observations were made on fish and fish-food organisms in a test stream eight months before, during and 19 months after treatment with DDT which was applied at one point in the stream at 0.4 ppm. Immature aquatic insect populations were reduced 99 percent following application and required 18 months to regain pre-treatment numbers. Immature aquatic insect populations below 0.75 mile downstream from the point of application regained 15 percent of the pre-treatment numbers 11 months after application. The diet of trout in this downstream area changed from immature aquatic insects to adult insects and aquatic vegetation. Trout populations in the first mile of treated water showed no significant change one year following treatment but below this point showed a 73 percent reduction.

INTRODUCTION

DDT is a widely used insecticide for the control of forest insects. Aerial application of this insecticide over millions of acres of forest lands has caused mortalities in aquatic organisms in the waters of these areas. Qualitative studies on the effects of DDT on aquatic organisms have been conducted by Adams et al. (1949), Hoffman and Surber (1949), Cope and Park (1957), and Warner and Fenderson (1962). Long range studies on the effects of DDT were reported in Canada by Ide (1957), Alderdice and Worthington (1959), Crouter and Vernon (1959), and Keenleyside (1959).

Aerial forest spraying at the rate of one pound of DDT per acre to control the spruce budworm in Montana started in 1952. In 1955, large areas of Yellowstone Park and the Gallatin National Forest were sprayed. During early winter of 1956, a heavy fish die-off was reported along the upper Yellowstone River. This stimulated the initiation of a cooperative study by the U. S. Fish and Wildlife Service, the U. S. Forest Service, the Montana Fish and Game Department and the Montana Agriculture Experiment Station to determine the effects of large-scale forest spraying on aquatic organisms (Graham and Scott, 1958). Another investigation was sponsored by the Montana Fish and Game Department. This had a twofold purpose: (1) to determine the effects of various concentrations of DDT under different physical and chemical conditions on the mortality of cold water fishes; (2) to determine the effects of a known concentration of DDT on fish and fish-food organisms in a test stream.

BIO-ASSAYS

The facilities of the State Fish Hatchery at Ovando, Montana were used for bio-assays which were conducted during the summers of 1957, 1958 and 1959.

Materials and Equipment

Bio-assays were conducted in modified hatchery troughs. Eight head troughs were used in holding the pre- and post-test fish. The rate of water flow to each of these troughs was regulated by individual faucets. Seven other troughs were used to conduct the experiments. In the early tests it was found that redwood troughs were unsatisfactory for DDT bio-assays. Analyses conducted by the Chemistry Department at Montana State College showed that redwood absorbed DDT from experiments using high concentrations and allowed it to leach out during low concentrations. Late in the experiments of the first year the troughs were painted with a chemical resistant paint. This reduced the leaching effect but was not entirely satisfactory due to chipping. During the last two seasons fiberglass troughs (210 liter capacity) were used. These were inserted into the redwood troughs with clearance at the head, sides and bottom. Overflow water from the head trough served as a water bath to reduce temperature fluctuation in the fiberglass troughs (Figure 1). Hardware cloth was used to cover the troughs and prevent fish from jumping out.

Centrifugal pumps rated at 17 gallons per minute were used to recirculate water in the fiberglass troughs. Plastic pipe was employed for recirculating water. The intake pipe was screened to prevent weakened

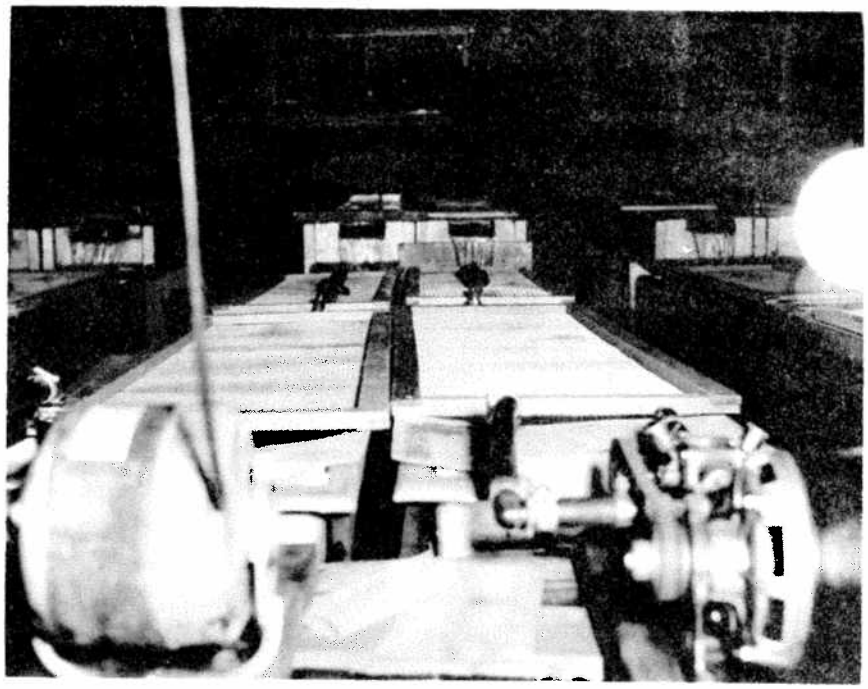

Figure 1. Unit experiment troughs and head troughs.

fish from being sucked into the pump. Test water was aerated by being forced under pressure through small holes in the end section of the ex-current pipe (Figure 2).

Two earthen ponds were used during the experiments. The larger had a surface area of about 2000 square feet with an average depth of 2.5 feet and was divided into six sections (Figure 3). The smaller had a surface area of approximately 250 square feet and was divided into three sections. Frames covered with plastic screen were used as dividers.

The water supply for the troughs and holding ponds was obtained from a spring. Physical and chemical water analyses showed the following ranges: temperature, 40 - 53 F.; turbidity, 0 - 35 ppm; pH, 7.6 - 8.2; methyl orange alkalinity, 180 - 280 ppm; total dissolved solids, 275 -

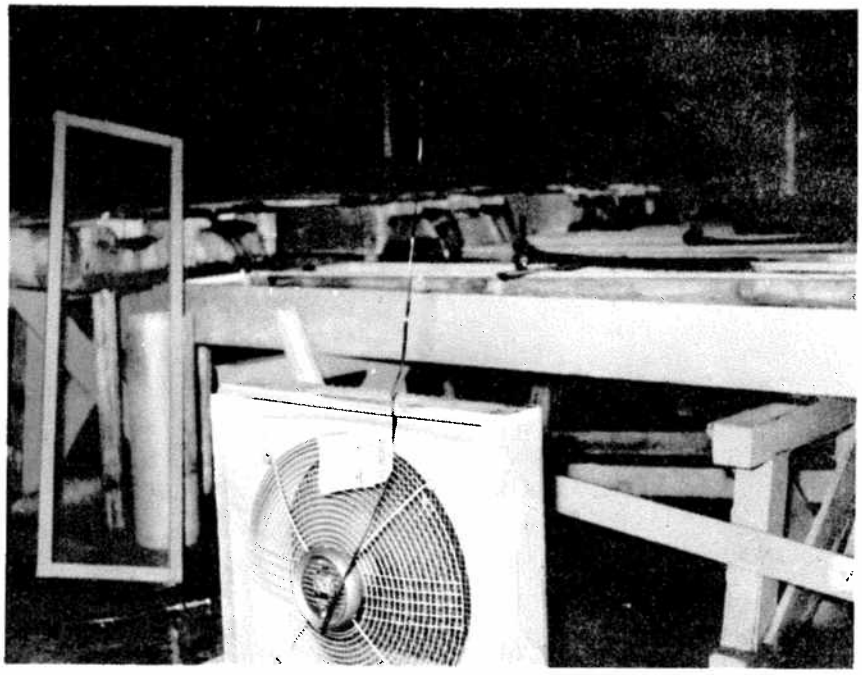

Figure 2. Cooling system used in unit trough.

330 ppm.

Both wild and hatchery fish were employed in the experiments. The wild fish included cutthroat trout (Salmo clarki), rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), brook trout (Salvelinus fontinalis), mountain whitefish (Prosopium williamsoni), longnose sucker (Catostomus catostomus), and largescale sucker (C. macrocheilus). Wild fish were collected with an electric shocker from unsprayed streams in Western Montana and transported to Ovando in a 250 gallon hatchery planting tank. Hatchery rainbow and cutthroat trout were obtained from the State Fish Hatcheries at Anaconda and Arlee. All wild and hatchery fish over four inches were kept in the holding ponds. Hatchery fry and fingerlings were held in the head troughs. Fish were held one week be-

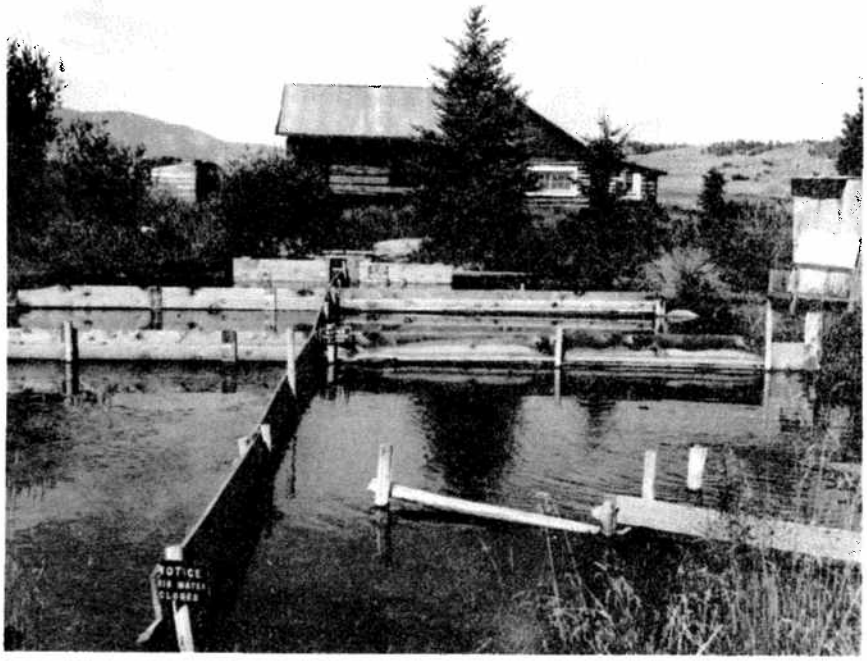

Figure 3. Holding pond used in experiments.

fore testing to allow recovery from shocking and/or transportation. During holding periods the fish were fed commercial dry concentrated food. The DDT solution used in the experiments was obtained from the U. S. Forest Service and contained one pound of DDT dissolved in 1.25 quarts of hydrocarbon solvent and diluted in fuel oil to make one gallon.

Methods

Bio-assay techniques were modified from Doudoroff, et al. (1951). Larger fish were seined from the holding pond and transferred to the head troughs for a 24-hour pre-testing hold. This allowed time for acclimation and also permitted the detection of injured or sick fish. Seven units (fiberglass troughs) including one control were run as a series. Several

series constituted an experiment. Test fish were transferred from the head trough to the unit troughs containing 200 liters of water. Then a known concentration of DDT was added to the water and recirculated for a 48-hour test period. At the start of each series pH and total alkalinity were determined. Oxygen and ammonia analyses were conducted at the start and at eight-hour intervals while temperature and the number of fish showing signs of distress were recorded hourly for each unit. When a mortality occurred, the weight and length of fish as well as the time of death (30 minute range) were recorded. Dead fish were put into labeled plastic bags and frozen for DDT analyses. At the conclusion of each series surviving fish were transferred from the unit trough to the head trough for a 24-hour post-test hold. After 24 hours, all surviving fish were weighed, measured, marked with either opercle or plastic jaw tags and released into a section of a holding pond for further observation. All post-test mortalities were recorded. Between each series, the contaminated troughs and equipment were thoroughly cleaned with hot soapy water followed by hot and cold water rinses.

Experimental Design. Many questions arose relative to the effects of DDT on fish from field studies conducted in Montana and during the bioassay operations. Experiments were designed to test the following: mortality of rainbow trout at various concentrations of DDT; relationship between size of test fish and mortality; variation in the mortality between hatchery-reared and wild fish of the same species; mortality of rainbow trout tested in fuel oil and in the solvent used for the DDT solution; comparative mortality of six species of cold water fish treated

in DDT; effects of various physical, chemical and biological factors on the mortality of fish tested in DDT. In addition to the immediate test mortality, experiments were designed to determine post-test mortalities. Fish (over four inches long) surviving the initial 48-hour test period were marked for later identification. Any mortality after the 48-hour test period was recorded as delayed. A 30-day delayed mortality period was employed in the 1957 and 1958 tests, while a six-month period was used in 1959. Fish in the latter group were observed for an additional 5.5 months after being moved from Ovando to the Blue Water Hatchery. Experiments were designed so that results could be treated statistically. Several statistical designs of analysis of variance were used to test the null hypothesis that the means of normal populations were equal; using independent samples of equal size from the populations and assuming that the populations had equal variances. The null hypothesis was rejected at the level of significance when the calculated mean square was greater than the tabular value obtained from the frequency (F) distribution tables for the appropriate degrees of freedom. The designs and distribution tables were taken from Ostle (1954) and Freund *et al.* (1960).

Experiment Limits. The first 25 units were used to establish standard procedures and set limits for the unit tests. Exceptions were made when a specific physical, chemical or biological factor in relation to the mortality was being tested. Limits set for the physical and chemical properties of the test water were as follows: temperature 50 - 70 F; turbidity 0 - 15 ppm; pH 7.6 - 8.2; methyl orange alkalinity 230 - 280

ppm; ammonia 0 - 1 ppm. If water in the test unit exceeded these limits the results were not used in analyses.

DDT (ppm by weight) dissolved in fuel oil was added to the water of the test unit. Samples were collected from the standard test units for DDT analyses at one hour and 48 hours, but due to cost of analyses only 10 selected samples were analyzed. Average results for the 48-hour samples were as follows: when DDT was added at the rate of 4 ppm, tests showed 0.24 ppm in the water; at 5 ppm - 0.33 ppm; 6 ppm - 0.6 ppm. These analyses consistently showed that only a small fraction of the DDT added to the water, actually entered it. Samples taken from units with 3 ppm DDT showed 0.56 ppm at one hour and 0.2 ppm after 48 hours. This was a 64 percent decrease. Alderdice and Worthington (1959) reported that only a small amount of the DDT added to the water, appeared in analyses. The DDT used in this study is expressed as the ppm added to the water, although actual concentrations in the water were known to be much less.

Effects of Various Concentrations of DDT on Mortality of Wild and Hatchery Rainbow Trout

The fish used for experiments were divided into three size groups as follows: 1.5 - 3.0 inches; 3.1 - 5.0 inches; 5.1 - 10 inches.

Rainbow Trout 1.5 - 3.0 inches. Four series of tests were conducted on this size group. Three of these were hatchery trout from Arlee and one was wild trout. All fish were tested within one week from time of collection or delivery from the hatchery. A total of 100 hatchery trout was used for each unit in the first series. One unit test was made at each

of the following concentrations: 0.05; 0.125; 0.25; 0.5; 1; 2; 3 ppm. All fish died within 24 hours. Only two fish died in the controls. In the second series 25 hatchery trout were used per unit. One unit test was made at each of the following DDT concentrations: 0.05; 0.125; 0.25; 0.5; 1 ppm. Again all fish died during the first 24 hours. No mortality occurred in the control. In the third series, another lot of Arlee hatchery rainbow trout was used. These were tested immediately upon arrival. Twenty-five trout were tested in each unit with five unit replications conducted at 0.125 ppm. All died within 24 hours. No mortality occurred in the control. In the fourth series 25 wild trout were tested in each unit. One at 0.5 ppm and another at 1 ppm. Mortalities were 5 and 7 trout respectively while no mortality occurred in the control.

Rainbow Trout 3.1 - 5.0 inches. Three series of tests were conducted for this size group. Arlee hatchery fish were tested in two series and wild trout in the other. In the first series 25 hatchery trout from one lot were tested in each unit. Seven unit replications were conducted at each of 0.5 and 1 ppm DDT. The mean unit mortalities were 21.8 trout at 0.5 ppm and 20.7 at 1 ppm. No mortality occurred in the control units. In the second series 25 hatchery trout from a different lot were tested in each unit. Seven unit replications were conducted at each of 2 and 3 ppm DDT. The mean unit mortalities were 16.3 trout at 2 ppm and 14.3 trout at 3 ppm. No mortality occurred in the control units. There was no significant difference between the mean mortalities within a series but there was a significant difference at the (F) 0.01 level between the mean mortalities of the two series using different lots of hatchery fish.

The surviving fish from each concentration in the second series were placed in separate sections of a head trough and held for 30 days to determine delayed mortality. Delayed mortality was 50 percent for trout tested at 2 ppm and 53 percent for those tested at 3 ppm, while control trout had a 6.0 percent mortality. In the third series 25 wild rainbow trout were tested in each unit. Two unit replications were conducted at 0.25, 0.5, 1, 2, 3, 4 and 10 ppm. The mean unit mortalities for the various concentrations were as follows: 2.5 trout at 0.125 ppm; 0.0 trout at 0.5 ppm; 3.5 trout at 1.0 ppm; 0.0 trout at 2 ppm; 3.5 trout at 3 ppm; 4 trout at 5 ppm; 5 trout at 10 ppm. No mortality occurred in the controls. No significant difference was found between the means of the various concentrations.

Rainbow Trout 5.1 - 10 inches. Three series of tests were conducted on hatchery trout of this size group and one series on wild trout. Hatchery trout from Anaconda were tested in the first series. Ten trout were tested in each unit with three unit replications at each of the following DDT concentrations: 0.5; 1; 3; 5; and 6 ppm. A wide range of mortalities (1 - 10 trout) occurred within the same concentration. The mean unit mortalities for the various concentrations were 6.9 trout at 0.5 ppm, 7.5 at 1 ppm, 7.3 at 3 ppm, 7.5 at 5 ppm and 7.2 at 6 ppm. No mortalities occurred in the controls. There was no significant difference between the means of the various concentrations. In the second series hatchery trout from Arlee were tested. Ten trout were tested in each unit with five unit replications conducted at the same concentrations as the first series. The mean unit mortalities for the various concentrations were

5.8 trout at 0.5 ppm, 6.4 at 1 ppm, 6.2 at 3 ppm, 6.0 at 5 ppm, and 5.9 at 6 ppm with no mortality in the controls. There was no significant difference between the means of the various concentrations. There was a greater difference in the mean unit mortality between the Anaconda hatchery trout (7.3) and Arlee hatchery trout (6.0) than between the levels of concentration within each series. This difference was significant at the (F) 0.05 level. The mean 30-day delayed mortality for the Anaconda trout was 0.8 bringing the mean total mortality to 8.1. The mean 30-day delayed mortality for the Arlee trout was 1.3 constituting a total mortality of 7.3. Arlee hatchery trout were tested in the third series. These were kept in the ponds at Ovando 45 days before being tested. Ten trout were tested in each unit with two unit replications at concentrations of 0.25 and 9 ppm. The mean unit mortalities were seven and eight fish respectively while no mortality occurred in the control. Wild rainbow trout were tested in the fourth series. Ten trout were tested in each unit with six unit replications at the following concentrations: 1; 2; 5; 10; 15 ppm. The mean unit mortalities for the various concentrations were 1.5 trout at 1 ppm, 0.5 at 2 ppm, 1.8 at 5 ppm, 1.0 at 10 ppm and 1.2 at 15 ppm. No mortality occurred in the controls. No significant difference was found between the means of the various concentrations. The mean 30-day mortality was 1.1 trout, bringing the mean total mortality to 2.3.

The DDT concentration had little or no effect on the mortality (Table I) in the experiments conducted. The average mortality at all concentrations for the three size groups of hatchery and wild rainbow trout tested showed the mortality to be higher for hatchery trout (66 -

Table I. The percent mortality and number of unit replications (parentheses) using wild and hatchery rainbow trout for various concentrations of DDT.

Size	Fish tested	DDT concentrations in ppm											
		0.05	0.125	0.25	0.5	1	2	3	4	5	6	10	15
1.5 - 3.0	Hatchery	100 (2)	100 (7)	100 (2)	100 (2)	100 (2)	100 (1)	100 (1)					
	Wild				28 (1)	20 (1)							
3.1 - 5.0	Hatchery												
	Series 1				87 (7)	83 (7)							
	Series 2						65 (7)	57 (7)					
	Wild		10 (2)		0 (2)	14 (2)	0 (2)	14 (2)	16 (2)			20 (2)	
5.1 - 10	Hatchery												
	Anaconda				69 (3)	75 (3)		73 (3)		75 (3)	72 (3)		
	Arlee (series 2)				58 (5)	64 (5)		62 (5)		60 (5)	59 (5)		
	Arlee (series 3)			70 (2)									80 (2)
	Wild					15 (6)	5 (6)			18 (6)		10 (6)	12 (6)

100 percent) than for wild trout (10 - 24 percent) (Table II). The mor-

Table II. Percent mortality and average time of first mortality for all wild and hatchery rainbow trout tested in DDT.

	Length of trout tested in inches					
	1.5 - 3.0		3.1 - 5.0		5.1 - 10	
	Hatchery	Wild	Hatchery	Wild	Hatchery	Wild
Percent mortalities						
48-hour period	100	24	73	10	66	12
30-day period	-	-	87	-	76	23
Average time (hours) of first mortality	10	22	28	32	32	40

tality of hatchery trout was highest for the 1.0 - 3.0-inch size group and decreased as the size increased. The mortality of wild trout was also highest in the small size group (1.0 - 3.0 inches) but there was no significant difference between the two larger size groups.

There was a direct correlation between the length of trout tested and the average time of first mortality. Trout 1.5 - 3.0 inches died 10 to 18 hours sooner than those 3.1 - 5.0 inches and 18 to 22 hours before those 5.1 - 10 inches. Hatchery trout died 4 to 12 hours earlier than wild trout of corresponding size.

Effects of Fuel Oil and Solvent on Mortality of Wild Rainbow Trout

An experiment was conducted to determine the mortality of trout tested with "number two" fuel oil used as the carrier for the DDT solution. Three units, each containing 10 trout (4.0 - 8.0 inches) were tested with 9.9 ml of oil. This approximates the amount of fuel oil found in a 15 ppm DDT concentration. No mortality occurred in these units.

Another experiment was conducted to determine the mortality of trout treated with the DDT solvent. Two series were conducted using 0.6 ml and 3.3 ml respectively of solvent. This was the approximate amount of solvent in 1 ppm and 5 ppm DDT concentrations. In the first series 10 trout were tested in each unit with six replications using 0.6 ml of solvent. No mortality occurred in these units. One month after testing the mean unit mortality was 0.7 trout. In the second series 10 trout were tested in each unit with six replications using 3.3 ml of solvent. The mean unit mortality was 0.7 trout and at 30 days it was 1.7 trout. No mortality occurred in the control fish.

Effects of Temperature, Turbidity and Alkalinity on Mortality of Wild Rainbow Trout Tested in DDT

Results from the bio-assays conducted on rainbow trout showed a wide range of mortalities in each concentration tested, however the DDT concentration had little or no effect on varying the mortality. Field studies also showed a wide variation even between sprayed streams in the same vicinity.

An experiment was designed to determine if the physical and chemical properties of the water influenced the mortality of trout exposed to 1 ppm DDT. A statistical 2^n factorial analysis of variance experiment was used to determine the effects of temperature, turbidity and alkalinity on the mortality of trout tested in 1 ppm DDT. Ten wild rainbow trout (4.0 - 9.0 inches) were tested in each unit. The two mean levels of temperature were 54.2 F (range 53 - 62 F) and 64.4 F (range 53 - 72 F). The first was

maintained by use of a water bath while the second was allowed to fluctuate without interference. Turbidities at 5.0 ppm and 80 ppm were used. The first was untreated water while the second was produced by the constant agitation of water containing 200 ml of silt obtained from a stream bed. Alkalinities (methyl orange) of 80 ppm and 240 ppm were employed. Water containing 80 ppm was transported from a nearby stream while that with 240 ppm came from a spring. Four unit replications were conducted at each level.

The main effects of temperature, turbidity and alkalinity were significant at the (F) 0.01 level in the 48-hour test period. The interaction of temperature-alkalinity was significant at the (F) 0.01 level while turbidity-alkalinity and temperature-turbidity-alkalinity were significant at the (F) 0.05 level (Table III). The interaction of temperature-turbidity was not significant. At the end of 30 days the main effects of temperature and turbidity were significant at the (F) 0.01 while alkalinity was significant at the (F) 0.05 level. The interaction of temperature-alkalinity and temperature-turbidity were significant at the (F) 0.05 level. Other interactions were not found to be significant. Treatment means for the factors tested were as follows: temperature low - 2.1, high - 4.1; turbidity low - 4.7, high - 1.5; alkalinity low - 4.3, high - 1.8. Results showed that increasing the temperature increased the mortality while increasing the alkalinity and turbidity decreased the mortality. Berck (1953) reported that a considerable amount of DDT was adsorbed by sediment.

Table III. Analysis of variance for temperature, turbidity and alkalinity on the mortality during a 48-hour and a 30-day test period on wild rainbow trout tested using 1.0 ppm DDT.

Source of variation	Degrees of freedom	Sum of squares		Mean square		F statistics	
		48-hour	one-month	48-hour	one-month	48-hour	one month
Replications	3	7.1	5.35	2.73	1.78		
Main effects	3						
Temperature	1	34.03	69.03	34.03	69.03	14.99	38.14
Turbidity	1	81.28	87.78	81.28	87.78	35.80	48.49
Alkalinity	1	52.53	9.03	52.53	9.03	23.14	4.99
Two-factor interactions	3						
Temp. and turb.	1	9.03	9.03	9.03	9.03	3.98	4.99
Temp. and alk.	1	30.03	13.78	30.03	13.78	13.22	7.61
Turb. and alk.	1	13.78	3.78	13.78	3.78	6.07	2.09
Three-factor interactions	1						
Temp., turb. and alk.	1	11.28	1.53	11.28	1.53	4.97	0.85
Error	21	47.66	37.91	2.27	1.81		
Total	31	286.72	237.22				

F .01 = 8.02
F .05 = 4.32

Effects of Feeding Aquatic Insects Treated with DDT on the Mortality of Hatchery Rainbow Trout

Field observations conducted on streams in forest areas treated with DDT showed that fish gorge themselves on drifting aquatic insects affected by the poison and also showed that aquatic insect populations were greatly reduced in numbers and volume over a period of several months. An experiment was designed to determine the effects of feeding aquatic insects treated with DDT on the mortality of trout. Dead and/or paralyzed aquatic insects (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera and Diptera) were collected from streams sprayed with DDT. These were kept frozen until used. Hatchery rainbow trout (6.5 - 9.5 inches) were tested in eight different ways (Figure 4). This design included: trout exposed to DDT in water and fed on insects killed by DDT; those exposed to DDT in the water but not fed DDT treated insects; and those not exposed to DDT in the water but fed DDT treated insects. The second phase of this experiment, in which the trout were starved for 215 days, was designed to determine if periods of reduced food availability would effect the mortality rate of trout under the various treatments.

Most of the trout fed aquatic insects treated with DDT appeared to force themselves onto the pond bank. This reaction persisted for about three days following feeding but did not occur in trout just treated with DDT and not fed treated insects. This behavior was also observed under field conditions after forest spraying.

The trout of this experiment were held at Ovando for 45 days after the initial tests and then transferred to the Blue Water Hatchery for an

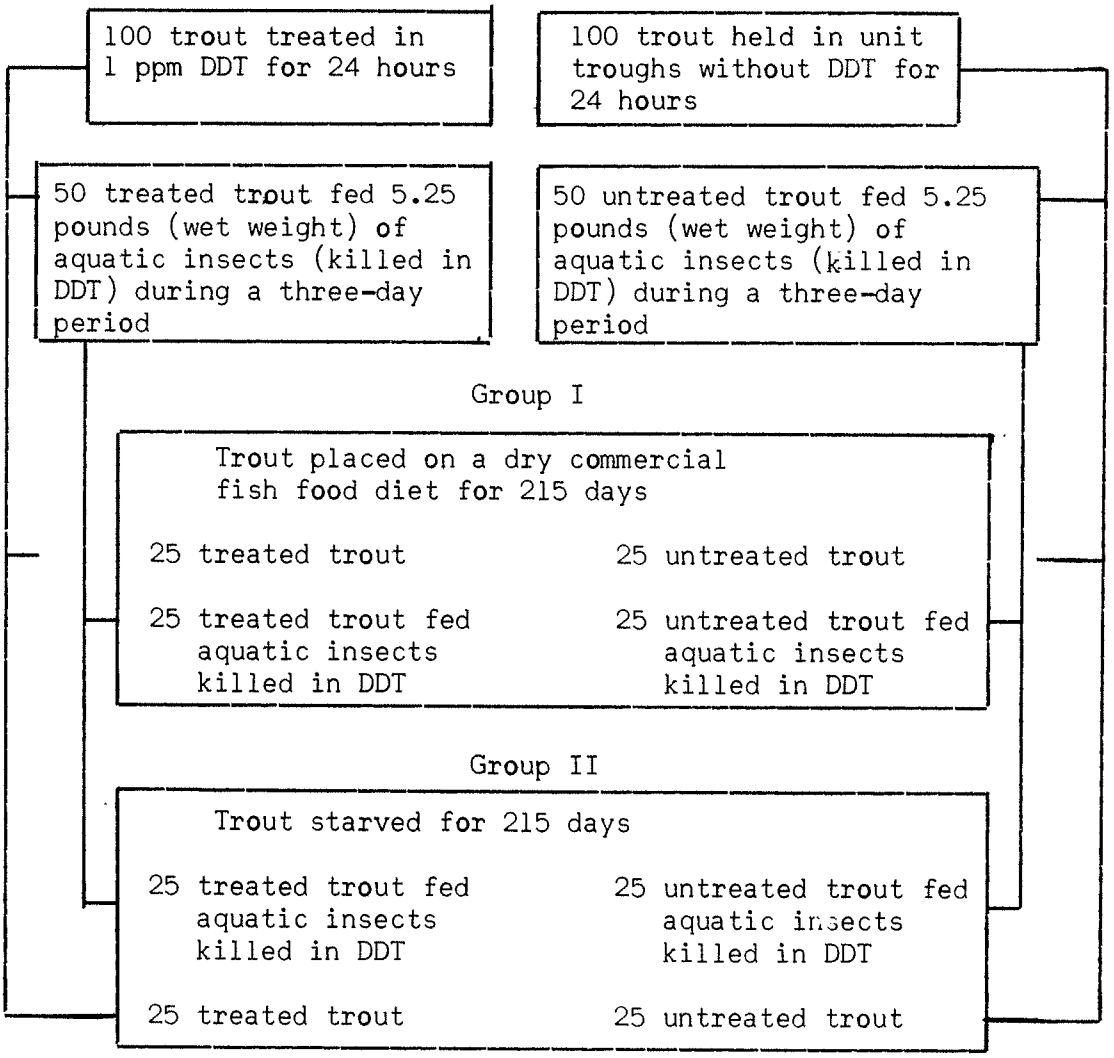

Figure 4. Design of feeding experiment for rainbow trout (6.5 - 9.5 inches) using aquatic insects previously treated with DDT.

additional 170 days. Twenty-four trout from this experiment disappeared soon after being placed in the holding ponds and were presumed eaten by mink. The mortality percentages do not include these fish.

The mortality of non-starved trout ranged from 17.6 to 35.2 percent for the various treatments while the starved group ranged from 39.8 to 83.6 percent (Table IV). Mortality for the controls were 4.5 percent for

Table IV. Percent mortalities of hatchery rainbow trout in feeding experiment.

Treatment	Series A	Series B
	Trout fed commercial food	Trout starved
No DDT in water	4.5	22.5
No DDT in water fed aquatic insects killed in DDT	17.6	39.8
Exposed to 1 ppm DDT for 24 hours	22.0	61.6
Exposed to 1 ppm DDT for 24 hours fed aquatic insects killed in DDT	35.2	83.6

the non-starved and 22.5 percent for the starved. Feeding DDT-killed insects increased mortalities of hatchery rainbow trout as follows: 13.1 percent for the non-starved group not exposed to DDT in the water; 13.2 percent for the non-starved group exposed to DDT; 17.3 percent for those starved for 215 days but not exposed to DDT; 22.0 percent for those starved and exposed to DDT. The starving of trout for 215 days increased mortalities as follows: 18.0 percent for the control; 22.2 percent for those fed DDT-killed insects but not exposed to DDT in the water; 39.6 percent for those not fed DDT-killed insects but exposed to DDT in the water; 48.4 percent for those fed DDT-killed insects and exposed to DDT in the water. The mortality of hatchery rainbow trout fed on DDT-killed

insects was significant at the (F) 0.01 level.

Effects of Algae Treated with DDT on Mortality of Hatchery Rainbow Trout

Chemical analyses of aquatic vegetation collected in forest areas treated with DDT showed that it contained 6.8 ppm DDT (Graham and Scott, 1959). An experiment was designed to determine the mortality of trout exposed to aquatic vegetation treated with DDT. Hatchery rainbow trout (6.5 - 9.5 inches) were divided into four lots. Two were exposed to 30 liters of filamentous algae previously treated with 2 ppm DDT for 48 hours while two lots were exposed to non-treated algae. The pattern of treatment is shown in Figure 5. The experiment had a duration of 200 days.

Trout mortalities for the lots exposed to DDT treated algae were as follows: 4.0 percent for those treated in water not containing DDT; 20.0 percent for those treated in water containing DDT. Mortalities for the lots not exposed to DDT treated algae were as follows: 8.0 percent for those treated in water not containing DDT; 24 percent for those treated in water containing DDT. There was no significant difference between the lots exposed to treated algae and those exposed to untreated algae.

A Comparison of Mortality for Six Species of Cold Water Fish Exposed to 1 ppm DDT

An experiment was designed to compare mortality of cutthroat trout (6.0 - 9.0 inches), rainbow trout (6.0 - 11 inches), brown trout (7.0 - 13 inches), brook trout (7.0 - 10 inches), mountain whitefish (8.0 - 13 inches), and longnose sucker (7.0 - 10 inches). Ten fish of one species

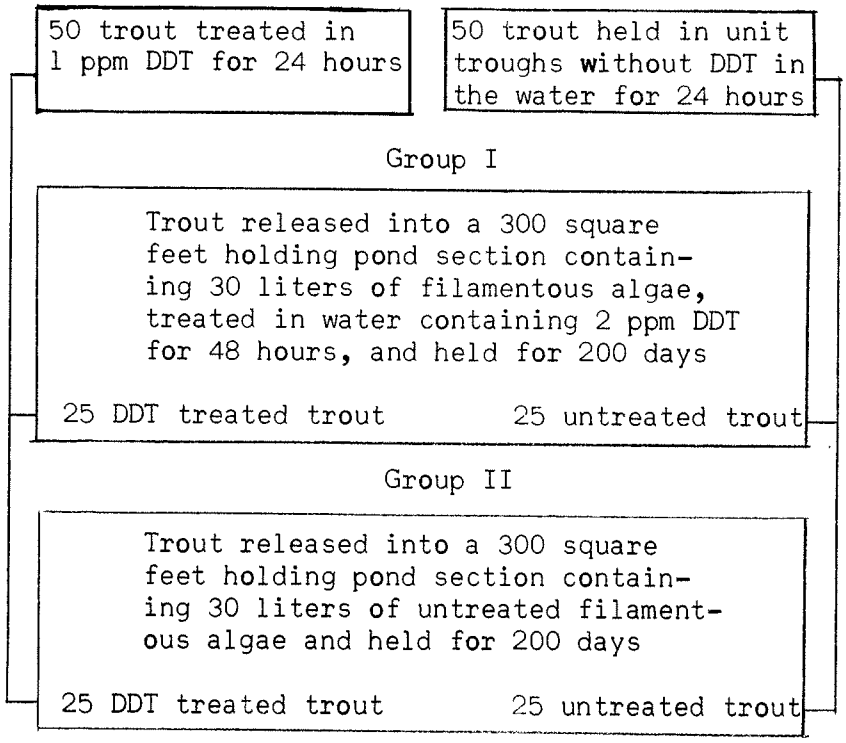

Figure 5. Design of algae experiment for rainbow trout (6.5 - 9.5 inches) using filamentous algae previously treated with DDT.

were tested per unit at 1 ppm DDT for 48 hours, with five unit replications per species. Fish were held at Ovando for 30 days post-treatment and then transferred to the Blue Water Hatchery for an additional five months.

The mean unit mortalities (Table V) during the 48-hour test period were as follows: longnose sucker - 94 percent; rainbow trout - 8 percent; other salmonids ranged from 0 - 2 percent. The mean unit mortalities 30 days following treatment were as follows: longnose sucker - 96 percent; all salmonids ranged from 15 - 19 percent except brook trout which was 2 percent. The mean unit mortalities six months following treatment were as follows: longnose sucker - 98 percent; mountain whitefish, rainbow trout,

Table V. Percent mortalities for six species of cold water fish tested in 1 ppm DDT for 48 hours.

Species	Duration		
	48 hours	30 days	Six months
Cutthroat trout	2	15	28
Rainbow trout	8	17	32
Brown trout	2	16	18
Brook trout	0	2	18
Mountain whitefish	2	19	38
Longnose sucker	94	96	98

cutthroat trout ranged from 28 - 38 percent; brook trout and brown trout - 18 percent. Control fish had a 4.0 percent mortality. The mean unit delayed mortality was highest for the mountain whitefish (36 percent) followed in order by cutthroat trout (26 percent), rainbow trout (24 percent), brook trout (18 percent) and brown trout (16 percent).

General Observations on Fish Exposed to DDT

Fish Reaction to DDT Poisoning. Early in the experiments it became apparent that all test fish showed similar behavior after exposure to DDT. They oriented themselves to the current and remained near the bottom immediately after initial exposure. Fish over 4.0 inches usually stayed close together at the lower end of the trough. After about two hours they spread out along the length of the trough but always near the bottom. The first sign of distress to DDT was movement toward the surface followed by a lack of positive response to the current. They then broke the water surface with the head partially extended and a few minutes later moved slowly back and forth throughout the trough, colliding with any objects

in their paths. Their movements became more rapid until violent thrashing and jumping occurred. This lasted from 10 to 30 minutes usually ending in death. The few fish that survived became paralyzed except for the operculum and did not respond to a pin-prick. Paralyzed fish were transferred out of the toxic water to head troughs for further observations and some lived for 10 days. In a few instances the entire tail region completely decomposed before movement of the operculum ceased. All fish that reached the violent stage died, however, many fish which showed only early symptoms of distress did recover.

When longnose suckers entered the violent stage a red slash appeared along the lateral line. This was observed in all individuals. Various color changes were noted in other species during the violent stage but never so pronounced.

Variation Between Lots of Fish Tested. Variations were observed between lots of fish from the same hatchery, between hatchery and wild fish of the same species and between wild fish of the same species collected from different areas.

Fish exposed to unfavorable conditions before testing had a higher mortality than those under favorable conditions. Wild fish held 30 days or more before testing showed higher mortalities than those used soon after capture. Wild rainbow trout from one field collection were divided into two lots. One was released into the holding pond while the second was held in a head trough where the water velocity required them to swim constantly. Three weeks later both lots were treated in 1 ppm DDT, using

10 trout (4.0 - 8.0 inches) per unit, with three replications from each lot. The mean mortalities were 0.7 for the holding pond lot and 5.6 for the head trough lot.

Sudden Temperature Change. After treatment all fish were transferred from the unit trough to the head trough and if temperature decreased more than 4 F, they thrashed about violently. This thrashing lasted about 15 minutes and approximately 50 percent of the fish died. Control fish were subjected to a 10 F temperature decrease without sustaining any loss.

Disease. Disease was much more prevalent in treated fish than in control fish. Furunculosis and fungus diseases were the most common. The greatest incidence occurred in mountain whitefish. Disease was more common in hatchery trout than in wild trout.

Spawning. Field observations indicated that some brown trout exposed to DDT in June died during the spawning period (October). Experiments designed to test this failed.

DDT Tissue Analyses. DDT analyses were made on dead and surviving fish treated with DDT. Fish from each unit were placed in separate plastic bags and refrigerated until the end of the test series. Unit samples were then ground, hand blended and kept frozen until analysed by the Chemistry Department of Montana State College.

The amount of DDT in the tissues of trout which died during tests ranged from 4.0 to 19 ppm while the tissue of fish that survived ranged from 5.5 to 23.7 ppm (Table VI). A tissue sample taken from fish killed in a unit with 0.5 ppm DDT had 10.3 ppm while one taken from a unit containing 6.0 ppm had 13.7 ppm. The variations in these observations

Table VI. Analyses of DDT in fish tissue collected from unit and surviving fish tested in bio-assays.

Concentration DDT in water 1/	Fish sample		Status when collected	ppm DDT in tissue
	Number	Species		
0.5	5	Rainbow trout	Dead (unit test)	10.3
0.5	1	Rainbow trout	Live (collected 63 days after test)	5.5
2.0	5	Cutthroat trout	Dead (unit test)	8.0
2.0	1	Cutthroat trout	Live (collected 66 days after test)	5.9
4.0	4	Mt. whitefish	Dead (unit test)	4.0
4.0	1	Mt. whitefish	Live (collected 69 days after test)	6.2
5.0	7	Rainbow trout	Dead (unit test)	19.0
5.0	3	Rainbow trout	Live (collected 1 day after test)	23.7
6.0	4	Rainbow trout	Dead (unit test)	13.7
6.0	2	Cutthroat trout	Dead (unit test)	15.3

1/ Calculated ppm of DDT suspended in oil added to water.

suggest that tests were either inadequate or wide individual tolerances existed.

TEST STREAM STUDY

Observations were made on fish and fish-food organisms in a test stream before, during and after treatment with DDT. The study started November 1957 and continued until April 1960.

Description of Area. The stream chosen for tests was Trail Creek which is a tributary to the Yellowstone River, in Park County. An 8-mile section without tributaries was used. This had an elevation of approximately 5380 feet msl and a gradient of 35 feet per mile. The study area was located in the transition between the upper Sonoran and Canadian life zones (Figure 6). Winter is severe in this area with prolonged periods

Figure 6. Study area located on Trail Creek.

of subfreezing temperatures and the stream frequently bridged over with ice or contained anchor ice. Melting snows caused flood conditions during the spring (April - June) each year of the study. Average stream measurements in the study section during low water were as follows: width 15 feet; depth 1.3 feet; surface velocity 1.9 feet per second; volume 30.1 cfs. There were numerous riffles and areas of slow shallow water but few pools and those over 3 feet in depth were rare. The principal bottom type was gravel followed in order by rubble, sand and silt. Water analyses at the low water period showed the following ranges: temperature 33 - 68 F; turbidity 15 - 80 ppm; oxygen 7.9 - 9.2 ppm; pH 7.8 - 8.0; methyl orange alkalinity 170 - 195 ppm; total dissolved solids 260 - 310 ppm. Algae was the only important aquatic vegetation in the stream.

Application of DDT. After eight months of pre-treatment sampling, the stream was treated with DDT on August 5, 1958. The chemical used was from the same stock employed in the bio-assays. To insure even distribution, the DDT solution was mixed with water and applied under pressure across a 3-foot swath at one location by use of a pump. It was applied at the approximate rate of one pound per surface acre, the same as that used in forest spraying. Based on the stream volume (28 cfs), this rate gave a concentration of 0.4 ppm DDT. It was added over a period of 232 minutes or the time required to treat a five-mile block flowing past the point of application.

Fish Population. Six 300-foot sections were sampled to determine the fish population before and after application of DDT. One section was located immediately above and another 600 feet above the point of appli-

cation. Four treated stations were at 0.05, 0.5, 1, and 2 miles respectively below (Figure 7). An electric shocker was used to collect fish and their total lengths were recorded. One census was made at each section as follows: five days before application (July 31, 1958); one month after application (September 7, 1958); one year after application (September 6, 1959).

Cutthroat trout was the most abundant salmonid in all censuses followed by brown trout and then rainbow trout. One longnose sucker and numerous longnose dace (Rhinichthys cataractae) were also taken. There were 18 percent more trout collected at one month post-treatment than in the pre-treatment sample. This probably resulted from an inadequate sample in the first census due to high turbidity. The two control sections and those treated at 0.05 and 0.5 miles showed no significant change in the fish population between the September 1958 and September 1959 sampling periods. However during this same period there was a 73 percent reduction in cutthroat trout and brown trout (Table VII) for the treated stations at 1.0 and 2.0 miles. Rainbow trout were present only in the 1959 sample. The average length of trout collected at the 1.0 and 2.0 mile stations in 1958 was 5.3 inches while in 1959 it was 7.0. Thirty-six trout under four inches were collected in 1958 compared to three in 1959.

Live Cars. Trout were placed in live cars at the various stations to determine the immediate effects of DDT. One control station was located 300 feet above the point of application while four treated stations were located 0.05, 0.5, 1, and 2 miles respectively below (Figure 7). The 82 rainbow and 44 brown trout tested were collected by shocking in the

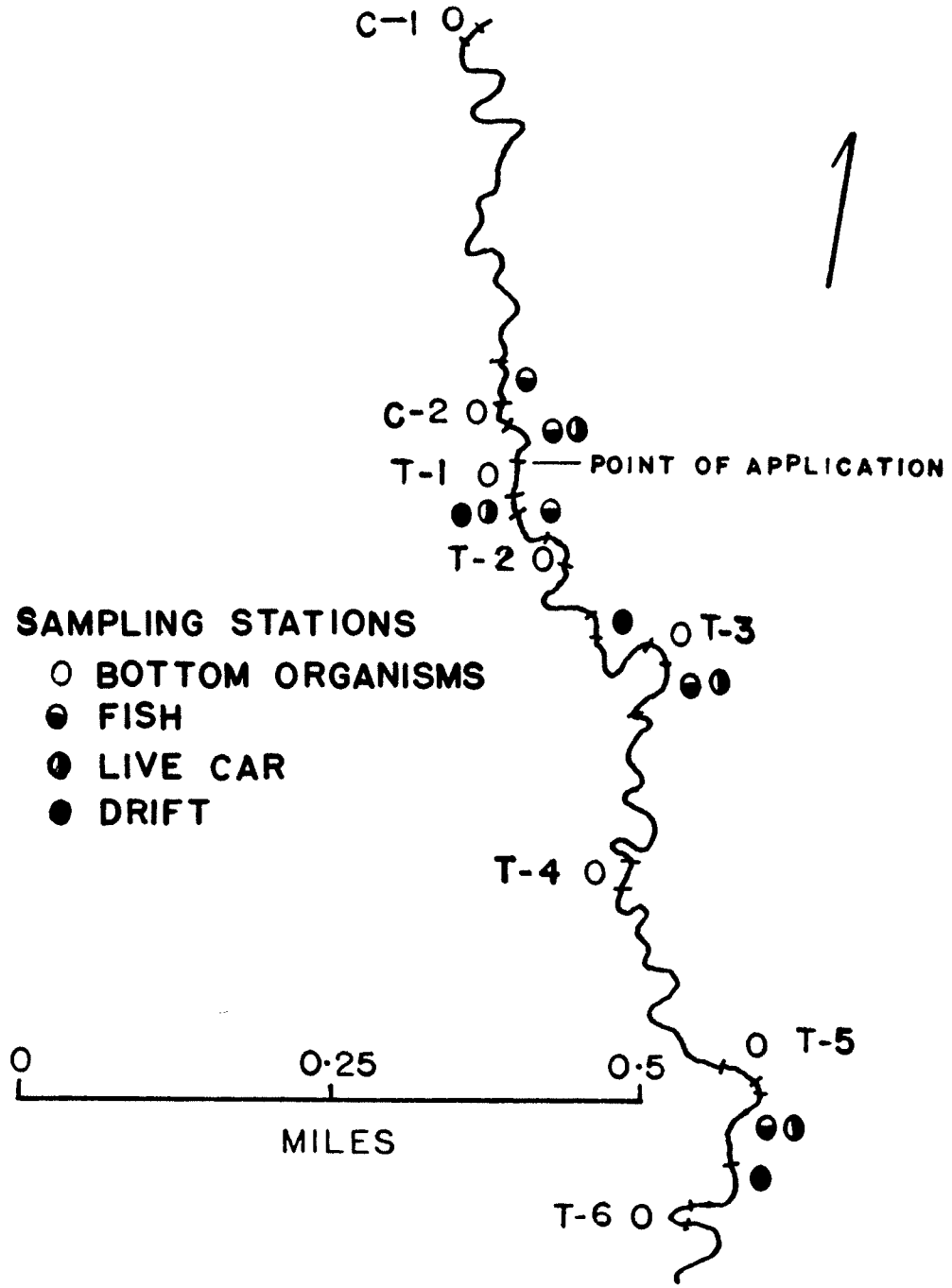

Figure 7. Study sections of Trail Creek, showing collection stations.

Table VII. Number of fish taken by shocking at various stations July 31, 1958, September 7, 1958 and September 6, 1959.

Date and species	Control stations		Treated stations			
	0.1-mile	0.05-mile	0.05-mile	0.5-mile	1.0-mile	2.0-mile
July 31, 1958						
Cutthroat trout	14	19	11	5	15	12
Rainbow trout	0	4	0	0	0	0
Brown trout	1	5	4	5	10	8
Total	15	28	15	10	25	20
September 7, 1958						
Cutthroat trout	27	25	12	6	19	9
Rainbow trout	3	7	0	1	0	0
Brown trout	2	8	6	8	33	13
Total	32	40	18	15	52	22
September 6, 1959						
Cutthroat trout	23	13	7	7	3	2
Rainbow trout	19	14	10	6	1	3
Brown trout	3	4	1	4	7	4
Total	45	31	18	17	11	9

Gallatin River drainage. These were placed in live cars one day before application (Table VIII). The number of trout per live car varied from

Table VIII. Trout held in live cars during DDT application and three days following.

Station	Rainbow trout Number	Rainbow trout Size range	Brown trout Number	Brown trout Size range	Number live cars	Percent mortality
Control	21	(4.3-11.5)	10	(6.2- 8.9)	2	0
Treated						
0.05 mile	24	(4.0-10.7)	14	(4.2-12.0)	2	17
0.5 mile	9	(5.2- 9.9)	6	(3.5-12.2)	1	0
1-mile	18	(4.0-12.5)	8	(3.2-13.6)	2	0
2-miles	10	(4.5-11.5)	6	(3.9-13.9)	1	0

13 to 19. All trout were held in live cars during application and for three days after and then their numbers were reduced to three rainbow trout and two brown trout. The latter were held for an additional 11 days. Surviving trout from both exposure times were measured, marked by the removal of the adipose fin and released at the stream station where exposed.

No mortality occurred in the control fish. The only mortality during the 3-day exposure occurred at the 0.5 mile station where two rainbow and one brown trout died during the third day. One rainbow trout died nine days after treatment at the 2-mile station during the 14-day exposure period.

Stomach Analyses. Trout stomachs were examined to determine changes in food habits following application of DDT. Samples were taken by angling in the control and treated sections from mid-August until November 1958 (11 collections) and again in March 1959 (3 collections). The contents of each stomach were identified and volume estimated to the nearest 10

percent. Twenty-six rainbow trout and 47 cutthroat trout were collected. Changes in stomach contents made it desirable to treat the data under three periods: August 1958; September-October 1958; March 1959.

The August 1958 sample contained 4 cutthroat and 1 rainbow stomachs from the control area and 7 cutthroat and 1 rainbow from the treated area. Adult insects made up 45 percent of the volume from the control compared to 78.5 percent for the treated area. Both contained ants and grasshoppers. Immature aquatic insects made up 46 percent of the control but only 9.5 percent of the treated. The volume of the control sample was dominated by Ephemeroptera followed in order by Trichoptera and Plecoptera. Trichoptera and Coleoptera were the only groups represented in the treated sample (Table IX).

The September-October sample included 7 cutthroat and 8 rainbow trout stomachs from the control area and 15 cutthroat and 8 rainbow trout stomachs from the treated area. The control stomachs contained 13.5 percent (volume) adult insects as compared to 36.5 percent for the treated. Ants and grasshoppers were common in both. The control contained 63 percent immature aquatic insects while less than one percent was found in the treated. Organisms in the control sample were similar to the August sample but only Trichoptera were found in the treated sample and these were almost wholly decomposed (probably dead before being eaten). Only 2.5 percent of the volume of control stomachs was aquatic vegetation while this made up 49 percent of the treated sample.

The March 1959 samples included 9 cutthroat and 4 rainbow trout from

Table IX. Stomach contents of trout collected in control and treated areas after application of DDT (August 5, 1958).

	August 1958		September-October		March 1959	
	Control	Treated	Control	Treated	Control	Treated
Number of trout in sample	5	8	15	23	13	9
Number of empty stomachs	0	1	1	5	1	2
Food organism	Average percentage of contents					
Adult and/or terrestrial insects	45.0	78.5	13.5	36.5	5.0	19.0
Immature aquatic insects	46.0	9.5	63.0	tr. 1/	81.0	8.5
Animal matter other than insects	3.5	5.0	15.0	7.0	9.0	tr.
Aquatic vegetation (algae)	tr.	tr.	2.5	49.0	3.0	64.0
Debris	5.5	7.0	6.0	7.5	2.0	8.5

1/ tr. means trace

the control area and 5 cutthroat and 4 rainbow trout from the treated area. The control stomachs contained 5.0 percent adult insects compared to 19.0 percent for the treated. Both groups contained adult water beetles. Aquatic insects made up 81.0 percent of the control but only 8.5 percent of the treated sample. Organisms in the control were similar to previous samples but only Diptera larvae were found in the treated lot. Vegetation constituted 3.0 percent of the control volume and 64.0 percent of the treated.

Six percent of all stomachs from control fish and 20.0 percent from those treated were empty.

Bottom Organisms. Eleven stations (50 - 100 feet in length) were established in riffle areas to determine population changes before and after treatment with DDT. Two control stations (C-1, C-2) were 0.5-mile and 300 feet respectively above the point of application. Nine treated stations were established. One was located at the point of application (T-1). The others (Figure 7) were below as follows (miles): T-2, 0.05; T-3, 0.25; T-4, 0.5; T-5, 0.75; T-6, 1; T-7, 2; T-8, 3; T-9, 6. Starting in January 1958 and continuing until April 1960, one square-foot of bottom was sampled bi-weekly (with a few exceptions) from the two control and upper eight treated stations. In addition, a 10 square-foot sample was collected from each station one day before application, six days after and one year following application. Numbers and volumes (water displacement) of each kind of organism were determined. Immature aquatic insects made up 99.9 percent of the volume. The remaining was comprised of Tur-

bellaria, Nematoda, Oligochaeta, Pelecypoda, Gastropoda and Arachnoidea.

Wide variations occurred among samples, even those taken at the same station less than one month apart. This may indicate the inadequacy of sampling. The January and February collections showed the smallest variation. Collections were averaged for each station to compare the composition by insect orders. Monthly collections were averaged to show seasonal changes in aquatic insect numbers at the following stations: C-1, C-2; T-1, T-2; T-3, T-4; T-5, T-6; T-7, T-8.

The numbers of aquatic insects in the 1958 mid-winter samples (Table X) contained 61.4 percent Ephemeroptera, 19.1 percent Trichoptera, 9.4 percent Plecoptera, 6.5 percent Coleoptera and 3.6 percent Diptera. During late April, May and June samples showed a reduction of more than 80 percent (Figure 8), probably resulting from flood conditions which lowered sampling efficiency. The August pre-treatment (10 square-feet) samples varied from 347 to 1511 aquatic insects representing five orders. Comparable samples collected at the treated stations six days after treatment ranged from 0 to 17 aquatic insects (Table XI) representing no more than two orders. At this time control samples ranged from 917 to 1232 aquatic insects representing five orders. The entire collection (90 square feet) in the treated area contained 19 Trichoptera, 18 Coleoptera, seven Diptera, three Plecoptera and two Ephemeroptera. This was less than one percent of the pre-treatment aquatic insect population.

During the recovery period in the treated area the following population changes were observed. Two weeks after treatment, 52 aquatic insects

Table X. Average number of aquatic insects per square foot collected bi-weekly during January and February 1958, 1959 and 1960.

	Ephemeroptera			Plecoptera			Coleoptera			Trichoptera			Diptera			Total		
Station	1958	1959	1960	1958	1959	1960	1958	1959	1960	1958	1959	1960	1958	1959	1960	1958	1959	1960
CONTROL																		
(C-1)	181	144	163	40	14	27	11	5	18	47	21	49	18	8	32	300	192	298
(C-2)	68	121	99	6	10	13	14	24	7	37	57	46	4	2	5	129	214	170
TREATED																		
(T-1)	207	47	85	40	19	16	14	1	6	73	5	32	8	11	8	342	83	147
(T-2)	72	104	80	7	0	23	5	0	9	32	0	42	2	48	9	118	152	164
(T-3)	35	8	162	9	0	42	10	0	2	9	0	29	2	5	2	65	13	237
(T-4)	22	37	53	3	0	11	2	0	1	15	0	10	0	2	1	42	39	76
(T-5)	48	9	91	6	0	20	4	0	2	5	0	11	0	4	0	63	13	124
(T-6)	107	2	96	21	0	17	1	0	1	7	0	14	8	4	7	144	6	135
(T-7)	96	1	133	16	0	55	13	0	3	25	0	33	4	10	3	154	11	227
(T-8)	206	0	150	6	0	14	30	0	6	74	0	50	22	16	24	338	16	244

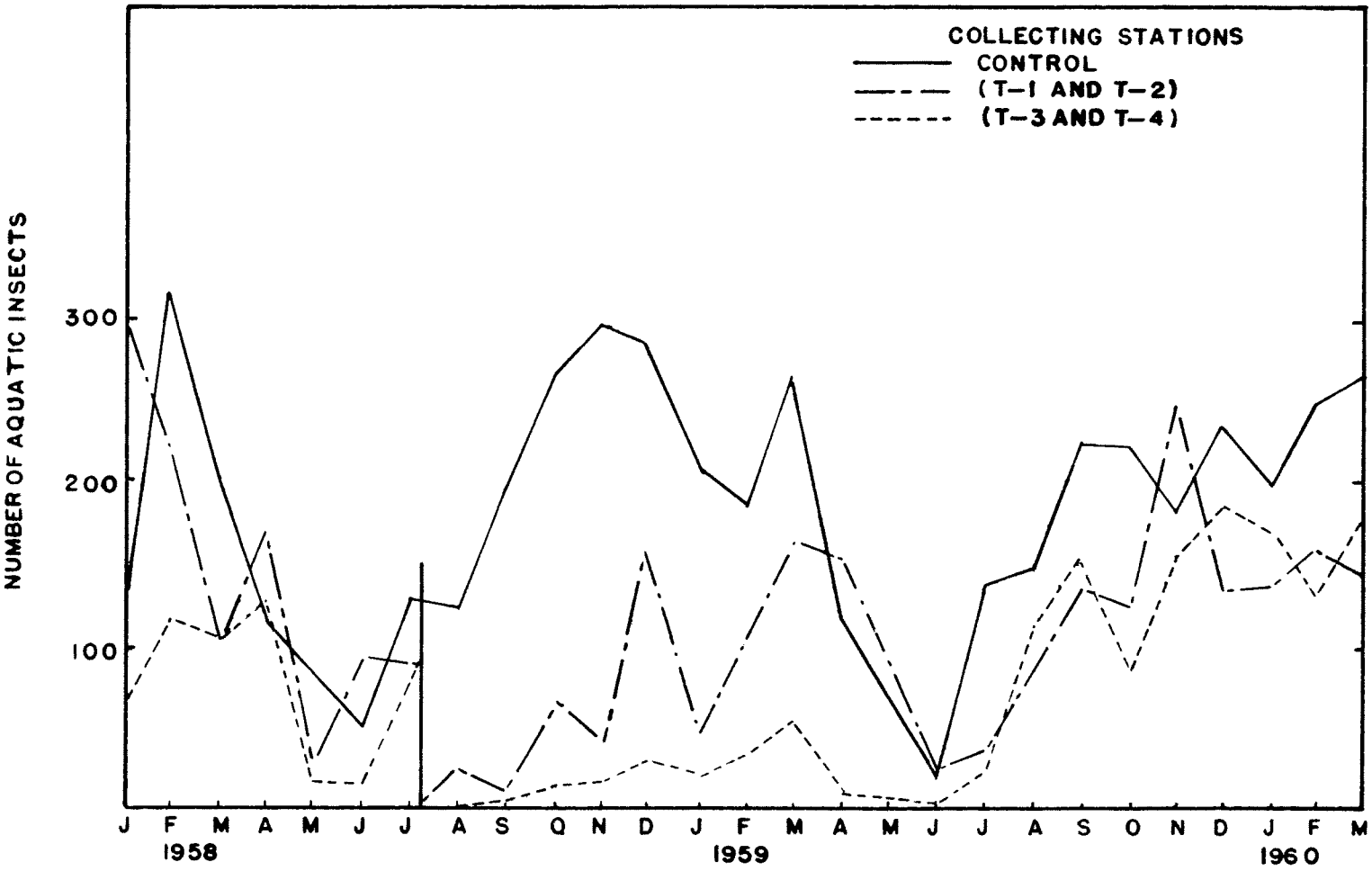

Figure 8. Average number of aquatic insects per square foot collected bi-weekly during 1958, 1959 and 1960.

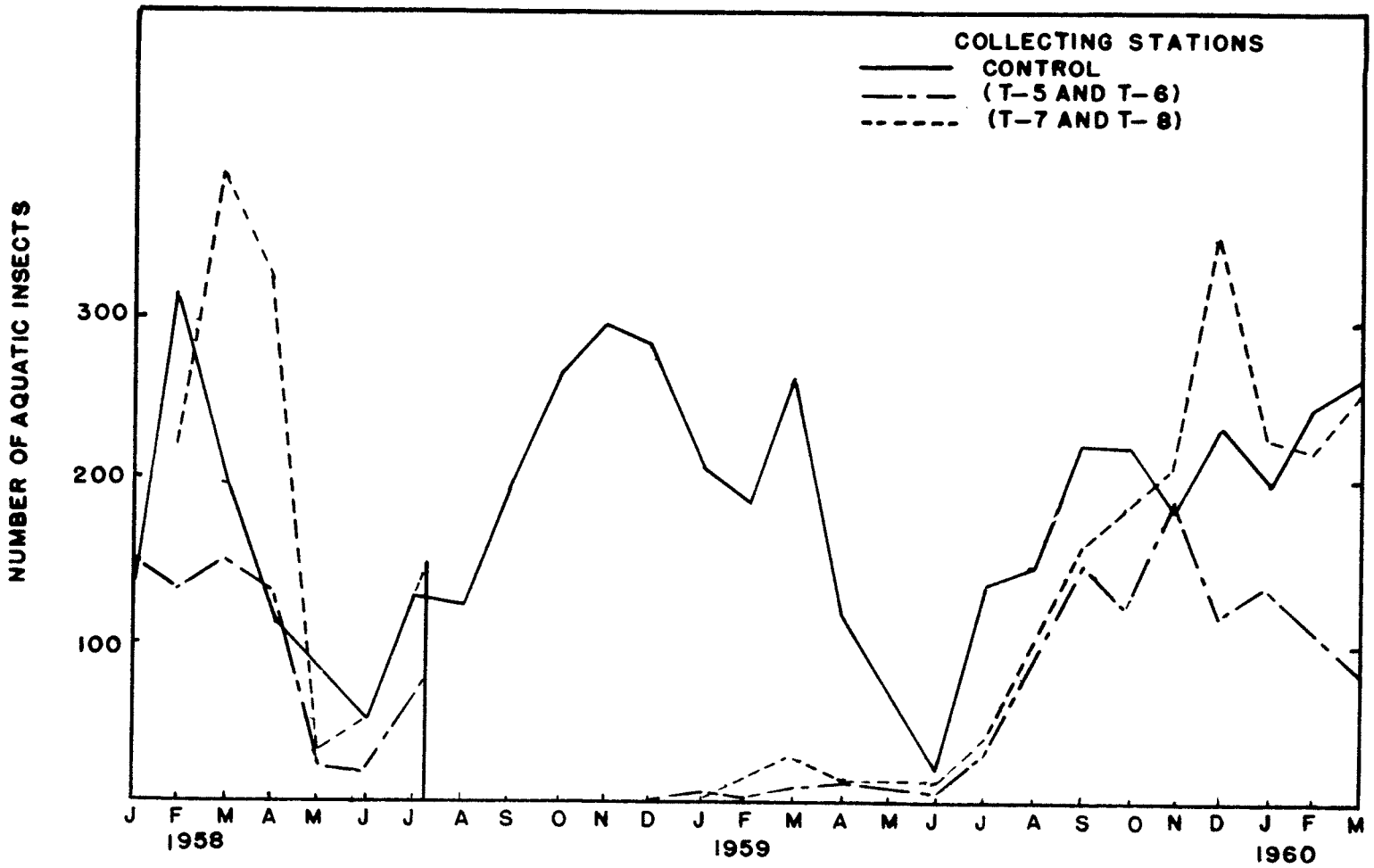

Figure 8, continued. Average number of aquatic insects per square foot collected bi-weekly during 1958, 1959 and 1960.

Table XI. The number of insects (by order) in a 10 square-foot bottom sample collected one day before, and at six days and at one year after application of DDT.

Station	Ephemeroptera			Plecoptera			Trichoptera			Coleoptera			Diptera			Total		
	Pre 1958	Post 1958	Post 1959	Pre 1958	Post 1958	Post 1959	Pre 1958	Post 1958	Post 1959	Pre 1958	Post 1958	Post 1959	Pre 1958	Post 1958	Post 1959	Pre 1958	Post 1958	Post 1959
CONTROL																		
0.5 mile	458	453	508	17	25	12	367	349	123	66	59	26	188	346	322	1096	1232	995
300 feet	142	286	321	9	23	18	130	215	125	44	52	46	204	341	292	499	917	802
TREATED																		
App-point	361	1	393	39	0	24	307	2	102	101	2	25	61	0	103	869	5	648
300 feet	247	0	258	22	0	16	39	1	85	23	1	36	15	1	78	346	3	475
0.25 mile	574	1	327	25	0	9	165	0	93	102	1	24	46	0	83	912	2	536
0.5 mile	323	0	274	16	0	0	180	1	16	75	0	9	42	0	13	636	1	284
0.75 mile	491	0	369	29	1	5	145	7	35	151	9	7	47	0	56	863	17	472
1 mile	327	0	266	37	0	10	42	2	44	122	0	9	28	1	103	556	3	432
2 miles	604	0	175	54	1	5	152	2	23	135	0	0	202	1	150	1147	4	354
3 miles	722	0	429	28	0	7	140	2	17	293	1	0	333	3	172	1516	6	624
6 miles	76	0	148	26	1	7	113	2	22	170	4	0	455	1	30	840	8	207

representing five orders, were collected at the T-1 station but all stations below were void of organisms. One month after treatment Ephemeroptera were collected at station T-2; and at three months at stations T-3 and T-4. After two months Diptera were collected at all treated stations. Four months after treatment, the samples at stations T-1 and T-2 contained 162 aquatic insects, 90 percent of which were Ephemeroptera and 5.0 percent Diptera. Collections 5 - 6 months after treatment (1959) contained Ephemeroptera at all treated stations and this order had returned to pre-treatment abundance at stations 1 - 4 but had only three percent of the pre-treatment numbers at stations below four. Collections contained 1.5 times more Diptera than the comparable 1958 collection. Trichoptera, Plecoptera and Coleoptera were absent at all stations below T-1. Seven months after treatment all treated stations showed a slight increase. The T-3, T-4 sample contained 59 aquatic insects composed entirely of Ephemeroptera and Diptera. Flood conditions encountered 8 - 10 months after treatment again lowered the sampling efficiency. Eleven months after treatment small recently hatched Plecoptera and Trichoptera were present at all treated stations. Twelve months after treatment Coleoptera were at all stations above T-7. The aquatic insects in the treated area were 60 percent of the comparable pre-treatment numbers and 25 percent of the pre-treatment volume. Ephemeroptera and Diptera were still the dominant forms. At 14 - 15 months following treatment, Plecoptera and Trichoptera made rapid gains at all treated stations below T-1. Coleoptera showed some increase but were still absent at stations T-7 and T-8. Sixteen months after treatment Coleoptera were present at stations T-7

and T-8. During mid-winter 1960 (17 - 18 months after treatment) the aquatic insect numbers consisted of 61.5 percent Ephemeroptera, 17.4 percent Trichoptera, 13.1 percent Plecoptera, 5.0 percent Diptera and 3.1 percent Coleoptera. This population was comparable to the 1958 pre-treatment numbers, kinds and volumes.

Repopulation of the treated area by aquatic insects probably took place in two ways. First, by gradual downstream movement where representatives of all insect orders present in the stream apparently moved downstream from the untreated area. Station T-1 was repopulated within one month after treatment. Ephemeroptera appeared progressively downstream and were present in all treated stations within six months after treatment. Second, by deposition of eggs into the treated area followed by a simultaneous appearance of recently hatched individuals at all treated stations. Diptera appeared at all stations two months after treatment and Plecoptera and Trichoptera at 11 months.

Drift Samples. Drift samples were taken to determine the immediate mortality of aquatic organisms following treatment. Collecting stations were established 0.05, 0.5, 1, and 2 miles respectively below the point of application (Figure 7). Each collection was made with a square-foot Surber sampler with its top edge held three inches below the water surface for five minutes. The number and volume of organisms were determined. One control collection was taken at each station three hours before treatment. The first treated collection was taken five minutes after the arrival of DDT at each station. The DDT arrival was easily detected by the appear-

ance of an oil slick, odor of the DDT carrier and hundreds of drifting insects. Three additional collections were taken at 30-minute intervals following this.

Each pre-treatment sample contained 0.1 ml of aquatic insects. Five minutes following treatment volumes ranged from 14.1 to 31 ml of dead and paralyzed insects (Table XII). The first group to be affected was Coleoptera followed in order by Diptera, Ephemeroptera, Plecoptera and Trichoptera. Greatest volumes of Diptera (7.9 ml) and Coleoptera (10.4 ml) were taken in the first treated collection. The greatest volume of Ephemeroptera was taken one hour after treatment while the greatest volumes of Plecoptera (30 ml) and Trichoptera (35.2 ml) were collected 1.5 hours after treatment. The greatest total drift occurred 1.5 hours after treatment. One sample at the 2.0 mile station had 125 ml of aquatic insects.

General Observations. The test area was patrolled during the day DDT was applied and daily for two weeks after. Bi-weekly patrols were then made until mid-December when ice cover prevented further observations.

Oil slicks caused by the fuel oil in the DDT solution were observed in the quiet waters for three days following application. One day after treatment mounds of dead insects (1 to 3 inches in depth) were observed in quiet water below riffle areas for a distance of nine miles below the point of application. No observations were made below this point because the entire stream was diverted for irrigation. The odor of these decomposing insects was pronounced for several days.

Table XII. Volume of aquatic insects taken in five-minute drift samples at treated stations.

Station	Time	Total volume	Percent (volume (ml) in parentheses)									
			Ephemeroptera		Plecoptera		Coleoptera		Trichoptera		Diptera	
0.5 mile	1100	14.1	28.8	(4.3)	19.5	(2.7)	19.2	(2.7)	21.9	(3.1)	11.0	(1.1)
	1130	65.8	69.1	(45.4)	10.6	(7.0)	2.5	(1.6)	15.3	(10.1)	2.3	(1.5)
	1200	102.0	59.6	(61.0)	22.5	(30.0)	1.2	(1.2)	15.3	(15.6)	1.1	(1.1)
	1230	112.4	44.5	(50.0)	23.1	(30.0)	tr.	tr.[1]	31.5	(35.2)	0.9	(1.0)
1.0 mile	1145	26.1	23.2	(6.1)	15.2	(4.0)	17.5	(4.6)	14.0	(3.6)	30.1	(7.9)
	1215	70.3	57.1	(40.1)	16.8	(11.8)	5.8	(4.0)	15.1	(10.6)	5.2	(3.7)
	1245	115.6	63.2	(73.0)	23.2	(26.8)	2.5	(2.7)	8.9	(10.3)	2.2	(2.4)
	1315	95.0	45.3	(43.0)	20.1	(19.1)	1.2	(1.1)	22.5	(21.4)	0.9	(0.8)
2.0 miles	1420	20.6	26.2	(5.4)	9.7	(2.0)	44.5	(9.2)	10.3	(2.1)	15.3	(3.2)
	1450	115.0	69.5	(79.9)	15.2	(17.5)	2.5	(3.0)	10.4	(11.9)	2.3	(2.6)
	1520	112.5	66.0	(74.3)	14.9	(16.8)	4.2	(4.7)	13.8	(15.5)	1.1	(1.2)
	1550	101.0	51.0	(51.0)	18.3	(18.3)	1.3	(1.3)	28.5	(28.5)	0.9	(0.9)
3.0 miles	1700	31.0	33.0	(10.2)	5.1	(1.6)	33.4	(10.4)	6.4	(2.0)	22.1	(6.8)
	1730	100.5	78.0	(78.0)	5.7	(5.7)	6.8	(6.8)	5.3	(5.3)	4.2	(4.2)
	1800	125.0	66.3	(82.9)	17.1	(21.4)	0.3	(0.4)	15.2	(15.2)	1.1	(1.1)
	1830	112.0	52.0	(58.2)	19.0	(21.2)	0.3	(0.4)	27.8	(31.1)	0.9	(0.9)

[1] Trace.

Five dead trout were collected in the treated area during the first two weeks. Small fish (fry) were closely observed but showed no apparent signs of DDT poisoning or decrease in numbers. In October, seven dead brown trout (11.0 - 12.1 inches in length) were found. During this period brown trout were observed spawning. No dead fish were found in the control area.

Trailing algae in the treated area developed rapidly and reached 3 to 4 inches in length during the month following application compared to approximately 0.5 to 1 inch in the control area.

SUMMARY

1. Bio-assays were conducted during the summers of 1957, 1958 and 1959 on 6000 cold water fish to determine mortality rates under different concentrations of DDT and varying physical, chemical and biological conditions.

2. The mortality rate of hatchery and wild rainbow trout varied little with DDT concentrations between 0.5 and 10 ppm.

3. Hatchery rainbow trout under 3.1 inches in length had a mortality rate of 100 percent at all concentrations. The mortality rate decreased as the size increased (66 percent for those over 5.0 inches). Wild rainbow trout under three inches had a mortality rate of 24 percent. Hatchery rainbow trout showed a 50 - 75 percent higher mortality than wild rainbow trout.

4. The mortality rate of wild rainbow trout treated in 1 ppm DDT was affected by temperature, turbidity and alkalinity and their interactions.

Mortality increased with an increase in temperature and decreased with an increase in turbidity and alkalinity.

5. The mortality rate of hatchery rainbow trout treated with 1 ppm DDT and those not treated in DDT was increased 13 - 22 percent by feeding aquatic insects treated with DDT.

6. Exposing hatchery rainbow trout to algae treated with DDT did not increase their mortality rate.

7. A comparison of six species of cold water fish over four inches in length tested in 1 ppm DDT showed that the longnose sucker had a mortality rate of 94 percent while rainbow trout, cutthroat trout, brown trout, brook trout and mountain whitefish had less than 10 percent.

8. Delayed mortality occurred in all species of fish treated in DDT throughout a six month observation period.

9. Factors that influenced the mortality rate of fish treated in DDT were as follows: source where collected; temperature change; disease; and method of handling before testing.

10. Observations were made on fish and fish-food organisms in a test stream eight months before, during, and 19 months after treatment with DDT. DDT was applied at one point in the stream at a rate of 0.4 ppm.

11. Trout populations in the first mile of treated water showed no significant change one year following treatment but below this point showed a 73 percent reduction.

12. Analyses of trout stomachs exhibited a change in diet after treatment from immature aquatic insects to adult insects and aquatic vegetation.

13. The immature aquatic insect population was reduced 99 percent in the DDT treated area within 6 days following application.

14. DDT was toxic to aquatic insects 9.0 miles below the point of application.

15. Immature aquatic insects regained pre-treatment numbers, kinds and volume 18 months after application.

16. Diptera and Ephemeroptera were the first to repopulate the treated area followed in order by Trichoptera, Plecoptera and Coleoptera.

17. Immature aquatic insect populations below 0.75 mile downstream from the point of application required 11 months to regain 15 percent of the pre-treatment numbers. It was in this same area that the food habits of the trout changed and a population reduction occurred.

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