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An Experiment In the Use of Derris Root (Rotenone) On the Fish and Fish-Food Organisms of Third Sister Lake [1]

[1] Contribution from the Michigan Institute for Fisheries Research.

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Introduction

The present experiment is part of a general investigation being conducted by the Institute for Fisheries Research, Michigan Department of Conservation, and the Zoology Department, University of Michigan, on the relationship between the fish and fish-food organisms of Third Sister Lake. This study is under the general direction of Dr. A. S. Hazzard, Director of the Institute, and Professor Paul S. Welch of the Zoology Department. Permission to use the lake was granted us by the University of Michigan School of Forestry and Conservation which is responsible for the administration of the Saginaw Forest tract upon which the lake is located.

The complete removal of fish from the lake was carried out for the purpose of observing what effect their absence would have upon the various fish-food constituents and to get more reliable figures on the kinds and abundance of fish present. It is planned to restore the fish population at the termination of this study.

There is little choice of tools when a complete removal of fish is desired and of those things tried, poisoning with rotenone is the most practical. The phase of the investigation reported here has only to do with the poisoning procedure and its effect on the fish and fish-food organisms. Other parts of this research will appear as they are completed.

Description of Third Sister Lake.--Third Sister Lake is roughly oval in outline with a surface area of approximately 10 acres and a maximum depth of 55 feet. It lies near the center of Washtenaw County in the Huron River drainage system. This lake has no permanent inlets nor outlets. Its water supply comes mainly from seepage and runoff originating in the surrounding moraines. The outlet which passes through a small marsh is dry most of the year, but may be an avenue of entrance during periods of high water for certain fishes in the Honey Creek and Huron River drainages less than 5 miles below.

About one-fourth of the shore immediately adjacent to the lake is firm and composed of sand and gravel; the remainder is very soft and peaty. The emergent and submerged vegetation zones cover approximately 35 per cent of the lake area and extend from the shore to about the 10-foot contour. The sides of the lake basin are steep, having a sharp "drop-off."

The bottom is composed of soft, finely divided organic material (pulpy peat) between the two and twenty-five foot contours. At depths greater than this the bottom consists of clay overlain by a thin coating of muck.

Third Sister Lake stratifies thermally and chemically during the summer. The average annual bottom temperature is approximately 4°C. and a thermocline develops with subsequent marked depletion of O_2 in the hypolimnion. The water is moderately soft having an average alkalinity of approximately 90 p.p.m. of $CaCO_3$ and a pH range of 6.8-8.4.

General procedure and methods of applying poison.--The first application of poison was made during the forenoon of May 6, 1941. While the water temperature on this date was far below the optimum desired for the best results, this time was chosen in order to remove the fish before they had spawned and before the aquatic plants were high or dense enough to prevent the recovery of dead fish.

Because of the unfavorable temperature conditions which existed in Third Sister Lake at the time of the first poisoning, there was reason to believe that the treatment had failed to kill all of the fish. Since complete removal was necessary in order to carry out the different phases of this investigation, careful checks were made between May 10 and August 1 by netting and through observations to determine if there were live fish still present in the lake. Gill nets were set on May 14 and during the ensuing three days captured five large bluegills including 3 females and 2 males, and one male pumpkinseed. On July 16 a large school of small bluegills or sunfish was observed and during the month that followed, these small centrarchids appeared in ever increasing numbers. We decided then to make another attempt at poisoning and this was accomplished on August 18 and 19.

The derris powder used was supplied by S. B. Pennick and Company, New York, and had a guaranteed rotenone content of 5 per cent. In the first application, 312 pounds of derris root was used, which amounts to approximately one part of powder to two million parts of water by weight. Four hundred pounds were used for the second application, making the concentration somewhat greater than the first.

The procedure of mixing and applying the poison was as follows: 10-15 pounds of derris powder were placed in a 50-gallon steel drum with one end removed; lake water was then added to fill the barrel about 3/4 full. An outboard motor was set in the barrel and mixing was attained by running the motor. This only takes a minute or so and the resulting mixture appears in color and consistency about like the usual chocolate "malted milk." This suspension was then placed in 10-gallon milk cans and poured from the side of a boat propelled by an outboard motor. In this manner it was well dispersed in the surface water by the churning action of the motor. The paths the boat followed were regular and overlapped until the entire lake was covered. Then the same procedure was followed crossing the first paths at right angles. The whole lake was covered four times in this manner. All of the shallow water near the edges was treated by means of a fire pump or by throwing the solution with a dipper.

In the second application the procedure was the same except that 85 pounds of derris were mixed into a batter and then placed in weighted burlap bags and dragged behind a boat in water at a depth varying between 15 and 25 feet.

Collections.--Samples of the fish, plankton and invertebrate animals were taken before, during and after the poisoning. Fish samples taken before poisoning were secured with gill nets, trap nets and by angling--and, of course, after poisoning all of the fish which came to the surface or which could be reached with dip nets on the lake bottom were recovered for a population study. This is described in another paper (Brown and Ball, 1942). Plankton was taken by means of a 10-liter plankton trap and bottom samples by the use of Ekman and Peterson dredges.

In addition to observations made on the fish and other organisms as they appeared under natural conditions, certain fish and invertebrate organisms were captures in the lake previous to poisoning and placed in cages at various levels in order to see what effects rotenone might have on them. Laboratory experiments using the treated lake water were also carried out to further test the effect of treated lake water on fish, tadpoles and several of the invertebrates.

The first poisoning

Conditions in lake.--Temperature and chemical analyses were made on Third Sister Lake 7 days before and 4 days after poisoning. Temperatures were also taken on the day before poisoning. These data are summarised in Table 1. The rather favorable conditions during the week previous to the poisoning made us believe that temperatures and other factors in general would be favorable for the experiment. However, the weather was cold and rainy for 4 or 5 days preceding the poisoning date and no more favorable on the day of poisoning, causing a drop in the surface temperature of 20°F. The entire surface layer of water down to 15 feet was affected by this change and early in the morning of the poisoning the lake had a maximum temperature of only 48°F. which occurred between the 5- and 10-foot contours. Four days later, after bright, warm weather the water temperatures had returned to levels about equal to those existing the week previous to poisoning. It would seem that the time we chose for our experiment was the most unfavorable from the point of view of temperature that existed during the spring period.

This experience was not without value, however, since it demonstrated that rotenone is not as effective when used in water with low temperatures.

Table 1
Temperature and chemical conditions in Third Sister Lake
before, during and after first poisoning

Depth, feet	Before April 29, 1941, 2:30 P.M. Air temperature 73°F.					During May 5, 1941, 6 A.M. Air temperature 46°F.	After May 8, 1941, 2:30 P.M. Air temperature 66°F.				
	Temperature, °F.	O_2, p.p.m.	CO_2, p.p.m.	M.O., p.p.m.	pH	Temperature, °F.	Temperature, °F.	O_2, p.p.m.	CO_2, p.p.m.	M.O. p.p.m.	pH
0	65	...	...	...	...	45	64	13.4	0	82	8.0
3	62	...	...	...	...	47	64	...	...	...	...
6	58	...	...	...	...	48	63	...	...	...	...
9	55	13.2	0	82	8.0	48	60	9.8	1	86	8.0
12	53	...	...	...	...	46	53	...	...	...	...
15	47	...	...	...	...	45	49	...	...	...	...
18	44	...	...	...	...	45	45	...	...	...	...
21	...	9.8	0	86	8.0	45	44	5.6	3	88	7.6
24	44	...	...	...	...	...	43	...	...	...	...
27	...	...	...	...	...	...	43	...	...	...	...
30	...	5.6	1	88	7.6	45	42	4.8	5	88	7.5
33	...	...	...	...	...	...	42	...	...	...	...
36	42	...	...	...	...	...	42	...	...	...	...
39	...	3.9	3	88	7.5	44	42	3.8	5	88	7.5
42	...	...	...	...	...	...	42	...	...	...	...
45	...	...	...	...	...	44	42	...	...	...	...
48	...	...	...	...	...	...	42	...	...	...	...
51	...	2.1	5	88	7.2	...	42	2.1	7	88	7.2
54	...	...	...	...	...	...	42	...	...	...	...
57	...	...	...	...	...	43	...	...	...	...	...
60	42	...	...	...	...	...	...	...	...	...	...

On the poisoning date the aquatic vegetation was still in the very early stages of spring growth. Some of the yellow water lily leaves had reached within a few inches of the surface but the pondweeds in general had not grown more than 4-6 inches above the bottom. The fish population in Third Sister Lake included the following species: Largemouth bass (Huro salmoides), bluegills (Lepomis macrochirus), pumpkinseeds (Lepomis gibbosus), green sunfish (Lepomis cyanellus), mud pickerel (Esox vermiculatus), bullheads (Ameiurus natalis), chub-sucker (Erimyzon sucetta), common suckers (Catostomus commersonnii), black-chinned shiner (Notropis heterodon), black-nosed shiner (Notropis heterolepis), common shiner (Notropis cornutus), golden shiner (Notemigonus crysoleucas), Iowa darter (Poecilichthys exilis), and mudminnow (Umbra limi).

Observations on fish.--The first poison was put in the lake at about 6:00 A.M. and the first dead and dying fish were observed 20 minutes later. There seemed to be little or no difference in the toleration of the different species of fish to the rotenone poison. However, the minnows and the young of the other fish appeared in larger numbers at the beginning. This may have been due to their greater susceptibility or to their location in the lake. Most of the smaller fish are in shallow water and would naturally come in contact with the poison sooner than those fish found at deeper levels.

Some of the fish floated to the surface immediately upon dying while others would sink or stay on the bottom.

Both small and large specimens reacted in this manner. The darters, of course, do not float as a rule because of the lack of a swim bladder; however, three darters were found floating the day following the poisoning. A great many of the fish never came to the surface even after decomposition set in. A number of specimens were observed each day for a week and were seen to decompose without leaving their original positions on the bottom. These were mostly small fish; the larger specimens seemed to float more readily and we believe that the fish recovered represent the majority of the larger fish. We are certain, on the other hand, that many small fish remained on the bottom and were never recovered. Thompson and Bennett (1939 a) have reported that a good many of the fish sink after being overcome by rotenone but they indicate that these bloat and rise to the surface a short time after.

Experiments with fish.--A careful check was made to determine the length of time the poison was present in quantities sufficient to kill fish at various depths. This was accomplished by placing live fish in minnow buckets or cages of approximately 500 cubic inches in volume and lowering them to different levels in the lake. Eight different locations well scattered over the lake were chosen in order to test for horizontal as well as vertical distribution of the poison. Cages containing bullheads, largemouth bass and bluegills captured in the lake before poison was administered were placed at the following depths (feet): 4, 6, 7, 9, 11, 16, 35, 40. The initial check was made approximately 8 hours after the first poison was introduced. All of the cages except the one at 40 feet were examined. This was kept as a control against the others which had to be brought up through the presumably more toxic surface water each time they were examined. We were not sure but that fish might be killed by their short exposure to surface water rather than to the poison at the levels which were being tested. We demonstrated to our own satisfaction in subsequent experiments that this short exposure had no serious effect on the fish. All of the fish were dead in the cages at 4 and 6 feet. A second examination was made at the end of 12 hours and the fish in the cage at 7 feet were found to be dead. At the end of 24 hours a third examination showed all of the fish suspended at levels between 9 and 40 feet to be alive and in good condition. At the end of 30 hours, however, the fish at 9 feet were all dead. The last examination was made on the fifth day following the application of derris root and all of the fish in the four deepest cages were still alive and apparently in good health. This would indicate that either the poison did not reach depths beyond 10 feet in concentrations sufficient to kill fish or that these fish were not affected by the poison because of the low temperatures prevailing there.

As a further check on the duration and extent of the poison in the lake, fish (pumpkinseeds, bluegills, largemouth bass and blunt-nosed minnows) seined from the Huron River were placed in cages and lowered to different levels. The method followed was to place 4-8 fish with representatives of each species in each trap. These traps were lowered quickly to a depth several feet below the desired level before being brought up to the level to be tested. This procedure insured the removal of all surface water which might otherwise have remained in the cages. Tests of this nature were made at regular intervals until all of the fish at all levels remained alive. When fish died, the approximate time before their death was recorded and they were replaced by living specimens. Examinations of the cages were made at either 10- or 24-hour intervals, or both. It did not seem advisable to check the fish oftener than this because it was necessary to haul them to the surface each time in order to see the results. Because of this, we do not have any information on the exact time the fish died but only know that death occurred sometime during the interval between examinations. It is fairly safe to assume, however, that the time required to kill fish used in these experiments was probably less than five hours. In other words, if they died at all from the effects of the poison, they did so within this time.

This experiment was started on May 7, 1941, and cages were placed at the following levels: 0-1, 3, 6, 9, 12, 15, 20, 25 and 30 feet. At the time of the first examination, 12 hours later, all of the fish were dead above 10 feet and all were alive below this level. As a matter of fact, the fish in the traps below 10 feet were still alive on May 21 when the experiment was terminated 13 days later.

On May 8, live fish were again placed in the four upper cages and these were dead when examined 10 hours later. The fish were again replaced the following morning (May 9) and all were found dead 24 hours later (May 10). Again they were replaced in the four upper cages and at the end of 10 hours part of the fish were dead in each cage except at the 9-foot level where all of the fish were alive. At the end of 30 hours (May 13) all of the fish in the three upper cages were dead. They were replaced for the final time on this date and all remained alive until the experiment was terminated 8 days later (May 21, 1941).

Additional experiments were carried out to test the dispersal and duration of the rotenone in lake water after removal from the lake. Samples of water were taken between the surface and the 30-foot levels with a Juday bottle and put into half-gallon glass jars. Fish were then placed in these jars and the time of their survival recorded under different temperature conditions. Four species of fish (largemouth bass, bluegills, pumpkinseeds and blunt-nosed minnows) averaging about 3 inches in total length were used in these experiments.

The temperature of the water in the jars was controlled within two or three degrees Fahrenheit except during the first experiment which was conducted at the lake shore.

The first series of water samples was taken 24 hours after the lake had been treated with derris root. One fish of each species was placed in a jar containing lake water soon after the experimental water was removed from the lake. The water was allowed to warm up to the air temperature (61°F.). The temperature and time were recorded at the beginning of the experiment and at the death of each individual. Two identical tests were made on the water from each depth and a similar test was made using surface well water as a control. The results are given in Table 2.

Table 2.--Time required to kill fish kept in treated lake water from various levels at increased temperatures

Depth of water sample, (feet)	Number of test	Temperature, °F., at start	Temperature, °F., at death	Time before death Hours - Minutes
Surface	1	45	61	0 - 46
	2	45	60	0 - 50
6	1	48	58	6 - 15
	2	48	62	2 - 10
	3	48	64	1 - 3
10	1	47	61	[1] No kill
	2	47	61	2 - 15
20	1	45	61	[1] No kill
	2	45	61	[1] No kill

[1] Fish lived for three weeks at which time the experiment was terminated. Water temperatures ranged between 58° and 64°F. during this time.

Water from the 20-foot level did not contain sufficient poison to kill fish even when the temperature was raised to 61°F. In one test on water from the 10-foot level, fish were killed in 2 1/4 hours and in another similar test none of the fish died even though they were held for three weeks. None of the water samples appeared to be toxic to fish until the water temperature had been raised at least to 57°F.

In these experiments, the fish died in the following order: Largemouth bass, bluegill, pumpkinseed, blunt-nosed minnow. We did not keep detailed records of the actual difference in time required to kill fish of each species but we do know the differences were small in most cases and not altogether significant.

In a second experiment, samples of lake water were taken at intervals of three feet from the surface to 30 feet, 48 hours after poisoning. These were then transported to the laboratory for the purpose of testing the vertical distribution of poison in the lake.

All samples were raised to a temperature of 69°-70°F. before fish were introduced, and kept at this temperature for the duration of the experiment. The elapsed time before death was recorded. Controls using tap water were set up in a similar manner. The size and species of fish used were the same as those described for the above experiment.

Three samples from each depth were tested simultaneously and an average of the data from each series is recorded in Table 3.

Table 3.--Time required to kill fish in treated lake water from various levels in Third Sister Lake

Depth, (feet)	Temperature, °F.	O_2 p.p.m. at time of death (average)	Time before death, minutes (average)
S	69	5.7	38
3	69	6.6	37
6	69	6.2	40
9	69	6.1	44
12	70	4.7	[1] ...
15	70	6.6	[1] ...
20	70	7.2	[1] ...
30	70	6.1	[1] ...
Control	70	5.5	[1] ...

[1] Where no time is given, fish were alive at end of experiment.

The species of fish used in this experiment showed the same sequence of death as in the first experiment. None of the fish died in water samples taken below nine feet. This is in agreement with observations made in the lake. As is shown by the above table, the oxygen in all of these experiments remained well above the critical point. The average time between the beginning of the experiment and the first symptoms of distress was 10 minutes, and the average time between distress and death was 30 minutes.

In order to test what effect aeration would have on the toxicity of lake water, the same samples used in the above experiment from the surface and at 9 feet were aerated vigorously for 30 minutes after the dead fish from the above experiment had been removed. Fish of the same species introduced into this aerated water died in 42 minutes. The water temperature was 70°F. and the oxygen 6.4 p.p.m. at the time of death.

A check was made to revive fish which had shown the first signs of distress in surface lake water. These fish were removed to tap water which was aerated throughout the experiment. None of the fish recovered although the period before death was 10 minutes longer than for those fish left in the surface lake water. Smith (1940) reports having revived trout which had lost their equilibrium due to rotenone poison. Most observers, however, have found that fish showing distress from poison will not revive on being transferred to untreated water.

Lake water samples were taken at intervals following the poisoning and experiments were conducted to test their toxicity to fish. On the sixth day following the poisoning, fish died in water from the six-foot level which had been raised to 78°F. but were not affected in this same water kept at 65°F. On the tenth day none of the water samples used showed any toxicity even when raised to 80°F. There is considerable variation in the time required for different waters treated with rotenone (derris root) to lose their toxicity. Leonard (1939) reports that with a concentration of 1 p.p.m., the water in his experimental aquaria was no longer toxic to bluegills and sunfish after 20-41 hours. Hamilton (1942) also reports the quick disintegration of the poison, and shows that with concentrations even as high as 10 p.p.m. his test solutions were no longer toxic to fish after 24 hours. Thompson and Bennett (1939) stocked Fork Lake in Illinois without loss of fish four days after poisoning. Vestal (1942) states that Gull Lake in California remained toxic for 12-26 days, and Smith (1940, 1941) reports that in Potter's Lake, New Brunswick, the water was still toxic 18 days after treatment and in the Nova Scotian Lakes it remained toxic for 30 days.

A brownish substance was found in plankton and bottom samples from the deep area of the lake soon after poisoning. A microscopical examination of this material left little doubt but that it was powdered derris. Some of this material was placed in a jar of lake water and this mixture was then warmed to 75°F. Fish were placed in this jar and suffered no ill effects.

Observations on fish food organisms.--Regular, bimonthly collections of plankton were taken from top to bottom in Third Sister Lake at 10-foot intervals during the entire period of this investigation. Partial counts have been made on these collections and the number of organisms per liter estimated in accordance with the usual methods. Identification of organisms was carried only to the larger groups except for about 10 predominant forms which were identified to genus.

Comparisons have been made between collections taken immediately before and after poisoning and, as well, between similar periods of the previous year. Only large and important changes in the plankton population would be recognizable by our methods but small differences would not have significance anyway because of the irregular, almost sudden, natural changes so characteristic of plankton populations.

Most of the phytoplankton groups showed little or no change following the introduction of rotenone, which could be attributed to the poison. A gradual decrease throughout the summer was noticeable for the Chroococcales with an accompanying increase in the diatoms (Bacillarieae) and Dinobryon. The dinoflagellate Peridinum completely disappeared 3 weeks after the first poison and did not reappear in collections until almost a year later.

In the zooplankton, noticeable reductions did occur, some of which we believe have significance. This was most obvious in the entromostracan genera of Daphnia and Diaptomous. Daphnia completely disappeared 2 days after poisoning and did not reappear in collections until 5 weeks after the second poisoning (Figure 1). Diaptomous disappeared along with Daphnia but reappeared a little sooner, i.e., one month after the second poisoning.

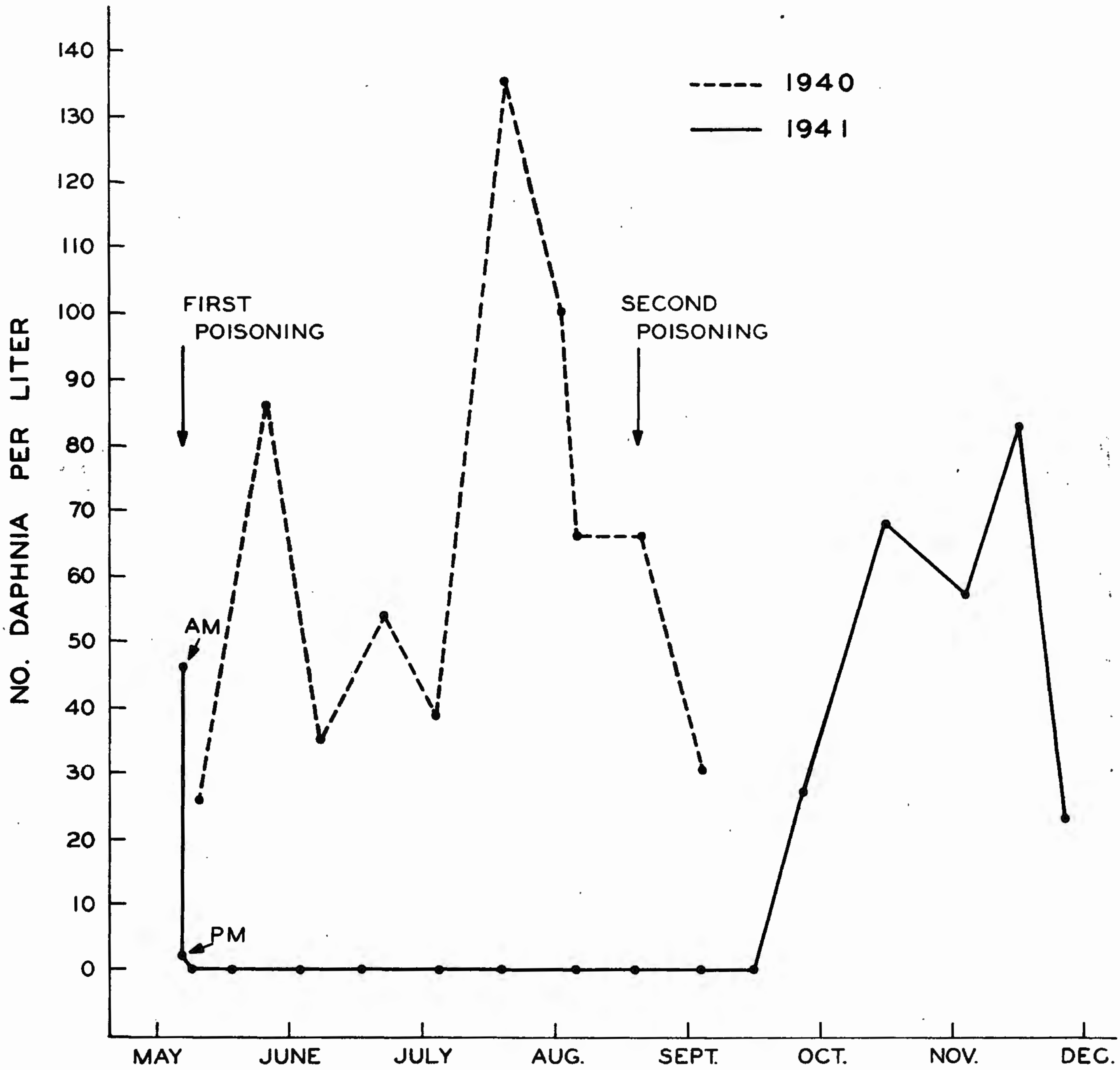

Figure 1.--Showing estimated number of Daphnia per liter before (1940) and after (1941) poisoning

Both of these organisms maintained more or less stable populations throughout this same period during the previous year. Cyclops showed a sharp decline immediately following each poisoning date but partially recovered in the interim. Collections made 6 weeks after the first poisoning and 4 weeks after the second showed about the same number of Cyclops recorded for the similar period of the preceding year. Epichura, which ordinarily appears very sporadically in this lake, did not show in any collections for the entire year after the first poisoning date. This may or may not be significant.

While rotifers were present in all collections following the poisoning the group as a whole appeared to be considerably reduced as compared with the same period of the previous year.

Observations were made on the aquatic invertebrate population before, during and after poisoning the lake. Regular sampling of an intensive nature was carried out for two years preceding poisoning, throughout the summer when the two treatments with rotenone were made, and for 6 months following the last poisoning. Samples were not numerous enough to detect small differences in populations but we believe they were adequate to show gross changes of aquatic invertebrates.

In general, our samples show no very significant changes before, during and after poisoning which could not be explained by the inadequacy of our method, emergence, migration or general seasonal variation. On the other hand, we do know that certain organisms were at least partially affected as shown by experiments and field observations at the time of each poisoning period.

Corethra were observed to be dying and coming to the surface within a short time after the first poison was introduced. The windward vegetation area of the lake was literally covered with dead *Corethra* larvae the day following the first poisoning. Field observations substantiated the experimental work described below in that large numbers of leeches, aeschnine dragonflies and tadpoles succumbed to the poison and were found dead in the shallow waters near shore. Meehean (1942) likewise observed that *Corethra* and leeches were readily killed by rotenone.

Experiments with fish food organisms.--An experiment to ascertain the effect of the poison on the fish food organisms was carried out at the lake during the poisoning. The organisms used in this experiment were collected in the lake prior to poisoning and placed in special screen wire cages. These cages were then distributed between the surface and the 6-foot level at several points on the lake. All cages were examined at intervals during the five days subsequent to poisoning. On the fifth day the experiment was terminated. The results appear in Table 4.

Table 4
The mortality of certain invertebrate organisms and tadpoles confined for five days to cages kept at various places in Third Sister Lake following the treatment of the lake waters with derris root

Organism	Depth of trap		
	Surface	3 feet	6 feet
Leeches	Dead	Dead	Dead
Snails (*Planorbis*, *Gyraulus*, *Physa*, *Amnicola*)	Alive	Alive	Alive
Amphipods (*Gammarus*, *Hyalella*)	Alive	Alive	Alive
Anisoptera nymphs			
Libelluline nymphs	Alive	Alive	Alive
Aeschnine nymphs	Dead	Dead	Dead
Gomphine nymphs	Alive	Alive	Alive
Zygoptera nymphs	Alive	Alive	Alive
Trichoptera larvae	Alive	Alive	Alive
Diptera larvae			
Chironomus	Dead	Dead	Dead
Corethra	Dead	Dead	Dead
Tadpoles	Dead	Dead	Dead

Practically all of the organisms that died did so within the first 24 hours after Derris Root was introduced into the water. The only organisms which were seriously affected included the aeschnine dragonflies, leeches, Corethra and tadpoles. The Chironomidae which lived about 48 hours probably died from unfavorable conditions resulting from the change of habitat rather than from the poison. The leeches and dragonflies were killed within 12 hours. Tadpoles (Rana catesbeiana) were also killed, although the time required was greater.

The second poisoning

Temperature and chemical analyses were made six days before, during, and 22 days after the second poisoning. These data are summarized in Table 5.

Table 5
Temperature and chemical conditions in Third Sister Lake before, during and after the second poisoning

Depth, feet	Before August 12, 1941					During August 18, 1941					After September 10, 1941				
	Temperature, °F.	O_2, p.p.m.	CO_2, p.p.m.	M.O., p.p.m.	pH	Temperature, °F.	O_2, p.p.m.	CO_2, p.p.m.	M.O., p.p.m.	pH	Temperature, °F.	O_2, p.p.m.	CO_2, p.p.m.	M.O., p.p.m.	pH
0	82	9.0	0.0	93	8.4	84	9.2	0.0	88	8.5	74	8.0	0.0	88	8.4
3	80	...	...	...	...	80	...	...	...	...	71	...	...	...	...
6	...	8.1	0.0	94	8.4	80	...	...	...	...	68	...	...	...	...
9	...	8.4	0.0	94	8.5	77	11.1	0.0	88	8.4	67	9.0	0.0	88	8.4
12	75	...	...	...	...	75	...	...	...	...	63	...	...	...	...
15	65	19.8	0.0	84	9.1	65	16.9	0.0	88	8.0	60	...	...	...	...
18	53	...	...	...	...	53	...	...	...	...	52	...	...	...	...
21	47	9.5	8.0	84	7.4	47	6.4	10.0	89	7.4	49	6.3	5.0	88	8.0
24	45	3.7	18.0	84	7.0	45	2.0	15.0	90	7.0	47	...	...	...	...
27	44	...	...	...	...	...	...	...	...	...	45	...	...	...	...
30	43	0.0	21.0	84	6.9	43	0.0	20.0	90	6.8	43	0.0	10.0	84	7.0
33	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
36	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
39	...	...	...	...	...	...	...	...	...	...	...	0.0	15.0	88	6.8
42	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
45	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
48	...	...	...	...	...	42	...	...	...	...	...	...	...	...	...
51	42	...	...	...	...	...	...	...	...	...	...	0.0	20.0	88	6.7
54	...	...	...	...	...	...	...	...	...	...	42	...	...	...	...

Surface water temperatures on August 18 and 19 were near the maximum for the summer and therefore were very favorable to poisoning. There was a zone of water, however, between 18 and 25 feet which still retained sufficient oxygen to support fish and which was rather cold (45°-53°F.) for the best effects of the poison. An attempt to increase the concentration of rotenone in this zone was made by dragging burlap bags containing derris through it.

The aquatic vegetation on this date was near its peak of abundance. This was particularly true for the pondweeds (*Potamogeton natans*, *P. amplifolius*) and the yellow water lilies (*Nuphar advena*).

Some observations made on the invertebrate organisms gave indication that with the exception of leeches, the general abundance of the various groups in the lake was about the same as in other summers. The leeches were undoubtedly drastically reduced by the first poison. The absence of *Gammarus* at this time of year in Third Sister Lake is not at all unusual. We do not know the reason. Aeschnine dragonflies were very abundant in spite of the apparent high mortality at the time of the first poisoning.

Tadpoles were extremely numerous--no doubt the result of spawning after the first poisoning and also because of the removal of the predatory fish.

The only fish observed to be present were bluegills and pumpkinseeds.

As a result of this poisoning, many thousand young bluegills and sunfish were killed. Part of these floated to the surface and part remained on the bottom, there to decompose and become engulfed by filamentous algae. The adult fish which were recovered included two largemouth bass, 12 large bluegills, one common shiner and one mudminnow.

Gill net sets and observations between the time of this poisoning and June of 1942 failed to show any fish left in the lake. However, in June of this year long-eared sunfish appeared. Since this species was not present at the time of the first poisoning, it is safe to assume that they came up the outlet during the extremely high water stages in June.

Experiments with fish.--The same methods employed in the first poisoning to determine the dispersal and effectiveness of the poison in the lake were used at the time of the second poisoning. The fish used in the experiments were collected in Huron River and included 3-4 inch bluegills, pumpkinseeds and largemouth bass. Cages containing representatives of each of these species were placed at the surface, 3, 6, 10, 15, 20, and 22 feet before the lake was poisoned. The cages were examined 24 hours after the poisoning. At this time the fish in the traps at and above the 15-foot level were all dead and those below the 15-foot level were all alive.

As previously described, powdered derris was distributed at depths between 18 and 25 feet. The cages were lifted again in 24 hours and all fish were dead in those below 15 feet.

A second series of experiments was conducted to test the relation between temperature and toxicity of the water at various depths in the lake. Samples of water were collected from the lake surface and at depth intervals of five feet down to and including 30 feet 48 hours after poison was introduced. These were taken to the laboratory and used in the experiments. Fish were introduced into several jars filled with water from the same sample. These jars were then maintained at different temperatures and records were kept of the length of time before the first signs of distress and at the death of the fish. The results of these experiments are given in Table 6.

Table 6.—The relation between temperature and toxicity of treated lake water
(LMB = Largemouth bass; PS = Pumpkinseed)

Sample depth	Sample	Species	Temperature, °F.	Elapsed time to distress of first fish, (minutes)	Elapsed time to death of last fish, (minutes)	Alive at termination of experiment
Surface	A	2-LMB 1-PS	45	...	...	5 hours
	B	2-LMB 1-PS	50	40	70	...
	C	2-LMB 1-PS	50	50	90	...
	D	1-LMB 1-PS	50	60	125	...
	E	3-LMB	50	40	60	...
	F	2-PS	50	120	210	...
	G	2-LMB	74	10	15	...
	H	2-PS	74	10	20	...
	I	2-Tadpoles	74	...	...	2 days
5 feet	A	1-PS 1-LMB	50	95	140	...
	B	2-PS	57	125	180	...
	C	2-LMB	57	20	35	...
	D	2-LMB	74	15	20	...
	E	2-LMB	74	15	25	...
	F	2-Tadpoles	74	...	...	2 days
	G	2-LMB 1-PS	45	...	...	5 hours
10 feet	A	2-PS	56	...	...	6 hours
	B	2-LMB	56	...	185	...
	C	1-PS	74	10	25	...
	D	2-PS	74	30	70	...
	E	1-PS	74	15	25	...
	F	2-LMB 1-PS	47	...	...	5 hours
	G	2-Tadpoles	68-72	...	...	2 days
15 feet	A	2-PS	50	...	240	...
	B	2-LMB	50	...	125	...
	C	2-LMB	74	10	24	...
20 feet	A	2-LMB	74	...	198	...
	B	1-PS	74	...	...	2 days
	C	1-PS	70	...	...	2 days
	D	1-PS	70	...	300	...
25 feet	A	1-PS 1-LMB	68-74	...	...	2 days
	B	1-PS 1-LMB	70-74	...	...	2 days
	C	2-LMB	75	...	...	2 days
30 feet	A	2-LMB	70-74	...	...	2 days
	B	2-PS	70-74	...	...	2 days

None of the experimental fish placed in water from the 25- or 30-foot levels died even at higher temperatures and none of the tadpoles died in any of the experiments. At any given temperature the elapsed time before the death of the fish progressively increased from the surface to 25 feet. This quite definitely shows that the surface water contains a higher concentration of the poison. At a temperature of 45°F. fish were not affected even when placed in surface water. In general, down to the 25-foot level, where fish died they did so more quickly at higher temperatures regardless of the depth of the sample. There was a marked difference between the time required to kill largemouth bass and pumpkinseeds under the same condition. At 50°F. pumpkinseeds survived 2½ hours longer than largemouth bass.

Water samples taken from the surface, 5, 10, 15 and 20 feet, 72 hours after poisoning, were used for laboratory experiments. Both largemouth bass and pumpkinseeds died in the water from the surface, 5-and 10-foot levels within 90 minutes (temperature 72°F.). Fish placed in water from the 15- and 20-foot levels were still alive at the termination of this experiment 2 days later (temperature 72°F.).

Experiments with fish-food organisms.--A number of experiments conducted during the first poisoning to test the effect of rotenone on some of the invertebrates were repeated during the second poisoning. Several species of invertebrates collected in the lake were confined to cages and placed just below the surface and at the 2- and 4-foot depths prior to poisoning. No *Chironomus* or *Corethra* larvae from the deep water were used in these experiments because it was known that these forms would not live for any length of time in the warmer surface water even before poisoning. No *Gammarus* was available in the lake at the time of the second poisoning and so was omitted from the experiments.

Cages were examined each day and the dead organisms were counted and removed. The following table (7) gives the results of these experiments.

Table 7.--Mortality of fish-food organisms when retained in treated lake water from the surface, 2-foot and 4-foot depths

Organism	Surface		2 feet		4 feet	
	Number of organisms	Hours to death	Number of organisms	Hours to death	Number of organisms	Hours to death
Leeches	...	12	...	...	...	...
Snails						
Planorbis	...	1/ ...	...	1/ ...	2	72
					4	1/ ...
Physa	...	1/ ...	...	1/ ...	...	1/ ...
Amnicola	...	1/ ...	...	1/ ...	...	1/ ...
Amphipods						
Hyalella	...	1/ ...	...	1/ ...	...	1/ ...
Anisoptera nymphs						
Libelluline	...	1/ ...	...	1/ ...	...	1/ ...
Gomphine	...	1/ ...	...	1/ ...	...	1/ ...
Aeschnine	4	1/ ...	...	3	48	1/ ...
	3	48	...	1	24	1/ ...
Zygoptera nymphs	...	1/ ...	...	1/ ...	...	1/ ...
Trichoptera larvae	...	1/ ...	...	1/ ...	...	1/ ...
Midges	...	1/ ...	...	1/ ...	...	1/ ...
Tadpoles	1	24	2	48	5	24
	3	72	2	1/ ...	1	1/ ...
	1	96	...	...	...	...
	1	1/ ...	...	...	...	...
Duration of experiment	8 days		3 days		4 days	

1/ All lived for duration of experiment

The results of these experiments are in most respects similar to those secured during the first poisoning except for the aeschnine dragonflies. As has been described above, all of the dragonflies of this group died very soon in the cages in the first poisoning experiments but this was not the case in the second poisoning. A few died at the end of 24 hours but most of them lived 48 hours or longer and several were still alive when the water was no longer toxic to fish. A few individuals were observed to survive high concentrations of derris near the dock where the powder and water were mixed.

From these experiments and observations on the invertebrates in the lake it is evident that certain organisms such as leeches, Corethra and aeschnine dragonflies may be seriously reduced by the treatment of the lake with derris powder. On the other hand, it is evident that most of the invertebrate organisms are not seriously affected by treatments of this kind.

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