STATE OF MICHIGAN

DEPARTMENT OF CONSERVATION
LANSING
P. J. HOFFMASTER, DIRECTOR

IFR #863

May 17, 1943

Memorandum to: P. J. Hoffmaster, Director
Michigan Department of Conservation

The attached copy of Institute for Fisheries Research Report No. 863, entitled "Experimental Work on the Toxicity of Michigan Oil-Well Brines to Fishes" by Carl L. Hubbs and George N. Washburn, reviews the progress that has been made during the past year in undertaking to determine the maximum concentration of oil-well brines that can be tolerated by species of fish that inhabit the area where this pollution occurs.

The report also suggests further research, some of which is now in progress, on other problems associated with the disposition of these brines.

Respectfully submitted,

F. A. Westerman

FISH DIVISION

FAW:PVP

INSTITUTE FOR FISHERIES RESEARCH
DIVISION OF FISHERIES
MICHIGAN DEPARTMENT OF CONSERVATION
COOPERATING WITH THE
UNIVERSITY OF MICHIGAN

ALBERT S. HAZZARD, PH.D.
DIRECTOR

ADDRESS
UNIVERSITY MUSEUMS ANNEX
ANN ARBOR, MICHIGAN

April 19, 1943

REPORT NO. 863

EXPERIMENTAL WORK ON THE TOXICITY OF MICHIGAN OIL-WELL BRINES TO FISHES

by

Carl L. Hubbs, Curator of Fishes, Museum of Zoology, University of Michigan
and
George N. Washburn, Fisheries Research Technician of the Institute for Fisheries Research, Michigan Department of Conservation

In recent years considerable attention has been focused on the pollution of Michigan streams by oil-well brines. In August of 1942, staff members of the Institute for Fisheries Research conducted a preliminary field investigation of brine pollution. This led to Institute Fisheries Report No. 805, "The Preliminary Inspection of Mansfield Creek, Arenac County, with Reference to the Salt Water Pollution."

In this report the pollution of Mansfield Creek was attributed to the large quantities of concentrated brines which escape into the stream. Mansfield Creek, a tributary to the Rifle River courses through an oil-producing area in the north-central part of Arenac County. Large volumes of brine are brought to the surface during oil pumping operations in this field, but most of this brine is collected in an intercepting line and then returned to its original substrata by pressure pump. Some of this brine escapes and possibly reaches the stream. However, the most definite and serious source of brine pollution is an uncapped flowing brine well. The estimated discharge of this well is 100 gallons per minute. The entire flow appears to reach Mansfield Creek through surface drainage and seepage. No fish could be found in the seriously polluted area.

At the request of P. J. Hoffmaster, Director of the Department of Conservation and Supervisor of Wells for the state of Michigan, the Institute for Fisheries Research has undertaken a series of tests to determine the maximum concentration of these oil-well brines which can be tolerated by species of fish that inhabit the region where this pollution occurs.

Two brine samples were secured for this study on August 1, 1942. One 5-gallon sample was taken from a return line at the Swanson-Romoor-Shephard lease and is referred to as the Swanson-Romoor-Shephard brine. This is a composite brine, derived from several oil wells. The other 5-gallon sample was taken from the flowing well (the Arenac Salt Company well). An additional 5-gallon sample was secured by conservation officer Milton Misner from this same well on January 18, 1943, to complete the experimental work.

Samples of these brines were analyzed by Arno Heyn, Teaching Fellow in Analytical Chemistry at the University of Michigan. His report follows:

CHEMICAL ANALYSIS OF MICHIGAN SALT BRINES

Results are indicated in milligrams per liter of brine.

1. Swanson-Romoor-Shephard Lease. Sec. 4, Clayton Township, Arenac Co.

Sample collected 7-21-42, 2:36 P.M., by Stream Control Commission.

Report:

Total solids (110° C.)	308,270
Specific gravity	1.189
pH	6.20
Chloride and Bromide (as Cl)	175,390
Bromide	612
Chloride	175,200
Sulfate	352
Bicarbonate	46
Calcium	14,030
Magnesium	3,316
Potassium	975
Sodium	91,385
Silica	less than 20
Fe and Al	0

To obtain conventional combinations, the value of sodium, being least certain, was changed to 91,030 in order to obtain a balance of positive and negative radicals. The small amount of bicarbonate was neglected.

Conventional Combinations:

Potassium bromide	911		
Potassium chloride	1,289		
Sodium chloride	231,350		
Magnesium chloride	12,985	as hexahydrate:	27,730
Calcium chloride	38,450	as dihydrate:	50,920
Calcium sulfate	499	as dihydrate:	631
Totals	285,484		312,831

2. Arenac Salt Co. Section 3, Clayton Township, Arenac County.

Sample collected 7-21-42, 2:50 P.M., by Stream Control Commission.

Report:

Total solids (110° C.)	315,090
Specific gravity	1.198
pH	5.79
Chloride and Bromide (as Cl)	180,600
Bromide	754
Chloride	180,300
Sulfate	155
Bicarbonate	37
Calcium	17,612
Magnesium	4,150
Potassium	1,240
Sodium	89,570
Silica	less than 20
Fe and Al	0

To obtain conventional combinations, the value of sodium, being the least certain, had to be changed to 88,440 in order to obtain a balance of positive and negative radicals. The small amount of bicarbonate was neglected.

Conventional Combinations:

Potassium bromide	1,123		
Potassium chloride	1,663		
Sodium chloride	224,770		
Magnesium chloride	16,250	as hexahydrate:	34,700
Calcium chloride	48,590	as dihydrate:	64,370
Calcuim sulfate	219	as dihydrate:	277
Totals	292,615		326,903

Analysis completed March 6, 1943
Ann Arbor, Michigan
(signed)
Arno Harry Albert Heyn, Teaching Fellow in Analytical Chemistry, University of Michigan.

FISH USED AND METHODS EMPLOYED

Seven species of fish assumed to be representative of the fauna of Mansfield Creek prior to its pollution, were used in these tests. Four, regarded as warm-water species, are black bullhead (Ameiurus melas), small-mouth bass (Micropterus dolomieu), pumpkinseed (Lepomis gibbosus) and brook stickleback (Eucalia inconstans). The three cold-water types tested were brook trout (Salvelinus fontinalis), brown trout (Salmo trutta fario) and rainbow trout (Salmo gairdnerii irideus). Since the fingerling trout proved to be unexpectedly resistant to the brine, and yet seemed to have been exterminated in the creek, it was thought that the eggs and fry might prove to be more susceptible than the older fish; consequently, tests were also run on these young stages.

The black bullheads provided particularly good experimental material; they were a very uniform stock because they were all taken as 2- to 3-inch fish, from a single school, and were therefore of the same age and parentage. The stock which had been collected in Susterka Pond, near Rawsonville, in August of 1942 was kept for several months without loss, throughout the period of the trials. These bullheads remained in good condition and grew rather rapidly in the stock tank.

The small-mouth bass were also of uniform stock. They were secured as 2- to 3-inch fish of this year's hatch, from the Federal Fish Hatchery at Northville, Michigan. These hatchery-raised fish held well in stock tanks.

The pumpkinseeds were all seined from the Huron River, about 4 miles west of Ann Arbor. They were 2- to 3-inches long and at least one year old.

The brook sticklebacks, 1½ to 2½ inches long, were seined from Mansfield Creek above the polluted section.

The fingerling brook, brown and rainbow trout were obtained from the Federal Fish Hatchery at Northville. Trout used in the experiments were preserved at death. Following are the size ranges in inches (total length) for each species; brook (22 fish) 3½-5½; brown (38 fish) 2 3/8-3 7/8; rainbow (32 fish) 2 3/8-3 7/8. The same hatchery supplied the eggs and fry as follows: brook trout eggs which had been kept 65 to 75 days, fourteen day old eyed eggs of rainbow trout and fourteen day old sac fry of the brown trout. These eggs and fry had all been reared at 39° to 40° F.

The warm water species were held in stock tanks at the Experimental Aquarium at the University Museums building. The cold-water forms were kept in live boxes in a spring-fed stream. All of the fish were fed at regular intervals. The mortality was not more than 5 per cent for any species.

All of the tolerance tests were conducted in the Experimental Aquarium. Dilution water used in preparing the various test concentrations was the filtered circulating Aquarium water, which in many tests has proven to be satisfactory for the maintenance of fish life. Due to changes caused by the regeneration of the filter, the pH varied from 7.2 to 7.9 and dissolved oxygen ranged from 6.9 to 8.5 p.p.m. The water was relatively soft (methyl orange alkalinity about 42 p.p.m.). The normal temperature for the aquarium-room water (80° F.) was maintained for all of the warm-water fish tests. For the cold-water species, the temperature was held at a 50° F. level by a cold-water jacket surrounding the test jars.

The experimental equipment consisted of wide-mouth two-quart and one-gallon glass jars, each vocered and each equipped with an air line. All of the warm-water species were tested in the two-quart containers in a one-liter medium. As the trout were larger, they were tested in the gallon jars, each containing 2.5 liters of solution.

All of the test solutions were pre-aerated for 20 minutes, to insure an adequate supply of dissolved oxygen from the start, and the aeration was continued throughout the run. Two fish were used in each test jar. The time of death was noted for those that died within the limits of the 96-hour test period. This is the time limit which in our experience has seemed most desirable for routine toxicity-threshold tests. When unexpected or inconsistent results were obtained at any given concentration, duplicate tests were made, and all of the data were tabulated. The trout eggs and fry were tested in lots of 50 for a 10-day period, in order to obtain reliable results.

The consistent tolerance limits were approximated by initial tests with widely different concentrations, and were more precisely fixed by trials with concentrations interpolated between those which killed and those which did not kill the fish within 96 hours. The consistent tolerance limit is the highest concentration at which all of the fish can be expected to survive (disregarding records of death that do not seem attributable to the toxic action of the test material). The values approximate the ascertained lethal thresholds at which some of the fish are killed. It should be borne in mind that the consistent tolerance limits refer to the actual results obtained under experimental conditions. It can not be assumed that the concentrations tolerated by the particular fish tested would prove innocuous in a stream. Some margin of safety is called for.

RESULTS

We conducted the first series of tests with the Swanson-Romoor-Shephard brine, using three warm-water species. The second series, using the same species, was run on the Arenac Brine. The results are expressed in Tables 1 and 2, and are then compared.

DISCUSSION AND INTERPRETATION OF TESTS SUMMARIZED IN TABLE 1.- Concentrations of stock brine above 100,000 p.p.m. (containing 85,222 p.p.m. of chloride salts) are highly toxic, bringing about death to the 3 species in less than one hour. When the fish were tested in the 300,000 p.p.m. brine sample, death was sudden, The effect was much like that of a shock: the fish lost balance and their respiratory movements ceased almost immediately. These symptoms were not so pronounced at lower concentrations (below 100,000 p.p.m.) and were not evident when the fish were placed in concentrations approaching the lethal threshold. All of the fish which succumbed before the termination of the test period revealed on examination, a highly inflamed condition of the gill filaments and hemorrhages in the thin fin membranes. These symptoms lead one to suspect that death is caused by an abrupt change in

TABLE 1. EXPERIMENTAL RUN WITH BLACK BASS, PUMPKINSEED AND STICKLEBACK IN DIFFERENT CONCENTRATIONS OF THE SWANSON*ROMOOR*SHEPHARD OIL*WELL BRINE

Experiment started August 8, 1942

Concentration of test samples			Number of fish	Fish used in tests and survival times, (Hrs.: Min.: Sec.)		
P.p.m. of stock brine	P.p.m. of total salts	P.p.m. of chloride salts		Bass	Pumpkinseeds	Sticklebacks
300,000	92,481	85,222	1	0:02:00	0:02:03	0:02:10
			1	0:02:03	0:02:07	0:02:31
			1	0:02:10		
			1	0:02:21		
200,000	61,654	56,814	1	0:02:45	0:02:51	0:03:00
			1	0:02:51	0:03:50	0:03:12
			1	0:03:01		
			1	0:03:12		
100,000	30,827	28,407	1	0:29:00	0:37:00	0:38:00
			1	0:31:00	0:44:00	0:46:00
75,000	23,120	21,305	1	05:18:00	04:15:00	05:40:00
			1	05:21:00	05:00:00	06:21:00
65,000	20,037	18,464	1	05:15:00	06:20:00	
			1	06:15:00	07:00:00	
55,000	16,054	15,624	1	18:45:00	37:00:00	19:30:00
			1	31:30:00	42:30:00	36:00:00
50,000	15,413	14,203	1	71:00:00	41:00:00	
			1	78:00:00	67:00:00	
45,000	13,872	12,783	1	24:30:00	27:00:00	69:00:00
			1	96+.. ..	30:15:00	96+.. ..
40,000	12,330	11,362	1	96+.. ..	83:00:00	42:00:00
			1	96+.. ..	96+.. ..	48:00:00
35,000	10,789	9,942	1	66:30:00	90:00:00	96+.. ..
			1	87:00:00	96+.. ..	96+.. ..
			2	96+.. ..		
30,000	9,248	8,522	1	21:10:00	96+.. ..	96+.. ..
			1	92:00:00	96+.. ..	96+.. ..
25,000	7,706	7,101	2	96-.. ..	96+.. ..	96+.. ..
20,000	6,165	5,618	2	96+.. ..	96+.. ..	96+.. ..
15,000	4,624	4,261	2	96+.. ..	96+.. ..	96+.. ..
10,000	3,082	2,840	2	96+.. ..	96+.. ..	96+.. ..
Control			2	96+.. ..	96+.. ..	96+.. ..

the osmotic pressure. Each species varied markedly in susceptibility to concentrations approaching the lethal threshold. This variation was probably due to individual physiological differences.

The consistent tolerance limits, approximately established, for these species of fish for a 96-hour test are as follows:

Smallmouth bass: 25,000 p.p.m. of stock brine, containing 7,706 p.p.m. of total solids of which 7,101 p.p.m. are chlorides.

Pumpkinseed: 30,000 p.p.m. of stock brine, containing 8,522 p.p.m. of chlorides.

Stickleback: 35,000 p.p.m. of stock brine, containing 9,942 p.p.m. of chlorides.

Of the 3 species tested with the Swanson-Romoor-Shephard brine the sticklebacks were the most resistant, the pumpkinseeds next and the bass the most susceptible. The percentage of difference of toleration between the 3 species is as follows: the sticklebacks tolerated 14 per cent more chloride than the pumpkinseeds did, and 29 per cent more than the bass. The pumpkinseeds tolerated 12 per cent more chloride than did the bass.

DISCUSSION AND INTERPRETATION OF TESTS SUMMARIZED IN TABLE 2.- Concentrations of stock brine above 100,000 p.p.m. are highly toxic, producing death in less than 45 minutes. Fish in these high concentrations exhibited the same actions and symptoms as previously described in the interpretation of data in Table 1. Again, these fish varied considerably in tolerance to concentrations that approached the lethal threshold. The thresholds are not clear-cut and a considerable range in individual tolerance can be expected. Consequently, the consistent tolerance limits as established are approximate. The sticklebacks were again the more resistant of the 3 species surviving in a stock concentration of 30,000 p.p.m. (containing 9,248 p.p.m. of total solids of which 8,738 p.p.m. were chlorides). The pumpkinseeds were next, tolerating 25,000 p.p.m. of stock brine, having a composition of 7,706 p.p.m. total solids of which 7,281 p.p.m. were chlorides. The bass were the most susceptible, being able to tolerate only a 20,000 p.p.m. concentration of stock brine containing 6,165 p.p.m. of total solids of which 5,825 p.p.m. were chlorides.

COMPARISON OF THE TOXICITY OF THE TWO BRINES

The three species of fish that were tested tolerated a somewhat higher concentration of the Swanson-Romoor-Shephard brine than of the Arenac brine. It was expected that the respective tolerance limits, as expressed in terms of the chloride content, would be approximately the same for the three species, since the chemical analyses of the two

TABLE 2. EXPERIMENTAL RUN WITH SMALLMOUTH BASS, PUMPKINSEEDS AND STICKLEBACKS IN DIFFERENT CONCENTRATIONS OF THE ARENAC SALT COMPANY BRINE

Experiment started August 8, 1942

Concentration of test samples			Number of fish	Fish used in tests and survival times, (Hrs.: Min.: Sec.)		
P.p.m. of stock brine	P.p.m. of total salts	P.p.m. of chloride salts		Bass	Pumpkinseeds	Sticklebacks
300,000	94,527	87,381	1	0:00:51	0:00:53	0:01:12
			1	0:00:55	0:00:56	0:01:22
			1	0:01:09		
			1	0:01:15		
200,000	63,018	58,254	1	0:01:31	0:01:45	0:01:59
			1	0:01:45	0:02:05	0:02:01
			1	0:02:30		0:03:10
			1	0:02:51		
100,000	31,509	29,127	1	0:17:00	0:18:00	0:38:00
			1	0:22:00	0:23:00	0:41:00
80,000	25,207	23,301	1			1:20:00
			1			2:05:00
75,000	23,631	21,845	1	2:10:00	2:20:00	2:00:00
			1	2:51:00	3:10:00	2:10:00
70,000	22,056	20,389	1			2:20:00
			1			2:40:00
65,000	20,480	18,932	1	4:47:00	5:10:00	
			1	6:10:00	7:40:00	
60,000	18,905	17,476	1			3:50:00
			1			4:15:00
55,000	17,329	16,020	1	36:30:00	35:00:00	
			1	37:15:00	38:30:00	
50,000	15,754	14,563	1	10:05:00	44:35:00	27:50:00
			1	58:10:00	62:00:00	28:10:00
45,000	14,179	13,107	1	48,10:00	72:00:00	
			1	96+.. ..	96+.. ..	
40,000	12,603	11,650	1	49:45:00	66:00:00	46:35:00
			1	96+.. ..	96+.. ..	51:20:00
35,000	11,028	10,194	1	96+.. ..	78:00:00	60:00:00
			1	96+.. ..	96+.. ..	71:00:00
30,000	9,452	8,738	1	51:10:00	67:00:00	96+.. ..
			1	96+.. ..	96+.. ..	96+.. ..
25,000	7,877	7,281	1	28:00:00	96+.. ..	96+.. ..
			1	96+.. ..	96+.. ..	96+.. ..
20,000	6,301	5,825	2	96+.. ..	96+.. ..	96+.. ..
10,000	3,150	2,912	2	96+.. ..	96+.. ..	96+.. ..
Control			2	96+.. ..	96+.. ..	96+.. ..

brines are very similar. The only difference that appears to be significant is the somewhat greater concentration of the Arenac brine. Owing to the wider range of concentrations over which some fish died and others lived, the consistent tolerance limits cannot be regarded as certainly established. Even if we assume that these limits are correctly determined, uniformly indicating, as shown in Tables 1 and 2, consistent tolerance limits of 5,000 p.p.m. greater for the Swanson-Romoor-Shephard than for the Arenac brine, still no difference in resistance to the salt content would be demonstrated. The tests were made at concentration differences of 5,000 p.p.m., and the limits may lie anywhere between those limits. On this basis the consistent tolerance limits to p.p.m. of chloride salts for the bass is more properly expressed as 7,101 to 8,522 p.p.m. for the Swanson-Romoor-Shephard brine and 5,825 to 7,281 p.p.m. for the Arenac brine. Since these values overlap for each species, the true consistent tolerance limits for the two brines may be identical-say at 7,200 p.p.m. for the bass.

The differences in toxicity (expressed in tolerance) for the two brine samples does not warrant additional tests with the Swanson-Romoor-Shephard brine, as it is a question as to the possible extent that this brine contributes to the pollution of the stream under study, whereby it is a known fact that the Arenac brine does directly contribute to the polution of this stream.

FURTHER FISH TOLERATION TESTS WITH THE ARENAC BRINE

Four additional species were tested with the Aronac brine. One of these was a woarm-water fish, the black bullhead, and three were cold-water forms, brook, brown and rainbow trout. In addition, as previously mentioned (page 4), various younger stages of the three species of trout were tested. Tables 3 to 7 present the results of these supplementary experiments.

DISCUSSION AND INTERPRETATION OF TESTS SUMMARIZED IN TABLE 3.- Concentrations of stock brine above 200,000 p.p.m. are quickly lethal, producing death to the bullhead in less than 2 minutes. The apparent cause of death is the sudden change in osmotic pressure. The gill filaments and the fin membranes become highly inflamed. The fish lost balance almost immediately on contact with the salt water and respiratory movements ceased a few seconds later. Concentrations between 43,000 and 100,000 p.p.m. of stock brine are only moderately toxic, requiring from a few to several hours to bring about death. Several observations were recorded at the time of the death of the individual fish. In a moderate concentration (50,000 p.p.m. of stock brine), the bullheads became quite dormant in a few hours, and this condition continued until nearly all of the activity ceased and the fish appeared to be dead. However, on an actual physical contact, such as pricking, the fish made a feeble response. Several times these stupified fish

TABLE 3. EXPERIMENTAL RUN WITH THE BLACK BULLHEAD IN DIFFERENT CONCENTRATIONS OF THE ARENAC BRINE

Experiment started December 7, 1942

Concentration of test samples			Number of fish tested	Survival times		
P.p.m. of stock brine	P.p.m. of total salts	P.p.m. of chloride salts		Hrs.	Min.	Sec.
300,000	94,527	87,381	1	...	...	58
			1	...	1	3
			1	...	1	12
			1	...	1	15
200,000	63,018	58,254	1	...	1	45
			1	...	1	56
			1	...	2	10
			1	...	3	...
100,000	31,509	29,127	1	...	49	...
			1	...	53	...
			1	...	57	...
			1	1	2	...
80,000	25,207	23,301	1	1	40	...
			1	2	10	...
75,000	23,631	21,845	1	1	55	...
			1	2	20	...
70,000	22,056	20,389	1	2	30	...
			1	2	50	...
65,000	20,480	18,932	1	3	15	...
			1	3	30	...
60,000	18,905	17,476	1	4	40	...
			1	7	25	...
55,000	17,329	16,020	2	11 to 19 hrs.		
50,000	15,754	14,563	1	18	55	...
			1	23	25	...
45,000	14,179	13,107	1	28	45	...
			1	31	40	...
43,000	13,548	12,524	1	28	45	...
			1	31	...	...
			1	45	...	...
			1	47	...	...
40,000	12,603	11,650	2	96+	...	...
35,000	11,028	10,194	2	96+	...	...
25,000	7,877	7,281	2	96+	...	...
20,000	6,301	5,825	2	96+	...	...
Control			2	96+	...	...

were seen to become active very suddenly, darting about the tank violently for several seconds, after which they became stiff and settled to the bottom. They were all tested immediately and found to be dead, for no stimulus would bring about a response. The bullheads were the only fish tested which exhibited these peculiar death reactions. The consistent tolerance threshold established by the bullheads was 40,000 p.p.m. of stock brine (containing 12,603 p.p.m. of total solids, including 11,650 p.p.m. of chloride salts).

SUDDEN SHOCK, SIMULATING DEATH, PRODUCED IN BULLHEADS BY HIGH CONCENTRATIONS OF BRINE

Some of the bullheads which had apparently been killed by a short exposure to strong concentrations of the Arenac brine recovered when replaced in fresh water. They had apparently suffered from shock, and would undoubtedly have soon died if left in the brine. Time had hardly sufficed for the absorption of much salt, and the shock is thought to have resulted from the sudden intense change in osmotic pressure. At lower concentrations the shock was less but absorption of salts relatively greater, because of the longer time of exposure. The possibility of reviving the fish with fresh water seems to stand in direct proportion to the concentration of brine (Table 4). The time required for revival, however, seems to be positively correlated with the time required to produce the shock that simulates death. These tests may bear some relation to the flash disposal of brine wastes.

TABLE 4. PERCENTAGE OF REVIVAL OF APPARENTLY DEAD BULLHEADS, WHEN CHANGED FROM SALT SOLUTIONS TO FRESH WATER

Concentration of brine		Number of fish tested	Average time required to produce shock			Average time required to revive	Percent of fish revived
P.p.m. of stock brine	P.p.m. of chlorides		Hrs.	Min.	Sec.	Min.	Percent
300,000	87,381	4	...	1	7	26	75
200,000	58,254	4	...	2	13	30	50
100,000	29,127	4	...	53	...	40	25
80,000	23,301	4	1	15	...	—	0
75,000	21,845	4	1	28	...	—	0

TOLERATION TESTS CONDUCTED WITH THREE SPECIES OF TROUT

Brook, brown and rainbow trout, in fingerling, egg and sac fry stages, were tested to determine their toleration to the Arenac brine. All of these experiments were conducted in one-gallon jars at 50° F. The duration of the test for the fingerlings was 96 hours (the same as for the warm-water species). For the eggs and sac fry, the toleration test period was extended to 10 days. The 3 species were tested simultaneously under identical conditions. Concentrations for the latter part of these tests were prepared with the new brine sample collected from the Arenac well in January. A total-solid and chloride analysis of this sample revealed very little difference in chemical composition. The analysis of the original Arenac brine sample indicated that it contained 315,090 p.p.m. of total solids, including 180,600 p.p.m. of Cl + Br. The analysis of the second sample revealed a concentration of 314,811 p.p.m. total solids including 180,510 p.p.m. of Cl + Br. Since the differences in the analyses is hardly significant, the computations for concentrations were based on the original analysis.

The results of these tests are dealt with in three tables: Table 5 treats the fingerlings; Table 6 deals with the eggs and sac fry; Table 7 compares the results for the three developmental stages.

DISCUSSION AND INTERPRETATION OF TESTS SUMMARIZED IN TABLE 5-Concentrations above 150,000 p.p.m. of stock brine are only moderately toxic, requiring over 1 hour to produce death. Concentrations from 80,000 to 100,000 p.p.m. have only a slight toxic effect upon the three species of trout. The brook trout, the most tolerant of the three species, were able to survive a 96 hour test period in a stock concentration of 80,000 p.p.m. (containing 25,207 p.p.m. of total solids including 23,301 p.p.m. of chloride salts). The brown and rainbow trout agreed in their consistent tolerance limit-70,000 p.p.m. of stock brine (containing 22,056 p.p.m. of total solids, including 20,389 p.p.m. of chloride salts). The brook trout were able to tolerate about 12 per cent more chloride than either the brown or rainbow. The three species of trout when subjected to brine concentrations approaching the consistent tolerance limits, exhibited sluggish behavior. They lost equilibrium after a 20 to 30 hour exposure, and the respiratory movement gradually became very slow (as low as 7 or 8 per minute), yet the fish continued to live in this stupor for many hours. This condition was most pronounced among the rainbow trout. Some of these motionless fish survived the 96 hour test period. A problem arises as to how these stupefied fish that survived the 96 hour test should be listed. For practical purposes they are dead. Attempts to recover them failed. They died within a few hours whether they were retained in the brine or were transferred to fresh water. They were listed as live, however, if they showed any sign of life.

TOXIC EFFECTS OF ARENAC BRINE ON EARLY DEVELOPMENTAL STAGES OF TROUT

During the experiments on the fingerling trout, the smaller of the two fish in each test jar was the first to die. These observations seemed to show that the smaller fish are the more susceptible to the brine. In order to determine whether younger fish or developing eggs may be still more susceptible, preliminary tolerance tests were conducted on eggs and on sac fry. Further experiments on the early stages are contemplated.

TABLE 5. EXPERIMENTAL RUN WITH THE BROOK, BROWN AND RAINBOW TROUT IN DIFFERENT CONCENTRATIONS OF THE ARENAC BRINE

Experiment started Dec. 18, 1942

Concentration of test sample			Number of fish tested	Fish used in test and survival times					
P.p.m. of stock brine	P.p.m. of total solids	P.p.m. of chloride salts		Brook trout		Brown trout		Rainbow trout	
				Hrs.	Min.	Hrs.	Min.	Hrs.	Min.
200,000	63,018	58,254	1	1	15	1	8	1	8
			1	1	22	1	27	1	23
150,000	47,269	43,690	1	3	55	2	10	2	40
			1	4	50	2	50	3	10
110,000	34,659	32,040	1	11	35	7	15	8	30
			1	17	...	15	...	13	45
108,000	34,029	31,457	1	20	...				
			1	22	40				
105,000	33,084	30,583	1					18	30
			1					26	10
103,000	32,454	30,001	1	20	45				
			1	23	15				
100,000	31,509	29,127	1	26	30	11	30	19	40
			1	32	15	17	50	21	10
94,000	29,618	27,669	1					22	45
			1					23	15
92,000	28,988	26,797	1			15	20		
			1			18	...		
90,000	28,358	26,214	1	36	10	21	...		
			1	44	...	25	20		
87,000	27,412	25,340	1	40	30	21	40		
			1	42	...	27	25		
86,000	27,097	25,049	1					46	...
			1					54	30
80,000	25,207	23,301	1	96+	...	52	...	78	...
			1	96+	...	96+	...	96+	...
70,000	22,056	20,389	2	96+	...	96+	...	96+	...
60,000	18,905	17,476	2	96+	...	96+	...	96+	...
50,000	15,754	14,563	2	96+	...	96+	...	96+	...
40,000	12,603	11,650	2	96+	...	96+	...	96+	...
30,000	9,452	8,738	2	96+	...	96+	...	96+	...
20,000	6,301	5,825	2	96+	...	96+	...	96+	...
Control	...	...	2	96+	...	96+	...	96+	...

Since Mansfield Creek has apparently been ruined as a trout stream, even where the brine concentration is the experimentally determined toxicity threshold for half-grown trout, it was early thought that the critical effect of the brine might be on some susceptible early stage in the development of the trout.

Various test concentrations were prepared, ranging from a point slightly above the consistent tolerance limits established for the yearling trout to a concentration of only a few thousand p.p.m. of chloride. Fifty fry or eggs were placed in each test concentration and the tests were continued for a period of 10 days. From time to time any dead eggs or fry present in the test jars were removed, to prevent the spread of disease and the contamination of the water. At the end of the test period, all samples were counted and the data recorded in terms of percentage of survival. Table 6 covers the data obtained during this test.

TABLE 6. PERCENTAGE OF SURVIVAL OF TROUT EGGS AND SAC FRY IN DIFFERENT CONCENTRATIONS OF THE ARENAC BRINE

Experiment started Feb. 2, 1943

Concentration of test samples			Species and percentage of survival for a 10-day period		
P.p.m. of stock brine	P.p.m. of total solids	P.p.m. of chloride salts	Brook trout eggs, developed 65-75 days	Brown trout sac fry, 8 days old	Rainbow trout eggs, developed 14-20 days
100,000	31,509	29,127	0	0	0
85,000	26,782	24,758	0	0	0
70,000	22,056	20,389	0	0	30
50,000	15,754	14,563	18	66	82
30,000	9,452	8,738	96	100	82
Control			98	100	88

DISCUSSION AND INTERPRETATION OF TESTS SUMMARIZED IN TABLE 6.--The percentage of mortality for the brook trout eggs was 100 per cent in stock brine concentrations of 70,000, 85,000 and 100,000 p.p.m. Only 18 per cent, however, were able to survive a concentration of 50,000 p.p.m. It appears that the consistent tolerance limits for the 65 to 75 day old eggs lies somewhere between 30,000 and 50,000 p.p.m. The brown trout sac fry reacted similarly, having a 100 per cent mortality in 70,000, 85,000 and 100,000 p.p.m. of stock brine. Likewise, the consistent tolerance limit fell between 30,000 and 50,000 p.p.m. Slightly different results were obtained for the rainbow trout eggs, which showed a mortality of 100 per cent in concentrations of 85,000 and 100,000 p.p.m., and of 30 per cent in 70,000 p.p.m. The consistent tolerance limits fell between 50,000 and 70,000 p.p.m. of stock brine.

The survival times recorded for the above tests were as follows:

Brook trout eggs in a 100,000 p.p.m. concentration		all dead in 24 hours
" " " " " 85,000 p.p.m.	"	" " " 48 "
" " " " " 70,000 p.p.m.	"	" " " 96 "
Brown trout fry in a 100,000 p.p.m. concentration		all dead in 24 hours
" " " " " 85,000 p.p.m.	"	" " " 48 "
" " " " " 70,000 p.p.m.	"	" " "120 "

The actual survival time for the early-stage rainbow trout eggs could not be accurately computed for a short period of exposure, because a color change of the egg was the only ready means of determining whether or not the enclosed embryo was alive or dead, and this color change, from translucent to white, is a rather slow process.

COMPARISON OF THE TOLERANCE OF YEARLING TROUT, SAC FRY AND DEVELOPING EGGS

Results from the brine tolerance tests conducted with fingerling, fry and developing eggs of the three species of trout, indicate that there are significant differences in tolerance. It was found that brook trout fingerlings were able to tolerate about twice the brine concentration that the brook trout eggs could survive (Table 7). Very similar results were found to hold true for the brown trout fingerlings and sac fry. Somewhat less difference was indicated for the rainbow trout yearlings and eyed eggs.

TABLE 7. TOLERANCE TO BRINE OF FINGERLING FTOUT, SAC FRY AND EGGS

Species	Developmental stages	Approximate consistent tolerance limits		
		P.p.m. of stock brine	P.p.m. of total solids	P.p.m. of chloride salts
Brook	fingerlings	80,000	25,207	23,301
"	65-70 day old eggs	30,000	9,452	8,738
Brown	fingerlings	70,000	22,056	20,389
"	sac fry	30,000	9,452	8,738
Rainbow	fingerlings	70,000	22,056	20,389
"	eggs	50,000	15,754	14,563

DIFFERENCES BETWEEN THE WARM-WATER AND COLD-WATER FISHES IN TOLERANCE FOR BRINE

The three species of trout tested were found to be capable of tolerating two to four times as strong a concentration of brine as the four warm-water fished could withstand (Plate 1). They were about twice as resistant as the black bullhead, and four time as tolerant as the smallmouth bass. At first tought this was a most unexpected result, for trout are always regarded as among the most susceptible of all fishes to pollution. There is, however, a biological reason for the high tolerance of the trout to chlorides. All or almost all of the salmonidae are euryhaline fishes, for those which live near the coast migrate back and forth between salt and fresh waters, and possess some mechanism which enables them to become adjusted to differences in salinity. Even the brook trout migrates to the sea where it has the opportunity to do so. The warm-water species studied are strictly fresh-water fishes, and for this reason presumably lack the ability of the salmonoids to tolerate salt water.

An alternative explanation for the greater toleration of the trouts to brine would be that they were tested at about 50° F., whereas the warm-water species were run at about 80°. It is a physiological principle that fishes become more susceptible to pollutants at higher temperatures, for the metabolic rate rises with the temperature (Powers, 1920). It was thought that the warm-water fish might be as resistant as the trout to brine if they were tested at 50° . This alternative explanation, however, was disposed of by the following experiment.

RELATION BETWEEN TEMPERATURE AND THE SUSCEPTIBILITY OF BLACK BULLHEADS TO THE ARENAC BRINE

To check the possiblity that the relatively great resistance of trout to brine was due to the fact that they were tested in cold water, experiments were run on the resistance of black bullheads to brine, when kept at 80° and at 50° F. Those to be tested at 50° were kept at that temperature for three weeks prior to the experiments. The experimental procedure was the same as had been used for the trouts.

The results of this test, given in Table 8, show that the black bullheads survived longer in the colder water,[1] but that the consistent tolerance limit remained at least approximately the same. The survival time at 50° was 2.7 times as long as at 80° , when the fish were in 60,000 p.p.m. of brine; 1.7 times as long in 50,000 p.p.m., and 1.6 times as long in 45,000 p.p.m. The factor probably approaches 1.0 at the toxicity threshold.

---

[1] McCay (1929) determined that bullheads when exposed to a certain concentration of ammonium carbonate survived 3 times as long at 10° to 13° C. as they did at 26° C. Powers (1920) found that the toxicities of certain chlorides (lithium, ammonium) to fishes increased with rise in temperature.

PLATE 1

RELATIVE TOLERANCE OF SEVEN SPECIES OF FISH TO THE ARENAC BRINE

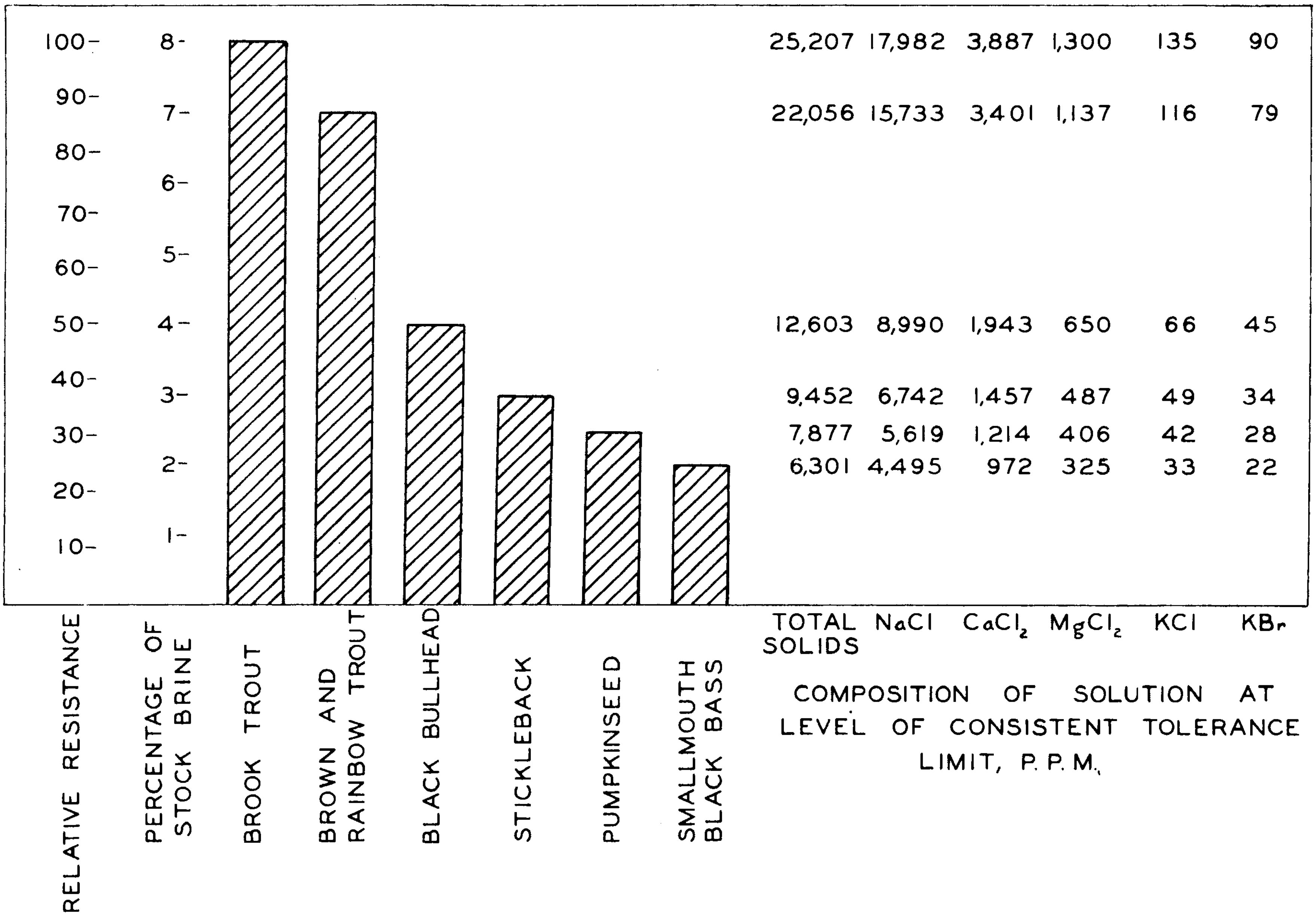

TABLE 8. SURVIVAL TIMES OF BLACK BULLHEADS IN DIFFERENT CONCENTRATIONS OF ARENAC BRINE, RUN AT 50° F. AND 80° F.

Concentration of brine sample			Number of fish tested	Test temperatures and average survival times			
P.p.m. of stock brine	P.p.m. of total solids	P.p.m. of chloride salts		80° F. [*]		50° F.	
				Hrs.	Min.	Hrs.	Min.
60,000	18,905	17,476	2	3	42	9	25
50,000	17,754	14,563	2	21	10	37	30
45,000	14,179	13,107	2	30	12	50	...
40,000	12,603	11,650	2	96+	...	96+	...

[*] Average from former tests (see Table 3).

CAUSES OF DEATH IN BRINE

It was not determined whether the fish that were killed by the Michigan oil-well brines died as a physical result of an increase in the osmotic pressure or a chemical result of some specific toxic factor, inherent either in one of the constituents or in the mixture of salts. As a result of experiments with the sand shiner (Notropis deliciosus) Garrey (1916) concluded that fresh-water fish are killed by salt concentrations that produce an Osmotic pressure greater than that of the fish's blood, but that they live in concentrations at that pressure or at a lower pressure. He determined that the limiting osmotic pressure was equivalent to six atmospheres, which is produced by a NaCl concentration of 0.7 per cent (7,000 p.p.m.).

The results of our tests indicate that the osmotic relations are not the sole factor in the killing of the fish by the oil-well brines. We might attribute to osmotic pressure the death of the smallmouth bass, which could tolerate about 6,700 p.p.m. of total salts, containing 4,500 p.p.m. of NaCl. Bullheads, however, tolerated 12,600 p.p.m. of total salts,[2] containing 9,000 p.p.m. of NaCl. The trouts tolerated much higher concentrations, far above the osmotic pressure of 7,000 p.p.m. (in terms of NaCl), supposed to be the limit that fresh-water fish can withstand. The consistent tolerance limits for the trouts ranged from 22,000 to 25,200 p.p.m. of total salts, with a NaCl content of 15,700 to 18,000 p.p.m. The trout in particular must have some mechanism that permits them to overcome the effects of high osmotic pressures in the surrounding medium. That such a mechanism operates when salmonoid fishes pass back and forth between fresh and salt water is evident from the physiological researches of Greene (1905). He found that the blood of king salmon had an osmotic pressure equivalent to 13,600 p.p.m. of NaCl when the fish were living in ocean water (almost 33,000 p.p.m. of salt), but that this value dropped to 10,380 p.p.m. when the salmon was in fresh water.

[2] Approximate values are given in this discussion; for more precise values refer to Plate 1.

We concluded therefore that chemical toxicity as well as osmotic pressure must be a factor in the killing of fish by oil-well brines. Whether the toxic effect is that of one salt or of a mixture was not determined. If, as might be expected, it is the mixture that is operative, the effect of the mixed salts might be an increase in toxicity (enhanced by synergetic action) or a decrease in toxicity (reduced by the antagonistic action of the cations). Sodium and calcium salts when mixed are known to be antagonistic, but the maximum antagonism is reached when the Ca content is 10 per cent that of the Na; at higher proportions of Ca the antagonism rapidly decreases and the synergetic action comes into effect (Powers, 1920). Since the Ca content of the oil-well brines tested is 38 per cent that of the Na (by weight), the toxicity can be expected to be at least as great as that of sodium alone.

Unfortunately most of the toxicity determinations on salts have been made with single salts or on combinations of two salts, not on such mixtures as the oil-well brines. The samples tested had the following percentage composition:

NaCl,	71 per cent	KCl,	0.5 per cent
$CaCl_2$,	15 " "	KBr,	0.3 " "
$MgCl_2$,	5 " "	Other salts,	8.2 " "

According to representative published accounts, salts differ markedly in toxicity (Table 9). A comparison of the data in Table 9 with that in Plate 1 will show that death of the fish tested by us could be attributed to the separate action of each of two or more of the constituent salts.

TABLE 9. REPRESENTATIVE DETERMINATION OF TOXICITY THRESHOLDS OF SALTS

Salt	Species of fish	Concentration (p.p.m.)	Time to kill	Author (date)
NaCl	Goldfish	10,000	4 to 7 days	Garrey (1916)
	do.	11,765	17 hours	Powers (1917)
	Golden shiner	10,000	97 hours	Wiebe, Burr and Faubion (1934)
$CaCl_2$	do.	5,000	143.5 hours	do.
	do.	10,000	27.6 hours	do.
	Sunfish	10,000	48 hours	do.
$MgCl_2$	Sand shiner	476	4 to 6 days	Garrey (1916)
	Golden shiner	5,000	96 hours	Wiebe, *et al.* (1934)
KCl	Sand shiner	373	12 to 29 hrs.	Garrey (1916)
	Goldfish	74.6	4 hrs. 40 min	Powers (1917)

FURTHER RESEARCHES ON BRINE POLLUTION

In as much as this series of toleration tests did not completely answer why the brine polluted stream in question has been eliminated as a trout stream, further research on this problem is needed. The special lines of inquiries are as follows:

1. Plans are progressing for a further detailed investigation to determine the effects of brines on early developmental stages of the trout. Special emphasis will be placed upon the eggs and young fry stages. Some stages may be particularly susceptible.

2. Tests are needed to determine the effects of brine on trout food organisms. Brine pollution may affect the availability of fish food.

3. Though some work has been done on the reaction of fishes to certain salts, it is advisable to investigate this problem further. It is possible that fishes may migrate out of areas polluted by non-lethal concentrations of brine. Such reactions could explain the absence of trout in Mansfield Creek. Two methods of studying this problem will be considered, one utilizing a gradient chamber in the laboratory and one performed in Mansfield Creek, using marked trout.

4. Another problem which needs to be investigated is to determine to what extent fish are able to acclimatize themselves to brine pollution.

5. Since only one type of Michigan brine has been studied, it may be desirable to carry on investigations with other types which may present disposal problems, for example, the highly concentrated magnesium brine wastes at Ludington, Michigan, which will be increasingly encountered in the expanding magnesium industry due to war demands.

6. Some work has been completed and more is contemplated as a contribution to the standardization of methods in the measurement of pollution. Hart, Doudoroff and Greenbank have prepared for early publication a detailed set of proposals for standardized tests. Preliminary results on Michigan oil-well brines, in tests conducted according to these proposed standard methods, are essentially in agreement with those herein reported.

SUMMARY AND CONCLUSIONS

1. Concentrations of brines containing more than 12,600 p.p.m. of total salts are definitely toxic to the four warm-water species (smallmouth bass, pumpkinseed, stickleback and black bullhead) in a 96 hour test period.

2. The bullheads were the most tolerant of the four species, surviving a 96 hour test period in about 12,600 p.p.m. of total salts.

The smallmouth bass were the most susceptible, tolerating only 6,300 p.p.m. of total salt.

3. The cold-water species, brook, brown and rainbow trout were found to tolerate from two to four times a greater brine concentration than were the four warm-water species. Of the three species of trout, the brook was the most tolerant, surviving in a concentration about 25,200 p.p.m. of salts. The brown and the rainbow trout were found to tolerate a concentration equal to 88 per cent of that tolerated by the brook trout.

4. The deleterious effect of brine on trout appears to vary with age and development. Preliminary results indicated that trout eggs and sac fry were about twice as susceptible as were fingerling trout.

5. The only appreciable effect of temperature on the tolerance of fish to brine was expressed by a longer survival time in lethal concentrations. The consistent tolerance limits were found to remain the same.

6. Fish exposed to high concentrations of brines (and appearing to be dead) were found to be shocked. Revival could be accomplished by the removal of the fish to fresh water.

7. The death of fish in brine is not wholly due to the increased osmotic pressure in the test medium. Specific toxicity is also involved.

8. The minimum dilutions of brine necessary to render it non-lethal for a 96 hour test period varied from 1:11.5 to 1:49 for the seven species of fish tested (Table 10).

TABLE 10. BRINE DILUTIONS CONSISTENTLY TOLERATED BY FISH

Species	Parts of dilution water (to 1 of brine)	Percentage of brine (by volume)
Brook trout	11.5	8
Brown trout	13	7
Rainbow trout	13	7
Black bullhead	19	4
Brook stickleback	32	3
Pumpkinseed	39	2.5
Smallmouth bass	49	2

The dilutions indicated cannot be taken as a safe limit for streams, unless some safety factor is applied. Some other species of fish or some stage in development may be more susceptible than those tested. Food organisms may be destroyed by concentrations below those lethal to fish.

Fish may react negatively to sublethal doses. Available evidence is not sufficient to fix the safety factor, without being arbitrary.

Tests performed by George N. Washburn and Carl L. Hubbs.

Supervision: Professor Carl L. Hubbs, University Museums; Dr. A. S. Hazzard, Director, Institute for Fisheries Research.

REFERENCES

Ellis, M. M. 1937. Detection and measurement of stream pollution. Bull. U. S. Bur. Fish., 22, pp. 365-437.

Garrey, W. C. 1916. The resistance of fresh-water fish to change of osmotic and chemical conditions. Am. Jour. Phys., 39, pp. 313-329.

Greene, C. W. 1905. Physiological studies of the chinook salmon. Bull. U. S. Bur. Fish., 24, pp. 455-465.

McCay, C. M. 1929. Studies upon fish blood and its relation to water pollution. Biol. Surv. St. Lawrence Watershed, 5th Ann. Rept. N. Y. Cons. Dept., Suppl. 20, p. 233.

Powers, E. B. 1917. The gold fish as a test animal in the study of toxicity. Ill. Biol. Mono., 4, pp. 127-193.

1920a. Influence of temperature and concentration on the toxicity of salts to fishes. Ecology, 1, pp. 95-112.

1920b. Antagonism and its possible utility in polluted waters. Trans. Am. Fish. Soc., 50, pp. 293-296.

Wiebe, A. H., Burr, J. G., Faubion, H. E. 1934. The problem of stream pollution in Texas with special reference to salt water from oil fields. Trans. Am. Fish. Soc., 64, pp. 81-86.

INSTITUTE FOR FISHERIES RESEARCH

By Carl L. Hubbs and George N. Washburn

Report approved by: A. S. Hazzard

Report typed by: G. Wood

Re-Typed 5-17-43 By: P. Ver Planck