Report 276 March 19, 1935

REPORT ON THE EFFECTS OF BRINE SOLUTIONS ON THE BLUNTNOSE MINNOW (Hyberhynchus notatus)

The following experiments were conducted to test the effect on fish life of a natural brine, pumped from certain oil wells in the Saginaw Valley, Michigan. The experiments were conducted by Mr. T. J. Powers and the Institute for Fisheries Research in the experimental aquarium of the Museum of Zoology, University of Michigan. A sample of the brine was brought to the Museum by Mr. Powers on February 26, 1935, and experiments were begun on this date. The following information was offered by Mr. Powers. The sample was obtained on January 23, 1935 from the Garfield-McCallum lease in Section 20, Porter Twp., Midland County. This is a typical "Dundee" brine, being pumped from oil-wells of depths averaging about 3620 feet. The natural salt concentration in this sample of brine is approximately the same as when pumped from the wells, and the brine is typical of the general Saginaw Valley district. The Garfield-McCallum lease is situated near the Pine River, a tributary of the Tittabawassee.

The chemical constituents (Mgs. per liter) of a sample of the brine were furnished by Mr. Powers as follows:

Specific gravity of brine	1.2189
Total solids	340,000
Calcium	41,800
Magnesium	8,212
Sodium and Potassium	80,100
Chloride	190,000
Free Carbon dioxide	none
HCO_3	88
CO_3	none
SO_4	90.5
Iodine	5.0
Bromine	1,700. ±

The fish, all Hyberhynchus notatus—the Bluntnose Minnow, were obtained on February 19 from an aquarium in the Science Building of the Michigan State Normal College by

courtesy of Professor T. L. Hankinson, and were kept in an aquarium in the Institute laboratory until February 26. These minnows, ranging in standard length from 28 to 49 millimeters, were mostly if not entirely, young of the previous summer. The minnows had been fed generously while in the Institute aquarium and were in a healthy condition for the initiation of the pollution experiments.

The experiments were begun on February 26 and concluded on March 12. The methods used were similar to those employed in previous pollution experiments conducted by Dr. Hubbs and other members of the Institute. Various dilutions of the brine were made up in two-quart jars. In each jar was put 500 cc. of water, taken from the general system of the aquarium, plus the amount of brine necessary to give the desired dilution. An aerated and a non-aerated medium were used for each dilution. Experiments were conducted at three different water temperatures (approximately 48, 72, and 84 degrees Fahrenheit). The 72° temperature was maintained by a constant room temperature; the 48° and 84° temperatures by submerging the jars in cold and warm water baths, respectively. The minnows used for experiments in temperatures of 48 and 72 degrees, were slowly acclimated (during a period of about 2 hours) to the temperature of the experiment in which they were used. The largest group of experiments were conducted at a water temperature of approximately 72 degrees, the same temperature as that of the large aquarium in which all of the minnows had been kept from February 19 to 26. Control experiments, both aerated and non-aerated, were conducted at each of the three temperatures. A single fish was used in each jar, and at the conclusion of the individual test, the minnows measured and discarded.

An analysis of a sample (taken on February 27) of the water in the general system of the aquarium revealed the following chemical constituents:

	Parts per million	Grains per gallon
N as NO_3	17.7	1.03
Solids, total	504	29.30
SiO_2 (Silica)	8	.46
Fe_2O_3	None	
Ca	23.5	1.37
Mg	7	.40
Na+K	160	9.35
Cl	13	.76
SO_4	70.8	4.13
HCO_3 (Bicarbonate)	352	20.50
CO_3 (Carbonate)	25	1.46
Hardness as $CaCO_3$	86	

Observations on the experiments were made as frequently as time would permit. The experiments, in the greater concentrations where the fish were expected to die within a few hours, were watched continuously. The experiments in the weaker concentrations could not be observed continuously; therefore the length of time which the fish lived in some of the experiments is known only within certain limits.

I. Experiments conducted at a water temperature of approximately 72° Fahrenheit (range of 68° - 76°).

Details concerning the methods used in these experiments and the results which were obtained are given in Table I. The results given in this table indicate that at this temperature, dilutions of one part of brine to thirty parts of water have a lethal reaction in less than fifteen hours; more concentrated dilutions of the brine are lethal in less than eight hours; and dilutions of 1:10 or stronger are lethal in 15 minutes or less. There was considerable individual variation in the reactions of the minnows to dilutions of 1:40 and to weaker concentrations; in general it appears that these concentrations had little effect on the minnows. Nine of the eleven specimens in these weaker dilutions, lived for three days or longer, and six of the eleven lived throughout the length of the experiment (over 11 days). It is quite probable that starvation was as potent a factor as the pollution in affecting the death of the specimens in these weaker concentrations—the minnows were not fed during the experiments and those which lived through the 11 day period, became markedly emaciated. The controls, which lived throughout the experiment, also showed the effects of starvation. It appears, therefore, that a dilution of one part of brine to 40 or possibly better to 50 or 60, parts of water is sufficient to protect these minnows from immediate lethal effects; or, in terms of chemical content, these fish appear to tolerate a chloride concentration of 4000 milligrams per liter and less.

In addition to the experiments conducted to test the lethal effect of certain dilutions, other experiments were carried on to determine the effect of short exposures to strong concentrations of the brine.

Table I. Methods and results of the pollution experiments conducted at a temperature of 72° Fahrenheit (range 68°-76°).

Aerated	No. of cc. of brine added to 500 cc. water	Dilution	Chlorides: Mgs. per liter	Standard length of fish in mm.	Reactions of fish time lapse before: First distress Hrs.:Min.	Critical condition Hrs.:Min.	Death Hrs.:Min.	Remarks
Yes	250	1:2	65,000	36	immediately	0:1	0:4	Stopped breathing in 1 1/2"
No	"	"	63,500	38	"	0:1	0:4	"
Yes	"	"	.....	29	"	0:1	0:2	...
No	"	"	.....	35	"	0:1	0:2	...
Yes	100	1:5	30,000	46	0:3	0:4	0:8	...
No	"	1:5	31,000	34	0:3	0:4	0:8	...
Yes	50	1:10	16,500	37	0:5	...	0:14	...
No	"	"	16,700	42	0:5	...	0:14	...
Yes	"	"	.....	37	0:10	...	0:15	...
No	"	"	.....	38	0:2	...	0:15	...
Yes	33.4	1:15	11,700	39	0:29	...	1:8	...
No	"	"	11,900	35	0:29	...	1:8	...
Yes	"	"	.....	40	0:29	...	1:6	...
No	"	"	.....	39	0:34	...	1:19	...
Yes	25	1:20	7,500	46	4:1	7:6	8:6	...
No	"	"	7,700	36	4:1	...	7:6	...
Yes	"	"	.....	38	1:18	2:33	3:33	...
No	"	"	.....	42	1:33	...	2:21	...
Yes	20	1:25	7,200	44	2:20	3:55	7:20	...
No	"	"	7,000	39	1:15	3:55	4:55	...
Yes	"	"	.....	34	1:37	4:12	4:27	...
No	"	"	.....	32	0:8	1:37	2:2	...
Yes	16.7	1:30	6,350	40	2:37	...	3:9	...
No	"	"	6,200	36	3:12	5:17	Between 5:32 and 15:2	...
Yes	"	"	.....	40	...	4:45	4:48	...
No	"	"	.....	40	4:46	...	8:21	...
Yes	"	"	.....	32	...	...	Between 1:30 and 3:30	...
Yes	12.5	1:40	4,100	38	...	...	Between 243:12 and 257:12	...
No	"	"	4,325×	32	...	...	2:32	Fish probably abnormal.
No[1]	"	"	....	35	...	...	333:6+	....
Yes	10	1:50	3,700	41	8:42	18:27	19:7	...
No[1]	"	"	3,700	39	...	...	331:27+	...
Yes	8.3	1:60	2,950	46	...	...	Between 72:47 and 74:33	...
No[1]	"	"	3,050	33	...	...	328:17+	...
Yes[1]	6.7	1:75	2,400	36	...	...	329:16+	...
No	"	"	2,450	39	...	...	Between 145:46 and 160:16	...
Yes[1]	5	1:100	2,010	28	...	...	328:18+	...
No[1]	"	"	1,800	33	...	...	328:18+	...
Yes[1]	0	Control (water)	12.0	32	...	...	331:27+	...
No[1]	"	"	12.0	30	...	...	331:27+	...

[1] These fish were found to be in a normal healthy condition at frequent observations throughout the duration of the experiments and at the time the experiments were terminated.

Two minnows (35 and 44 millimeters in length) put in 1:5 dilutions of the brine (aerated and non-aerated samples), were distressed after 1 minute; two minutes after their introduction into the brine they were transferred to fresh water in which they lived only 8 minutes. Two more minnows (32 and 36 mm. in length) were subjected to 1:5 dilutions of the brine for 1 minute, during which they showed no ill effect from the pollution, and were then transferred to jars of fresh water; after 329 hours and 32 minutes in the water (aerated and non-aerated), these fish were still in a normal, healthy condition and the experiment was terminated. Thus a 2-minute exposure to 1:5 dilutions of the brine resulted in fatality for the first two minnows, while a 1 minute exposure did not result in death for the latter two.

Two fish (29 and 32 mm. in length) were exposed to 1:10 dilutions for 3 minutes and then were transferred to water. The 29 mm. specimen in the aerated water died after being in the water for 6 hours and 25 minutes; the other minnow was normal after 32 hours and 48 minutes at which time the experiment was terminated.

An experiment was conducted to determine if these minnows, subjected to a weak concentration of the brine for a considerable period of time, could build up an appreciable degree of immunity to stronger concentrations. The procedure and results of this experiment are indicated by the following tabel:

Fish introduced into 1:100 dilutions	Addition of brine: 2 cc. at 2:30 P.M., II:27 — After lapse of	2 cc. at 2:30 P.M., II:27 — Resulting dilutions	2 cc. at 5:30 P.M., II:27 — After lapse of	2 cc. at 5:30 P.M., II:27 — Resulting dilutions	3 cc. at 8:40 A.M., II:28 — After lapse of	3 cc. at 8:40 A.M., II:28 — Resulting dilutions	4 cc. at 1:37 P.M., II:28 — After lapse of	4 cc. at 1:37 P.M., II:28 — Resulting dilutions	Time of death	Life after last addition
8:45 P.M. II:26 [1]	20 hrs. 45 min.	1:71	21 hrs. 45 min.	1:56	38 hrs. 55 min.	1:42	45 hrs. 52 min.	1:31	Between 12:01 and 8 (A.M.) III:1	Between 10 hrs., 24 min. and 18 hrs., 23 mi
7:25 [1] P.M. II:26	19 hrs. 5 min.	1:71	20 hrs. 5 min.	1:56	37 hrs. 15 min.	1:42	42 hrs. 12 min.	1:31	Between 6 and 7:45 (P.M.) III:1	Between 28 hrs., 23 min. and 30 hrs., 8 min.

[1] Chloride content of 8000 mgs. per liter. Fish 43 mm. in standard length. Sample aerated.

[2] Chloride content of 1825 mgs. per liter. Fish 46 mm. in standard length. Sample not aerated.

After a concentration of 1:31 was obtained, one fish lived between 10 and 18 hours, and the other between 28 and 30 hours. The specimens subjected to 1:30 dilutions (Table I) lived, on the average, considerably less than 10 hours. Although not too great an emphasis should be put on this simple experiment, it suggests that some degree of immunity can be obtained by the fish if first subjected to weak, non-lethal, dilutions.

II. Experiments conducted at a water temperature of approximately 84° Fahrenheit (range 79° to 85°)

The methods and results of these experiments are given in the following table:

Aerated	No. of cc. of brine added to 800 cc. water	Dilution	Standard length of fish in mm.	Reactions of fish time lapse before: First distress Hrs.:Min.	Critical condition Hrs.:Min.	Death Hrs.:Min.	Remarks
Yes	50	1:10	33	0:7	...	0:13	...
No	"	"	32	0:7	...	0:13	...
Yes	"	"	38	0:9	...	0:12	...
No	"	"	36	0.10	...	0:11	...
Yes	20	1:25	32	...	0:28	1:59	...
No	"	"	32	0.19	...	0:28	...
Yes	"	"	41	...	...	Between 1:42 and 3:42	...
No	"	"	36	3:42	...	5:17	...
Yes	10	1:50	39	18:12	...	20:37	...
No[1]	"	"	35	...	...	147:22+	...
Yes	"	"	38	...	...	23:8	...
Yes	0	Control (water)	43	...	...	42:20	...
No[1]	"	"	37	...	...	147:30+	...
Yes[1]	"	"	37	...	...	100:39+	...

1 These minnows were in a healthy, normal condition at frequent observations made during the experiments and at the termination of the experiments.

III. Experiments conducted at a constant water temperature of 48° Fahrenheit

The methods and results of these experiments are given in the following table:

Aerated	No. of cc. of brine added to 500 cc. water	Dilution	Standard length of fish in mm.	Reactions of fish time lapse before: First distress Hrs.:Min.	Critical condition Hrs.:Min.	Death Hrs.:Min.	Remarks
Yes	50	1:10	35	0:3	0:15	0:22	...
No	"	"	49	0:3	0:15	0:38	...
Yes	"	"	37	0:0	0:22	0:38	Immediate distress, then recovered.
No	"	"	40	0:0	0:22	0:42	"
Yes	20	1:25	39	1:40	3:20	Between 3:20 and 5:20	...
No	"	"	38	1:40	...	9:24	...
Yes	"	"	37	1:2	...	3:23	...
No	"	"	40	3:25	7:28	Between 7:28 and 10:28	...
Yes	10	1:50	35	...	17:53	20:58	...
No	"	"	35	20:18	41:53	56:43	...
Yes	"	"	38	27:17	...	43:2	...
Yes	0	Control (water)	38	...	...	34:0	...
No	"	"	37	20:25	...	21:55	...
Yes[1]	"	"	36	...	...	105:34+	...
No	"	"	34	0:39	...	...	Fish apparently abnormal, or method faulty
No	"	"	37	...	71:15	72:30	...

[1] This fish was found normal at frequent observations during the experiment and at the termination of the test.

There was a correlation between the length of time required for a given dilution to kill fish, and the temperature of the solution. The experiments with those dilutions (1:10, 1:25 and 1:50) which were used at the three temperatures showed that it required the greatest amount of time to kill fish at a temperature of 48°, and the least amount of time to kill them at a temperature of 84°. This effect of temperature on the killing capacity of the brine indicates that the liberation of the brine into natural waters would be less harmful in winter than in summer.

The minnows subjected to dilutions (especially 1:50, and 1:25) at a temperature of 48°, could have been expected justly to live longer than they actually did in these tests.

The high mortality among the controls at this temperature suggests that the change (within a few hours) in the water temperature from 72° to 48° was harmful to the minnows. The effect of this change in temperature probably supplemented and enhanced the lethal effect of the brine.

In general a longer period of time was required for the weaker concentrations of the brine to kill the minnows in the non-aerated samples than in the aerated ones. There seems to be two possible explanations for this difference:

(1) Due to the high specific gravity of the brine, it does not mix readily with water. To obtain a homogeneous mixture the samples were shaken; however, this shaking may not have been sufficient to achieve its purpose. If the samples were not mixed well, the minnows in non-aerated jars might have been able to avoid the regions of heavy concentration of the brine; in the aerated samples the constant bubbling of the air must have affected, in a very short period of time, a homogeneous mixture. If this was the condition, then the results obtained from the experiments with aerated samples are more significant.

(2) The constant agitation of the water by the air bubbling through it, or the greater oxygen supply in the aerated samples, might have affected in these minnows a greater activity and metabolism. A greater activity of the minnows in the aerated samples would have resulted in a more rapid absorption of the toxic salts and hence in more rapid death.

If these were the prevailing conditions, by which this difference in the length of time required for aerated and non-aerated samples to kill the fish is explainable, then the results obtained from the experiments with aerated samples would apply to swift, turbulent streams, and the results obtained with the non-aerated samples would be applicable to very sluggish streams. The opinion is here given that the more rapid succumbing of the minnows in aerated samples was due in part to each of the above causes: the exposure of the minnows in the aerated samples to homogeneous mixtures of the brine, and the greater activity of the fish in the aerated samples resulting from the constant agitation of the medium or the greater oxygen supply.

Both of the above factors must operate in natural waters. Brine emptied into a sluggish stream would flow or settle along the bottom, leaving the upper strata much more suitable to fish life than the lower; and in contrast, if emptied into a turbulent stream, the brine would mix rather thoroughly with water making all parts of the stream of equal potency in effect on fish life. Also the greater activity and metabolism of fish in turbulent water would result in a more rapid succumbing of the fish to the brine.

In the application of the results of these experiments to the disposal of the brine waste in natural waters, the difference between the effect of brine on fish in quiet waters and in turbulent waters should be subordinated to the difference in effect of the various dilutions. The disposal of the brine during the "high-water", winter season, when a weak dilution can be obtained in cold waters, is desirable even though the streams at this time are more turbulent and the fish more active.

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