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CHRONIC EFFECT OF LOW pH ON FATHEAD MINNOW SURVIVAL, GROWTH AND REPRODUCTION

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Abstract—Fathead minnows (*Pimephales promelas* Rafinesque) were continuously exposed to reduced pH levels of 4.5, 5.2, 5.9, 6.6 and 7.5 (control) during a 13-month, one-generation test. Survival was not affected, even at the lowest pH tested. Fish behavior was abnormal, and fish were deformed at pH 4.5 and 5.2. Egg production and egg hatchability were reduced at pH 5.9 and lower, and all eggs were abnormal. A pH of 6.6 was marginal for vital life functions, but safe for continuous exposure. Free carbon dioxide, liberated by the addition of sulfuric acid to reduce the pH, may have had an unknown effect. The fish did not become accliminated to low pH levels.

INTRODUCTION

THE PROBLEM of acid mine drainage in the eastern part of the United States and the Water Quality Act of 1965 requiring water quality standards for receiving waters have increased the need to determine acceptable pH extremes for desirable aquatic organisms. A recent report by the EUROPEAN INLAND FISHERIES ADVISORY COMMISSION (1969) summarized the literature regarding the effect of lowered pH on aquatic organisms and emphasizes the need for information on long-term effects on desirable aquatic life.

Data on the effects of acid waters on aquatic organisms are difficult to interpret because a reduction of pH produces changes in other water characteristics, especially free CO_2, alkalinity, and the solubility and form of metals. Field observations in acid waters caused by mining operations are confused by the presence of iron and aluminum, in particular. Likewise, acid waters caused by drainage from such areas as peat bogs also contain many soluble acidic organic compounds. Indeed, the effect of decreased pH alone cannot be measured; the effects of other factors can only be minimized.

The review cited above identifies many characteristics common to unpolluted water, but only the concentration of free CO_2 has a significant effect on lethal acid pH ranges. LLOYD and JORDAN (1964) found that 20 mg l^{-1} or more of free CO_2 increased the toxic effects of reduced pH (TL_{50} pH higher). Most lethal pH values from laboratory data reported in the literature are below 4.0. The report also states that growth of fish is known to be less in acid waters, but that no evidence is available to suggest that the effect is direct. Effects on the food supply, and perhaps the bacterial decomposition rate (involving nutrient availability), are implicated as significant factors in reduced growth rates.

The work described here was performed to measure the effect on reproduction and growth of fathead minnows (*Pimephales promelas* Rafinesque) of continued exposure

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to decreased pH during their entire life cycle. Fatheads are important forage fish and are good bioassay animals. Concentrations of CO_2 were above the level known to affect hydrogen-ion toxicity and may have contributed to the effects observed. The CO_2 was allowed to remain, however, since CO_2, as indicated by total acidity, is not rapidly lost from streams receiving acid mine drainage, but returns to normal slowly as reaeration occurs over several miles of the affected reach of the stream (U.S. Environmental Protection Agency, *unpublished data*). By leaving the free CO_2 in the water, extant conditions were more nearly simulated.

MATERIALS AND METHODS

The test procedure was similar to that described by MOUNT and STEPHAN (1969). A proportional diluter (MOUNT and BRUNGS, 1967) was used as a metering device, and each of four water-delivery tubes operated a chemical metering apparatus (MOUNT and WARNER, 1965) that added sufficient diluted sulfuric acid during each cycle of the diluter to produce the desired pH. Proportional dilution of the most acid concentration to produce lesser concentrations was physically impractical in this water. After acid addition, two mixing chambers were necessary to mix the acid and water adequately. Nominal pH values in the test chambers were 4.5, 5.2, 5.9 and 6.6.

Test water was prepared by mixing hard water from a spring emerging from limestone strata with carbon-filtered, de-ionized Cincinnati tap water (from Ohio River) to produce a nominal total hardness of 200 mg l^{-1}. The water was stored in a 5000-gal underground, polyester-lined tank that was filled weekly and aerated continuously. A secondary tank provided water while more was being mixed. Flow rate through each test chamber was increased as the fish grew and become more active; the turnover time in each chamber was decreased from approximately 10 to 5 h during the test and the tank size was 12 × 24 in., with a water depth of 6 in.

Photoperiod was maintained at 16 h for the first 7 months, but because spawning did not begin promptly in all chambers, the photoperiod was reduced to 12 h and increased 1 h each week for 4 weeks. (Subsequent work has demonstrated that a constant photoperiod is acceptable.) A 16-h photoperiod was maintained thereafter, and the light intensity was sufficient for moderate algal growth.

The exposure was begun in August 1966 with seventy 1-day-old fry, hatched from laboratory-spawned eggs, in each of five tanks. The pH was gradually and equally reduced in the four test tanks, and as the pH reached the nominal value in each tank, no further reduction was made. Ten days were required to reach pH 4.5. A tank of untreated test water served as the control (nominal pH 7.5). After 60 days each of the five test chambers was subdivided by a plastic screen, and 20 fish were selected at random from each treatment and randomly assigned to two groups of 10 and returned to the divided chamber. The test lasted for 13 months and was terminated on September 16, 1967.

Commercial trout food was fed daily, *ad lib.*, and live organisms in the water supply supplemented this diet.

As the fish neared maturity, three one-half sections of asbestos-cement tile, 4 in. in diameter and 4 in. long, were placed in a row in each duplicate chamber as spawning substrate for the fish. Eggs, spawned on the under surface, were removed and counted before 10 a.m. each day, and any number of eggs was considered a spawning if spawning activity was not apparent. Eggs were incubated in small screen-bottomed cups

(MOUNT, 1968), and hatchability was determined as the number of living fry from 50 eggs at 7 days. Two groups of 50 fry were placed in 6 × 6 in. compartments located in one corner of each tank and their growth measured for 30 days. Measurement was made to the nearest millimeter after aspirating each fish into a glass tube.

The test chamber with a nominal pH of 4.5 was monitored continuously on a circular chart recorder that also activated a relay interrupting the acid supply to that chamber at a pH of 4.2 and permitting the acid to flow again when the test chamber pH had reached 4.4. This protection was essential because a change in water hardness of 1–2 per cent, or an error in water delivery of 0.5 per cent, would cause the pH to fall to 4.1, a pH lethal to the fish, even after 10 months of exposure. The pH in the chambers maintained nominally at 5.2, 5.9 and 6.6 was recorded about three times each hour by switching a flow of water from the chambers to the electrode and recording the pH on another meter. The system employed siphons and needle valves so that no aeration occurred until the water had passed the electrode. The pH in the four test chambers and the control was measured each weekday also by means of grab samples and a different pH meter.

Since the TL_{50} to fatheads was close to 4.0 but not definitely known, two acute tests were performed in the same system after the chronic test to determine the lethal pH. Duplicate tests with 10 and 20 subadult fish, respectively, at three pH values were completed. The pH was recorded three times per hour during this test.

TABLE 1. CHEMICAL ANALYSES OF TEST WATER IN FOUR EXPERIMENTAL TANKS (NOMINAL pH 4.5, 5.2, 5.9 6.6) AND THE CONTROL, BASED ON WEEKLY GRAB SAMPLES

Item	Number of samples	Nominal pH 4.5	5.2	5.9	6.6	Control 7.5
Dissolved oxygen (mg l^{-1})	50	7.2 ± 1.1*	8.6 ± 1.4	7.9 ± 1.6	7.2 ± 1.3	7.1 ± 1.2
Alkalinity (mg l^{-1} as $CaCO_3$)	50	3.9 ± 0.6	10.7 ± 3.2	41.6 ± 9.1	102.0 ± 15.4	155.6 ± 4.4
Acidity (mg l^{-1} as $CaCO_3$)	50	74.7 ± 11.3	63.3 ± 10.4	52.6 ± 6.9	27.2 ± 4.5	10.4 ± 2.4

* Mean and standard deviation.

Dissolved oxygen, alkalinity, and acidity of the test water were determined weekly from grab samples (TABLE 1). Water hardness averaged 200.6 mg l^{-1} as $CaCO_3$, with a standard deviation of 2.9 mg l^{-1}, and the weekly mean temperature for the test was 22.3°C with a standard deviation of 1.04° and a range of 20°–24.5°. The quality of the pH control system as measured by continuous recording (pH 4.5) tests three times per hour (pH 5.2, 5.9, 6.6), and grab samples is shown in TABLE 2. Daily pH means were estimated ones, and the agreement was ±5 per cent. (Arithmetic means for pH,

TABLE 2. MEASURED pH VALUES IN THE TEST CHAMBERS (UPPER VALUE BASED ON CONTINUOUS RECORDING FOR pH 4.5 AND THREE TIMES PER HOUR FOR 5.2, 5.9 AND 6.6; LOWER VALUE BASED ON GRAB SAMPLES) (SEE TEXT CONCERNING USE OF ARITHMETIC MEANS)

	Nominal pH				
Item	4.5	5.2	5.9	6.6	Control 7.5
Number of measurements	358	356	354	356	—
	87	85	86	84	234
Mean	4.60	5.24	5.93	6.59	—
	4.66	5.23	5.95	6.65	7.58
Standard deviation	0.18	0.18	0.09	0.11	—
	0.22	0.17	0.12	0.12	0.13
Range	4.2–5.5	4.4–7.4	5.6–6.3	6.1–7.0	—
	4.2–5.7	4.7–5.5	5.7–6.2	6.3–6.9	7.2–8.0

although not normally used, were employed in this instance because they appeared to portray conditions accurately enough for the intended purpose.) Extreme values were easily observed on the charts. The pH values of 4.5 and 5.2 were very difficult to control because the water's buffering capacity was exhausted, and any change in rate of loss of CO_2 from the test chambers also altered the pH.

RESULTS

Survival of fathead minnows was affected little, if at all, by 13 months continuous exposure to lowered pH (TABLE 3). However, at pH 4.5, the lowest level tested, behavior of the young fish was abnormal for several months. When the pH approached 4.3 or 4.2, they showed stress (surface swimming and hyperactivity) even after 11 months acclimation to pH 4.5. The fish appeared somewhat deformed, the heads in particular seeming smaller than normal.

The fish at pH 5.2 were somewhat hyperactive, and the males especially had an abnormal "hunch-backed" appearance. Their heads were smaller than normal in proportion to their bodies. The females were heavy with eggs and were sexually mature based on gross examination, but did not spawn (TABLE 3).

At pH 5.9 the fish appeared nearly normal, but a few showed mild symptoms of the deformities common at lower pH values. The males were not as brightly colored as those in the control and seemed to be more affected than the females. The number of eggs per female was reduced, and all eggs were abnormal. They had fragile shells, lacked turgidity, and were difficult to remove from the spawning tile for hatchability tests.

No abnormal effects were observed at pH 6.6 except for a possible reduction in eggs produced, as compared to the control (TABLE 3). The onset of spawning was not affected at either pH 6.6 or 5.9, but hatchability was reduced at 5.9, apparently because of the fragility of the eggs. This fragility has ecological importance, however, because such eggs when left with the male were smashed and destroyed by his normal cleaning actions.

Two groups of 50 eggs from each of three different control spawnings were placed

Table 3. Survival, growth and reproduction of fathead minnows after chronic exposure (13 months) to low pH

Item	Nominal pH 4.5 A	4.5 B	5.2 A	5.2 B	5.9 A	5.9 B	6.6 A	6.6 B	Control 7.5 A	Control 7.5 B
Survival (%)	90	70	80	70	90	80	80	70	80	90
Number of males	5	4	2	2	2	2	3	2	3	2
Number of mature females	4	3	6	5	7	6	5	5	5	7*
Mean length at termination (mm)										
Male	70	70	79	80	77	78	68	64	69	72
Female	59	60	60	61	60	60	55	58	55	53
Spawnings/female	0	0	0	0	1.0	1.2	3	4	5.4	4.2
Hatchability (%)	—	—	—	—	55 (4)†	29 (3)	78 (7)	82 (10)	79 (11)	78 (15)
Total number of eggs produced	0	0	0	0	708	398	1061	1971	4837	2882
Number of eggs per female	—	—	—	—	101	66	210	394	968	480

* One was immature.

† Number of spawnings tested in parenthesis.

in the control and in pH 4.5 to test hatchability. For the three sets and average of 74 per cent hatched in the control (range (42–94 per cent) and 1 per cent (range 0–2 per cent) at pH 4.5. Most mortality occurred in the first 48 h of incubation. The low pH greatly delayed the hatching time of the few eggs that did survive until hatching, and the embryos were grossly deformed. Similar comparisons at pH 5.2 were made for 10 different egg masses; the control hatchability averaged 82 per cent (range 42–94 per cent) and that at pH 5·2 averaged 67 per cent (range 16–98 per cent). At pH 5·2 many deformities occurred, and the normal hatching period of 4–5 days was lengthened to 6–7 days.

Fry survival and growth were variable, thus reducing the value of this measurement of effect. The available data show no effect of pH on growth (TABLE 4); the lack of spawning at lower pH values prohibited growth measurements. The small number of spawnings, low hatchability, and egg fragility made it impossible to conduct duplicate tests of the growth of fry from eggs produced by fish at pH 5.9.

The TL_{50} values in the acute tests were 4.05 and 4.2. In one test all fish died in the chamber maintained between pH 3.6 and 3.8, while all survived in pH 4.5–4.6. In the other test 20 per cent survived pH 4.1–4.3 and none died at 4.5. Since exact lethal levels were not essential and pH control was very difficult, no further refined testing was done.

TABLE 4. GROWTH AND SURVIVAL OF TWO GROUPS OF FATHEAD MINNOW FRY PRODUCED AND GROWN AT VARIOUS HYDROGEN-ION CONCENTRATIONS FOR 30 DAYS

		Nominal pH				
Item	Group	4.5	5.2	5.9	6.6	Control 7.5
Survival (%)	1	—	—	62	92	46
	2	—	—	—	70	46
Final length (mm)						
Mean	1	—	—	10	10	13
	2	—	—	—	10	11
Range	1	—	—	7–14	7–14	11–19
	2	—	—	—	7–16	9–14

DISCUSSION

Clearly, a pH of 5.2 or less is unacceptable for this species and water type. The reduction in eggs per female and in hatchability at pH 5.9 indicates that this level is marginal, a view further supported by incompleted tests not included here. The lack of true duplicates in the exposure prohibits valid statistical comparisons.

The results of these tests support earlier published work (EUROPEAN INLAND FISHERIES ADVISORY COMMISSION, 1969) in refuting the commonly held belief that fishes become acclimated to low pH levels. Fish in this test did not acclimate even after 12 months exposure. When the pH approached 4.2 in the tank at nominal pH 4.5 as it occasionally did, the fish showed marked distress comparable to that displayed by control fish exposed to the same pH. The marked effect on spawning at nonlethal pH levels is important ecologically and could easily be overlooked in field studies because of recruitment from adjacent stream areas. The lack of growth effects noted by others in natural waters is consistent with the absence of growth effects found in this study. Furthermore, the lower productivity attributed in the literature to reduced food could be equally attributed to reduced egg production. The tolerance of several aquatic insects to low pH (Bell and Nebeker, 1969; Bell, 1970) suggests that decreased productivity is not likely to be related to reduced insect populations.

Water quality standards allowing pH values lower than 6 will not provide protection for this species. Observations of Ellis (1937) that 97 per cent of the locations having good fish populations had pH values between 6.7 and 8.6 are consistent with these laboratory data. The evidence collectively supports the need to maintain pH at or above 6.5. Even though sustained fish populations may exist at lower pH levels, production will most likely be reduced.

The reproductive impairment noted at pH levels at least two units higher than lethal pH levels, and yet the absence of growth effects, illustrates the need for additional chronic (full life cycle) tests with other important species to develop data for establishing water quality standards.

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