PRELIMINARY STUDIES ON THE TOLERANCE OF AQUATIC INSECTS TO LOW PH[1]

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ABSTRACT

The mature larvae of 10 species of aquatic insects (*Boyeria vinosa* Say, *Ophiogomphus rupinsulensis* Walsh, *Pteronarcys dorsata* (Say), *Taeniopetryx maura* (Pictet), *Acroneuria lycorias* (Newman), *Isogenus frontalis* Newman, *Ephemerella subvaria* McDunnough, *Stenonema rubrum* (McDunnough), *Brachycentrus americanus* (Banks), and *Hydropsyche betteni* Ross) were tested in the laboratory to determine their relative tolerance to low pH. The pH at which 50% died after 96 hours ($TL_m{}^{96}$) was recorded as the lethal pH. These values ranged from pH 4.65 for mayflies to pH 1.50 for caddisflies.

In recent years, attention has focused on the water pollution problem caused by acid mine drainage. The problem is not new, being present in the United States since colonial times and the development of the first coal mines. The seriousness of the problem is increasing because of the ever-growing number of mines, operational and abandoned, in the coal mining regions of the United States. Western Europe's coal mining regions are plagued by the problem, and in South Africa acid waters are widespread.

In the United States, primary attention has focused on abatement of the acid waters. An investigation of the literature showed that few laboratory studies have dealt with the effects of low pH on the biota of a stream, particularly the bottom fauna. One of those was by Stickney (1922), who conducted a series of laboratory experiments on the relation of a species of dragonfly to acid and temperature. Some valuable field studies have been conducted. Warner (1968) has done some field work on the effects of acid mine drainage on the bottom fauna of streams in West Virginia and Pennsylvania. Parsons (1956) conducted an ecological survey of a central Missouri stream polluted by acid mine drainage. Jewell (1922) studied the fauna of Big Muddy River in the coal fields of southern Illinois. Cowles and Schwitalla (1923) investigated the hydrogen-ion concentration of a creek, its waterfall, swamp and ponds. Shoup (1948) listed general ecological conditions of two acid streams in Virginia, and Bick et al. (1953) conducted an ecological reconnaissance of a naturally acid stream in southern Louisiana.

This paper summarizes the results of acute, short-term 96-hour tests ($TL_m{}^{96}$) used in screening 10 aquatic insect species to determine

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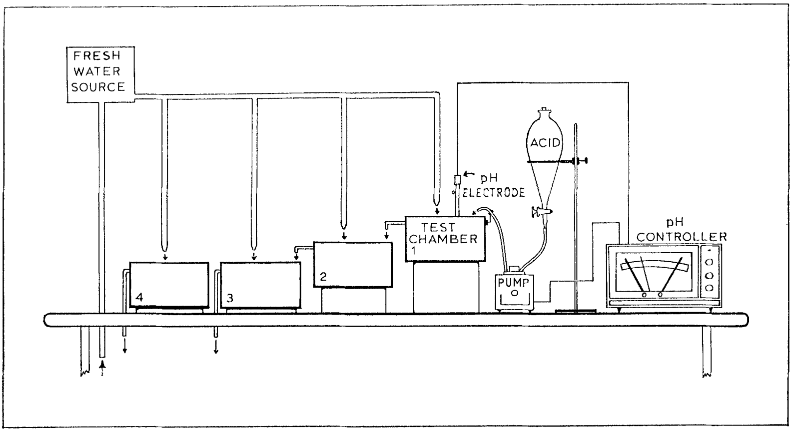

FIG. 1. Diagram of test chambers and pH regulation system.

their relative tolerance to low pH. The 96-hour TL_m (Standard Methods, 1960) was used as the measure of effect in these tests. This work will lay a foundation for further long-term studies dealing with the effects of low pH on factors such as molting, adult emergence, reproduction, and long-term survival. The test species include the dragonflies *Boyeria vinosa* Say and *Ophiogomphus rupinsulensis* Walsh; the stoneflies *Pteronarcys dorsata* (Say), *Taeniopteryx maura* (Pictet), *Acroneuria lycorias* (Newman), and *Isogenus frontalis* Newman; the mayflies *Ephemerella subvaria* McDunnough and *Stenonema rubrum* (McDunnough); and the caddisflies *Brachycentrus americanus* (Banks) and *Hydropsyche betteni* Ross.

METHODS AND MATERIALS

All tests were conducted in oval stainless steel tanks measuring 24 inches (60.5 cm) long, 9 inches (23.0 cm) wide, and 4 inches (10.0 cm) deep, similar to those used by Nebeker and Lemke (1968). The oval tanks were used as artificial streams where various water flows could be maintained. Concentrated hydrochloric acid was used throughout the study. The pH was controlled by a model 10C Corning (Corning Glass Works) pH meter.[2] This instrument is designed to control pH by use of two movable contacts on the dial of the pH meter which are electrically connected to a centrifugal pump containing a rubber tube through which the stock solution flows. The output of the meter is a load relay whose contacts are actuated whenever the meter reading and

[2] Mention of commercial products does not constitute endorsement by the Federal Water Pollution Control Administration of the United States Department of the Interior.

the set point value coincide. The contacts are preset at a given pH and the stock solution flows into the test tank through the pump until the meter reads the preset pH. At this point contact is broken and the pump stops, preventing further inflow of the stock solution until the pH changes. This instrument maintained a pH of ± .05 pH unit over the test period.

Four oval test tanks were utilized. The tanks were graded in elevation (Fig. 1) and, by utilizing gravity flow, water from the highest tank would flow through the drain into the second tank, and from the second tank into the third tank. The fourth tank was used as a control tank. The pH electrode was submerged in the first tank to control the pH of that tank and partly control the pH in tanks 2 and 3.

A constant, controlled amount of water was allowed to flow into tanks 2 and 3 to dilute the flow of acidic water from tanks 1 and 2, thereby raising the pH to test values in those tanks (e.g., tank 1, pH 2.5; tank 2, pH 3.0; tank 3, pH 3.5). The volume of water increased from tanks 1 to 3, but velocity of flow was maintained at a constant rate. An overflow drain was located in tanks 3 and 4 to maintain the water level and discharge displaced water. The water used for all testing was obtained from Lake Superior via Duluth city water lines. Carbon filters dechlorinated the water just prior to use. Water chemistries throughout the testing period were quite stable. Dissolved oxygen was maintained at a level of 90 to 100% saturation, and water temperatures ranged from 11° to 13° C. Table 1 lists the acidity and alkalinity at the various pH values used in the tests.

The test organisms (Table 2) were collected from local trout streams near the National Water Quality Laboratory, Duluth, Minnesota; all were mature larvae and nymphs. They were placed in acclimation tanks for one week at 11° C and pH 7.8 ± .1 to ensure that healthy specimens could be chosen from the material collected. Natural

TABLE 1. Acidity and alkalinity of water at various pH values (expressed in ppm) used to test pH tolerance of aquatic insects.

pH	Acidity	Alkalinity
7.0	3.0	26.0
6.5	13.0	25.0
6.0	9.0	12.0
5.5	18.0	5.0
5.0	15.0	2.0
4.5	27.0	0.0
4.0	38.0	0.0
3.5	33.0	0.0
3.0	63.0	0.0
2.5	111.0	0.0
2.0	400.0	0.0
1.5	692.0	0.0
Control (pH 7.8)	3.0	42.0

TABLE 2. pH values at which 50% of the test species died after 96 hours' exposure (TL_m^{96}) in pH-tolerance tests of aquatic insects.

Species tested	Individual test TL_m^{96} values	Mean TL_m^{96}
Brachycentrus americanus	1.80	1.5
(caddisfly)	1.50	
	1.21	
Hydropsyche betteni	3.10	3.15
(caddisfly)	3.00	
	3.35	
Taeniopteryx maura	3 07	3.25
(stonefly)	3.20	
	3.48	
Boyeria vinosa	3.35	3.25
(dragonfly)	3.18	
	3.22	
Acroneuria lycorias	3.74	3.32
(stonefly)	3.32	
	2.90	
Stenonema rubrum	3.15	3.32
(mayfly)	3.41	
	3.40	
Ophiogomphus rupinsulensis	3.31	3.5
(dragonfly)	3.48	
	3.71	
Isogenus frontalis	4.04	3.68
(stonefly)	3.20	
	3.80	
Pteronarcys dorsata	4.12	4.25
(stonefly)	4.73	
	3.90	
Ephemerella subvaria	4.55	4.65
(mayfly)	5.05	
	4.35	

substrate and a flow were maintained in the holding streams. Nebeker and Lemke (1968) have shown that the aquatic insects can live for several weeks without feeding, so test organisms were not fed during the four-day test. Survivors of the tests were held for several weeks after the tests and little mortality occurred. Ten specimens were used for each test.

If any deaths occurred in either of the duplicated controls, the entire test was discarded. If the test pH deviated by more than 0.25 pH units from the desired pH, the test was also terminated. Normal deviation did not exceed ± .05 pH unit. To monitor the test water continuously and keep a permanent record, a strip chart recorder was coupled to the pH meter.

The pH values at which 50% of the organisms died were obtained by using a modification of the straight line graphical interpolation method as outlined in Standard Methods (1960).

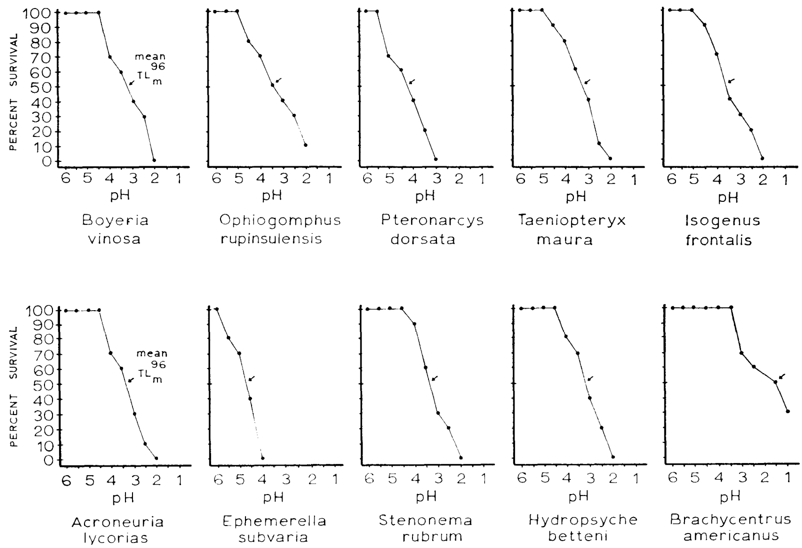

FIG. 2. Percent survival of 10 species of aquatic insects after 96 hours' exposure to low pH.

To find the approximate lethal pH, testing began with an initial survey series of pH values ranging from 7.0 to 1.0. Since only three pH values were available during each 96-hour test, three consecutive tests were necessary to obtain the final TL_m value. Final pH tests were triplicated to be statistically comparable, and their mean was plotted as the final TL_m value for each species tested (Table 2). Tests were statistically compared by the group comparison or 't' test and the differences between the samples were not found to be significant for the conditions of the test.

RESULTS

Mature larvae and nymphs of 10 species of aquatic insects tested to determine their relative tolerance to low pH showed a decided difference in tolerance. In general, all test species were fairly tolerant. The caddisfly *Brachycentrus americanus*, an extremely abundant insect in certain areas, proved to be the most tolerant with a 96-hour TL_m of 1.5. Another caddisfly, *Hydropsyche betteni*, a widely distributed free-living form, was found to tolerate a pH of 3.15. The winter stonefly *Taeniopteryx maura* and the dragonfly *Boyeria vinosa* exhibited a 96-hour TL_m of 3.25. The larger, widely distributed stonefly *Acroneuria lycorias* had a 96-hour TL_m value of 3.32. The mayfly *Stenonema rubrum* proved to be moderately tolerant with a 96-hour TL_m of 3.32 also. Somewhat less tolerant was the dragonfly *Ophiogomphus rupinsulensis*, commonly found in the slow, quiet reaches of a stream, with a 96-hour TL_m of 3.50. The stonefly *Isogenus frontalis* was moderately tolerant, with 50% dying at pH 3.68. The largest North American stonefly species, *Pteronarcys dorsata*, had a 96-hour TL_m value of 4.25. The least tolerant of the species tested was the small, spring-emerging mayfly *Ephemerella subvaria,* which exhibited a 96-hour TL_m of 4.65.

DISCUSSION

In general, aquatic insects are tolerant of acid conditions, at least for periods of less than one week; future work should deal with long-term effects on molting, growth, and reproduction, as well as survival for specified periods of time.

In our studies we found that *Boyeria vinosa* could not tolerate pH values lower than 3.25 for even short periods of time. Field data of Warner (1968) and Bick et al. (1953), who collected this organism from acid streams with a pH value of around 4.0, would indicate that it is tolerant of these low pH values at least for short periods. Another dragonfly, *Ophiogomphus rupinsulensis,* which we found to be only moderately tolerant, has not been reported from areas where acid pollution is a problem. Like *Boyeria vinosa* it could probably tolerate low pH values if they were of short duration. Apparently the dragonflies were some of the most numerous organisms in the acid stream investigated by Bick et al. (1953). The pH in that stream ranged from 4.0 to 6.8. The stoneflies, in general, can tolerate low pH values of short duration. They have been collected from minimum pH values of 3.1 (Warner, 1968) which lasted just a few hours. In our studies, stoneflies were only moderately tolerant. Long-term exposure at pH values of 3.5 would completely eliminate them. Mayfly survival would depend on the genera found in the stream. The species *E. subvaria* which we used was comparatively sensitive, as were other species observed by other workers. Most species in the genus *Ephemerella* would probably be eliminated by pH values near 4.5, while some species of the genus *Stenonema* probably could tolerate pH values of around 2.0 for short periods. Bick et al. (1953) listed the genera *Stenonema, Baetis, Blasturus, Callibaetis,* and *Paraleptophlebia* as being present in pH values of 4.0 to 5.0. Berner (1950) found that nymphs of *Stenonema smithae* are not greatly affected by low pH. These values are apparently of fairly short duration. Caddisflies generally are a very tolerant group. Free-living forms such as *Hydropsyche betteni* have been collected from streams with pH values of 4.0 to 5.7. Such case-building caddisflies as *Brachycentrus americanus* are very tolerant to low pH, as shown by the value of 1.5 obtained during this study.

In all of the bioassays conducted, the test organisms died at pH values below those normally found in the field. Conditions in the field differ from those in the laboratory and ecological factors exist in the streams which may alter the results, but the values given do constitute a valid comparison between the relative toxicity of the low pH tested and the tolerance of the test organism.

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ANTHONOMUS ROBINSONI BLATCHLEY, A SYNONYM OF AMALUS HAEMORRHOUS (HERBST) (Coleoptera: Curculionidae)[1]

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During recent examination of types of the Blatchley species of *Anthonomus*, it was found that *Anthonomus robinsoni* Blatchley, 1916, is a junior synonym of *Amalus haemorrhous* (Herbst, 1795) (NEW SYNONYMY).

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