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EFFECT OF LOW pH ON THE SURVIVAL AND EMERGENCE OF AQUATIC INSECTS

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Abstract—Mature larvae and nymphs of 9 species of aquatic insects (dragonflies, stoneflies, caddisflies, and mayfly) were tested in the laboratory at pH values from 1.0 to 7.0. The TL_{50} values (pH at which 50 per cent of the organisms died) at 30 days ranged from pH 2.45 (*Brachycentrus americanus*) to pH 5.38 (*Ephemerella subvaria*). The range at which 50 per cent of the insects emerged was pH 4.0–5.9. The 9 species tested were all more sensitive to low pH during the period of emergence.

INTRODUCTION

THE NEED for criteria in establishing water-quality standards is critical to the conservation of aquatic life. Long-term toxicity tests are a part of determining these criteria because they indicate relative species sensitivity and lethal concentrations over long periods of time. This information can then be used to help establish the requirements for the well-being of aquatic life.

Recent information on pH toxicity to fish was summarized by the EUROPEAN INLAND FISHERIES ADVISORY COMMISSION (1969), but information on pH toxicity to aquatic invertebrates is very limited. BELL and NEBEKER (1969) established $TL_m{}^{96}$ values for 10 species of aquatic insects, and STICKNEY (1922) studied the relation of a species of dragonfly to acid and temperature. Most studies of the effects of pH on aquatic invertebrates have been ecological field studies. PARSONS (1956, 1968) conducted surveys of a central Missouri stream polluted by acid mine drainage. HARP and CAMPBELL (1967) studied the distribution of a midge species in mineral acid waters. JEWELL (1922) studied the fauna of Big Muddy River in the coal fields of southern Illinois. Two programs carried out in Pennsylvania report in detail the biological and chemical conditions of several streams polluted by acid mine drainage (PENNSYLVANIA DEPARTMENT OF HEALTH, 1967; DINSMORE, 1968).

This paper summarizes the results of chronic (30-day) pH tests used to determine the long-term tolerance and effects on emergence of nine species of aquatic insects. The test species were 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 mayfly *Ephemerella subvaria* (McDunnough); and the caddisflies *Brachycentrus americanus* (Banks) and *Hydropsyche betteni* (Ross). These species were used because of their widespread distribution, their value as fish food, and the ease with which they could be obtained in sufficient numbers.

MATERIALS AND METHODS

A modification of the proportional diluter of MOUNT and BRUNGS (1967) was utilized in these experiments. The modifications included the use of five dipping birds, instead of one, to deliver the toxicant and the use of an electrically operated solenoid valve to start or stop the water flow into the top row of water cells.

All tests were conducted in ellipsoid stainless steel tanks 60.5 cm long, 23.0 cm wide and 10.0 cm deep, similar to those used by BELL and NEBEKER (1969). They were used as, artificial streams in which various water flows could be maintained. Twelve tanks were used for testing: 10 were connected by glass tubing to the diluter, where the flow from each of the five mixing chambers was split to provide duplicate tanks for each pH value, and two served as control tanks, one for each of the five experimental tanks. All tanks received water from a 40 l. stainless steel reservoir located above the diluter.

A stainless steel heat-exchanger was fitted into the water reservoir to provide a constant water temperature. Hot water (140°F) from the laboratory boilers flowed through the heat-exchanger and maintained the test water at 18.5°C ± 0.1. Temperature was controlled by using a solenoid valve in conjunction with an immersion thermoregulator unit in the water reservoir preset at 19.0°C. Water temperatures decreased approximately 0.5°C from the reservoir to the test tanks. To prevent sudden surges of hot water through the heat-exchanger a needle valve was placed in line just ahead of the solenoid valve and adjusted so that water flowed at a moderate velocity through the heat-exchanger unit.

The water used for testing, obtained from Lake Superior via Duluth city water lines, was dechlorinated with carbon filters just prior to use. The chemical make-up of the test water, as determined by standard methods (AMERICAN PUBLIC HEALTH ASSOCIATION, 1965) was: total available chlorine, 0.001–0.003 ppm, pH 7.58–7.81, total alkalinity 39.5–40.0 ppm, total hardness 44.0–45.0 ppm, calcium hardness 35.0–37.0 ppm, chlorides 2.6 ppm, nitrate nitrogen 0.28–0.45 ppm, total phosphates 0.005–0.006 ppm, and total acidity 0.7–1.5 ppm. Water-chemistry data for the experimental conditions are shown in TABLE 1. Since oxygen levels of the test water were at or near saturation, the test water was not aerated.

TABLE 1. MEAN PHYSICAL AND CHEMICAL DATA OF WATER USED IN EXPERIMENTAL AND CONTROL TANKS

pH	Temperature (°C)	Dissolved oxygen ($mg\ l^{-1}$)	Free CO_2 ($mg\ l^{-1}$)	Total acidity ($CO_2 + H_2SO_4$) ($mg\ l^{-1}$)	Total alkalinity ($mg\ l^{-1}$)	Total hardness ($mg\ l^{-1}$)
1.0	18.5	8.47	21.9	647.0	0.0	44.0
2.0	18.5	8.72	19.7	393.0	0.0	44.0
3.0	18.5	7.95	16.7	59.0	0.0	44.0
4.0	18.5	8.36	13.0	42.0	0.0	44.0
5.0	18.5	8.93	7.9	15.0	2.0	44.0
6.0	18.5	8.26	3.7	7.6	12.0	44.0
7.0	18.5	8.70	2.2	2.1	26.0	44.0
7.8 (Control)	18.5	9.01	1.5	1.5	42.0	44.0

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 1 week at 11–14°C and pH 7.8 ± 0.1 to insure that healthy specimens could be chosen from the material collected. Natural substrate and a flow of 0.2 ft s^{-1} were maintained in the holding streams. All organisms were fed biweekly during the tests. The carnivorous species were fed

TABLE 2. THE pH VALUES AT WHICH 50 PER CENT OF THE TEST SPECIES DIED AFTER 30 DAYS' EXPOSURE (30-DAY TL_{50}) AND 50 PER CENT EMERGED SUCCESSFULLY

Species tested	30-day TL_{50}	50 per cent successful emergence
Brachycentrus americanus (caddisfly)	2.45	4.0
Hydropsyche betteni (caddisfly)	3.38	4.7
Taeniopteryx maura (stonefly)	3.71	5.0
Acroneuria lycorias (stonefly)	3.85	5.0
Ophiogomphus rupinsulensis (dragonfly)	4.30	5.2
Boyeria vinosa (dragonfly)	4.42	5.2
Isogenus frontalis (stonefly)	4.50	6.6
Pteronarcys dorsata (stonefly)	5.00	5.8
Ephemerella subvaria (mayfly)	5.38	5.9

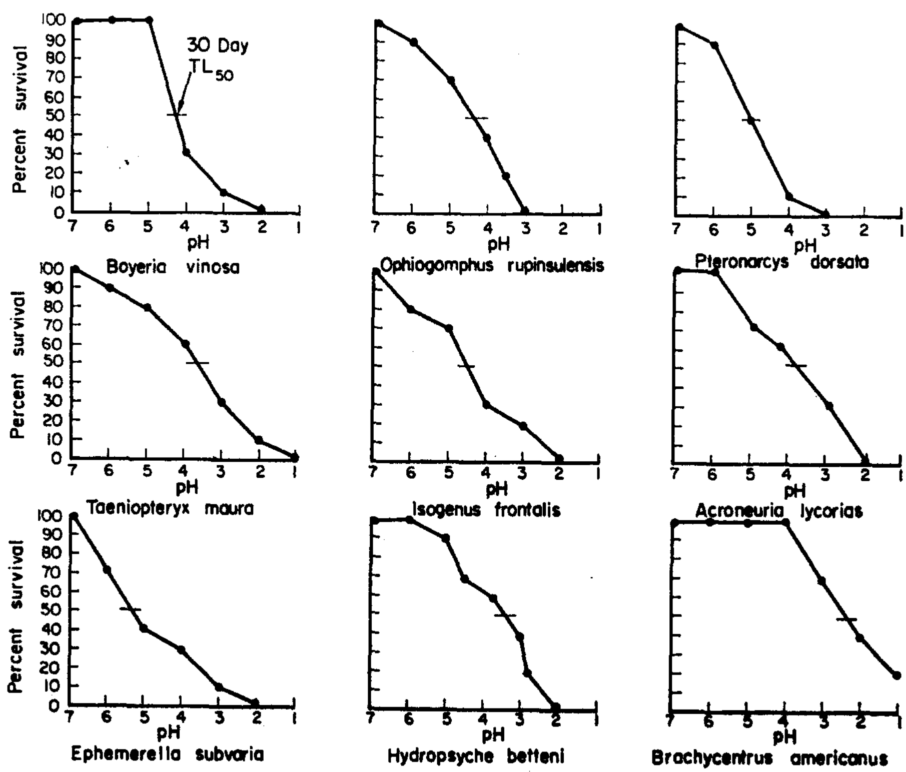

FIG. 1. Percentage survival of 9 species of aquatic insects.

larvae of the caddisfly *Hydropsyche betteni;* the herbivores were fed aspen leaves (*Populus tremuloides*) or a nettle powder broth.

Twenty specimens (10 to each duplicate test tank) of each species were used for testing at each pH value. All nine species were tested simultaneously, and each species was isolated in a cylindrical stainless steel screen wire cage (20-mesh 0.009 wire, 1.04-mm opening) which was 60 mm in diameter and 150 mm high (FIG. 1). This arrangement not only permitted simultaneous testing, but also provided additional substrate and more convenient observation of the test specimens.

Concentrated sulfuric acid was used as the toxicant. To obtain proper pH levels in the test chambers the acid was diluted with distilled water to various concentrations in the stock bottles. Approximately 4–6 ml H_2SO_4 entered the system when the diluter cycled. During the 30-day testing period experimental tanks were maintained at pH 1.0–7.0, to an accuracy of $\pm$ 0.2 pH units, with control tanks at pH 7.8. A Model 12 Corning pH meter* was used to measure pH values daily; accuracy of the electrode is rated at $\pm$ 0.002 pH units. If the test pH deviated by more than 0.4 pH units from the desired level, the test was terminated. If any deaths occurred in either of the duplicated controls, the entire test was terminated. The pH value at which 50 per cent of the organisms died (TL_{50}) was obtained by using a modification of the straight-line graphical interpolation method (AMERICAN PUBLIC HEALTH ASSOCIATION, 1965). Tests were compared statistically by the group comparison or '*t*' test. The differences between the duplicated tests were not found to be significant for the conditions of the test.

RESULTS

In general, all test species were fairly tolerant of low pH during the 30-day tests (TABLE 2, FIG. 1). The most tolerant species was *Brachycentrus americanus*, a widespread and important fish-food organism, with a 30-day TL_{50} of pH 2.45. The widely distributed free-living caddisfly *Hydropsyche betteni* tolerated a pH of 3.38. The stoneflies *Taeniopteryx maura* and *Acroneuria lycorias* had closely associated TL_{50} values of 3.71 and 3.85, respectively. The dragonfly *Ophiogomphus rupinsulensis*, commonly found in the slow, quiet reaches of a stream, was somewhat less tolerant with a TL_{50} of 4.30. Another dragonfly *Boyeria vinosa* was found to tolerate a pH of 4.42 for 30 days. The stonefly *Isogenus frontalis* was moderately tolerant, with 50 per cent dying at pH 4.50. The largest North American stonefly species, *Pteronarcys dorsata*, had a 30-day TL_{50} of 5.00. It typically inhabits larger streams where widely fluctuating pH values are unknown. The least tolerant of the species tested was the small, spring-emerging mayfly *Ephemerella subvaria*, which exhibited a 30-day TL_{50} of 5.38.

Broadly speaking, as the pH of the water decreases, the percentage of aquatic insects which emerge successfully also decreases (FIG. 2). If an arbitrary 50 per cent successful emergence value is used as a criterion, six of the nine species fall between pH 5.0 and 5.9. These include *Boyeria vinosa*, pH 5.2; *Ophiogomphus rupinsulensis*, pH 5.2; *Pteronarcys dorsata*, pH 5.8; *Taeniopteryx maura*, pH 5.0; *Acroneuria lycorias*, pH 5.0; and *Ephemerella subvaria*, pH 5.9. With the exception of *E. subvaria*, these species are moderately tolerant with regard to their 30-day TL_{50} values. At the ex-

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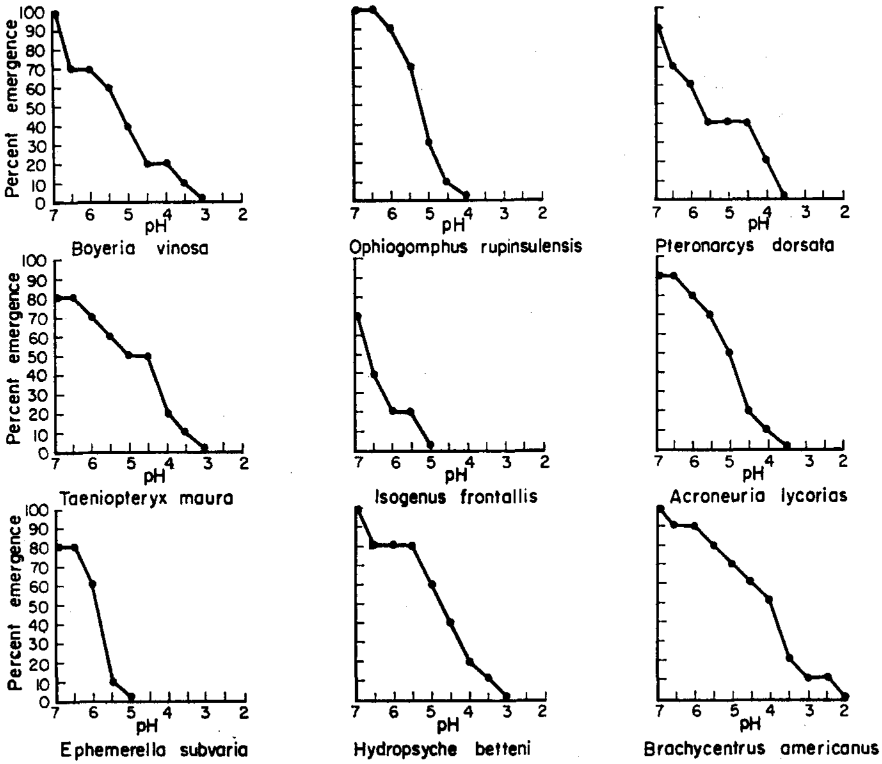

FIG. 2. Effects of low pH on emergence of 9 species of aquatic insects.

tremes of the pH values tested fall *Isogenus frontalis* with a 50 per cent successful emergence at pH 6.6, *Hydropsyche betteni* at pH 4.7, and *Brachycentrus americanus* at pH 4.0. The caddisflies *H. betteni* and *B. americanus* were the most tolerant of low pH values of the nine species tested.

To consider the H^+ ion as the limiting factor in this study, it was necessary to eliminate other factors which may also be limiting. Carbon dioxide, for example, has been found to be toxic to *Corethra* sp. and *Tendipes* sp. larvae in concentrations of 800–900 mg l^{-1} (EGGLETON, 1931). Tendipedid larvae have been collected from waters with as much as 61 mg l^{-1} CO_2, where they live with no apparent deleterious effects (CURRY, 1965). In this study maximum CO_2 concentration was 21.9 mg l^{-1}, an amount believed to be well below harmful levels. Sulfates are another possible limiting factor. Since sulfate concentrations of 1200–2000 mg l^{-1} are not harmful after 30 days to certain stoneflies (Plecoptera), dragonflies (Odonata), caddisflies (Trichoptera), and mayflies (Ephemeroptera) (BELL, unpublished data), the maximum sulfate concentration in this study of 523 mg l^{-1} is considered well below the limiting range.

DISCUSSION

The data obtained during this study shows that aquatic insects differ markedly in pH tolerance. In general, the caddisflies are very tolerant of low pH, the stoneflies and dragonflies are moderately tolerant, and the mayflies are fairly sensitive. With a 30-day

TL_{50} value as high as pH 5.38, it is apparent that mayflies will be somewhat limited in numbers and species composition under prolonged acid conditions.

Several excellent field studies dealing with the effects of acid waters on stream biota are useful for comparison of laboratory and field data (PENNSLVANIA DEPARTMENT OF HEALTH, 1967; DINSMORE, 1968; PARSONS, 1968). These studies describe in detail the chemical and biological conditions over a 4–27 month period. In Pennsylvania the mayfly *Ephemerella* sp. was not collected from waters with a pH lower than 5.5 (DINSMORE, 1968); in the present study a 30-day TL_{50} of pH 5.38 was established for the mayfly *E. subvaria.* Mayflies subjected to pH values below 5.3–5.5 for extended periods of time would be under continual stress and would probably not be able to survive and reproduce. In Cedar Creek in central Missouri the dragonfly *Ophiogomphus* sp. was present in a "normal" pH range of 4.2, but was completely eliminated when the pH dropped to 3.4 during an acid flow and apparently did not reappear afterward (PARSONS, 1968). In this study a 30-day TL_{50} of 4.3 was established for *Ophiogomphus rupinsulensis*. PARSONS (1968) found that during an acid flow nearly all the bottom forms were completely eradicated, except for a few dipterans and neuropterans, and in many instances they did not reappear after the acid flow had ceased. Elimination by low pH or lack of occurrence below a given pH was also true for all the test species in this investigation.

Under low pH conditions the emergence of aquatic insects appears to be the most critical stage of the life cycle. The pH at which 50 per cent successful emergence takes place ranges from 0.52 to 2.10 pH units higher than the 30-day TL_{50} value among the nine test species.

In terms of water-quality criteria, safe pH levels for aquatic insects will vary from family to family, but the value will need to be one that will allow the greatest possible percentage of successful emergence. Based on the data presented here, a value of pH 5.5 or higher will permit at least a 50 per cent successful emergence. With a few exceptions the aquatic insects are more tolerant of acid conditions than fish and if the recommendation of the EUROPEAN INLAND FISHERY ADVISORY COMMISSION (1969) of a pH range from 6.5 to 9.0 for fish be accepted, the survival and emergence of aquatic insects will be assured.

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