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ABSTRACT

Safe concentrations of dissolved oxygen for survival and adult emergence of larvae of nine species of aquatic insects, including mayflies, stoneflies, caddisflies, and midges, ranged from 0.6 mg/liter for the midge *Tanytarsus dissimilis* to slightly less than saturation for the emergence of *Ephemera simulans* (18.5 C). All species tested were less tolerant of low oxygen concentrations for 30 days than for 96 hours (90% of *E. simulans* survived 4 mg/liter for 96 hours, but no adults emerged successfully). Long-term studies are essential for the accurate assessment of oxygen stress in aquatic insects.

INTRODUCTION

Data that led to the establishment of state and federal water-quality standards for dissolved oxygen were obtained largely from field and laboratory observations of fish species (Doudoroff and Shumway, 1970). Information is also available to indicate that other aquatic organisms, especially aquatic insects, generally exhibit responses to low oxygen concentrations similar to those of fish (Tarzwell, 1965). Cold-water mayflies and stoneflies cannot tolerate dissolved oxygen concentrations much below 5 mg/liter (Gaufin, 1971). Other insects such as mosquitoes, which are not dependent on the dissolved oxygen content of the water, can live where the oxygen is near zero at all times.

Ambühl (1959) and Phillipson (1954, 1956) showed that certain insects can do well at oxygen concentrations below 5 mg/liter, but that oxygen content of water cannot be used as an absolute value without taking other things into consideration, such as temperature or water velocity. Britt (1955) found that dissolved oxygen concentrations as low as 0.7 mg/liter in Lake Erie resulted in massive kills of the mayfly *Hexagenia limbata*. Curry (1965) in his summary of environmental requirements for the midges indicates that many midge species can tolerate oxygen concentrations down to 1.0 mg/liter.

Most species can tolerate short periods of low oxygen stress that occur in streams, but little information is available dealing with long-term effects of chronic low oxygen concentrations on survival, growth, and adult emergence. Insects that do not use atmospheric air directly, such as mayflies, stoneflies, and caddisflies, and that respire with gills or direct cuticular exchange are subject to the same problems as fish. Few studies have been conducted to establish the actual long-term requirements of these important aquatic animals.

This study was designed to establish safe levels of dissolved oxygen for common species of aquatic insects known to be important as fish-food organisms. Ninety-six-hour tests, 30-day survival tests, and long-term tests (1–9 months) to determine effects of low oxygen on adult emergence were conducted.

MATERIALS AND METHODS

The aquatic insects tested during this study were larvae of the stoneflies *Pteronarcys dorsata* (Say) and *Acroneuria lycorias* (Newman); the mayflies *Hexagenia limbata* (Serville), *Ephemera simulans* Walker, *Ephemerella subvaria* McDunnough, *Leptophlebia nebulosa* (Walker), and *Baetisca laurentina*; the caddisfly *Hydropsyche betteni* Ross; and the midge *Tanytarsus dissimilis* Joh. Twenty animals were tested at each dissolved oxygen concentration, and all tests were duplicated.

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TABLE 1.—*Calculated 96-hr LC50 values and confidence limits ($p = .05$) for eight species of aquatic insects subjected to low dissolved oxygen concentrations*

Species tested	96-hr LC50 mg/liter	Confidence limits mg/liter	Temperature (C)
Pteronarcys dorsata (stonefly)	2.2	1.8–2.7	18.5
Acroneuria lycorias (stonefly)	3.6	3.4–3.8	14.0
Hexagenia limbata (mayfly)	1.4	1.2–1.6	18.5
Baetisca laurentina (mayfly)	3.5	3.1–3.9	18.5
Leptophlebia nebulosa (mayfly)	2.2	1.8–2.7	18.5
Ephemerella subvaria (mayfly)	3.9	3.7–4.1	18.5
Tanytarsus dissimilis (midge)	<0.6	–	18.5
Hydropsyche betteni (caddisfly)	2.9	2.7–3.1	21.0
Hydropsyche betteni	2.6	2.4–2.7	18.5
Hydropsyche betteni	2.3	2.1–2.5	17.0
Hydropsyche betteni	1.0	0.9–1.1	10.0

Raw Lake Superior water was used in all tests. General water-chemistry analyses were conducted weekly, or at greater intervals if the values remained relatively constant. Oxygen concentrations were measured at least daily by the sodium azide modification of the Winkler method (American Public Health Association, 1965). Variation of oxygen concentrations in the test chambers (± 0.1 mg/liter) was not significant. The pH was 7.5–7.8; total alkalinity (as $CaCO_3$), 39–41 mg/liter; total hardness (as $CaCO_3$), 43–46 mg/liter. Temperature was carefully controlled and was held at 18.5 C for most tests (Table 1).

The oxygen concentrations ranged from 0.6 mg/liter to saturation (18.5 C) and were prepared with the aid of a nitrogen stripping column, 30 ft high, made of 4-inch PVC-plastic pipe filled with 15-mm diameter glass balls. The oxygen depleted water was then introduced at the top of a tilted glass reaerator, similar to the reaerator of Brungs (1971), which was partitioned with standard microscope slides so the water cascaded from top to bottom (Fig. 1). As the water flowed down the reaerator it successively regained its original oxygen concentration. Calibrated tubes (125 ml H_2O/min) were used to siphon water from the reaerator partitions containing the desired oxygen concentration and deliver it to the test chambers (Fig. 1).

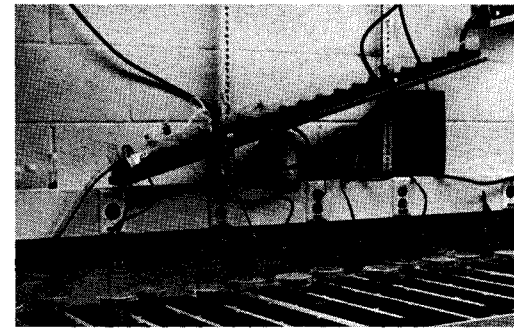

FIGURE 1.—Test system showing (a) reaerator for reaerating degassed water, (b) siphon lines for delivering selected dissolved oxygen concentrations, and (c) test streams containing screen cages housing the test species.

Six duplicate test streams were used. Mud from the river where the specimens were collected was used as a substrate for the two burrowing mayfly species and was placed in the bottom of the test streams to a depth of 25 mm; the water depth was maintained at 60 mm. The other species tested were held in stainless steel wire cages rather than on the mud substrate. Because of the higher water flow requirements of *Hydropsyche betteni* the cages holding it were placed directly in the oxygen ladder where the same water depth was maintained.

The burrowing mayflies and *Hydropsyche betteni* were fed a combination of finely chopped soaked maple leaves, powdered commercial fish food, and powdered dried wheat leaves. Maple and aspen leaves and aspen stems were placed in cages as food for the herbivorous insects. The predatory stoneflies were fed small blackfly and caddisfly larvae.

Deaths in each concentration were recorded after 96 hours and 30 days, and the results were plotted on logarithmic probability paper. The effect of low dissolved oxygen was calculated as the median lethal concentration (LC50) with confidence limits determined by the statistical method of Litchfield and Wilcoxon (1949).

Eight species were tested for survival after 96 hours exposure to reduced oxygen concentrations (Table 1) so that comparative values could be obtained for the design of future long-term studies. Five species were tested for 30

days, and four species were successfully carried through adult emergence. All animals in the 96-hr and 30-day tests were restricted from the water surface so that they could not obtain atmospheric oxygen. I conducted tests with the stonefly *Pteronarcys dorsata* to determine the effects of low oxygen on survival and behavior under two different conditions: (1) where the larvae had access to the water surface (test cage half submersed), and (2) where the larvae had no access to the water surface (test cage completely submersed).

The natural photoperiod of Duluth, Minnesota, was maintained by means of fluorescent bulbs with a light intensity of 60 ft-C at the water surface.

RESULTS AND DISCUSSION

The tolerance of the aquatic insects of low oxygen concentrations varied significantly among species. The midge *Tanytarsus dissimilis* was by far the most tolerant, with a 96-hr LC50 of less than 0.6 mg/liter (Table 1). The small mayfly *Ephemerella subvaria* was the least tolerant, with a 96-hr LC50 of 3.9 mg/liter. *Hexagenia limbata* had an average 96-hr LC50 of 1.4 mg/liter. The effects of temperature on the 96-hr LC50 are shown in Table 1 for the caddisfly *Hydropsyche betteni*; 50% survived at 2.9 mg/liter at 21 C, whereas at 10 C 50% survived a little more than a third that concentration (1.0 mg/liter).

Thirty day tests give a better indication of the long-term effects of low oxygen concentration on aquatic insects. All species tested were less tolerant of low oxygen concentrations for 30 days than for 96-hr (Table 2). Figure 2 illustrates the 30-day survival of *Ephemera simulans* (LC50 = 4.5 mg/liter) and compares it with 96-hr survival and the percentage of successful adult emergence (where all had emerged or died). The percentage mortality with time at each concentration is also shown in Figure 3.

TABLE 2.—*Calculated 30-day LC50 values and confidence limits (p = .05) for four species of aquatic insects subjected to low dissolved oxygen concentrations at 18.5 C*

Species tested	30-day LC50	
	mg/liter	Confidence limits mg/liter
Pteronarcys dorsata (3-year-old larvae)	4.8	4.3–5.3
Pteronarcys dorsata (2-year-old larvae)	4.4	4.0–4.8
Baetisca laurentina	5.0	4.5–5.6
Ephemera simulans	4.5	3.8–5.3
Tanytarsus dissimilis	<0.6	–

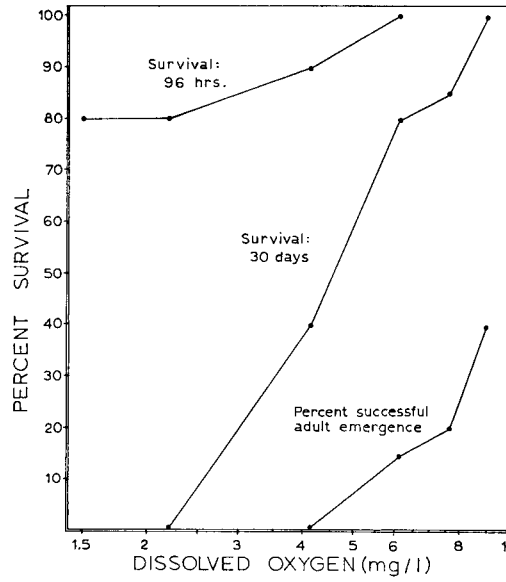

FIGURE 2.—Percentage survival and successful adult emergence of *Ephemera simulans* exposed to low concentrations of dissolved oxygen.

Pteronarcys dorsata had an average 96-hr LC50 of 2.2 mg/liter (Table 1) and an average 30-day LC50 of 4.6 mg/liter (Table 2), when tested in cages that prevented access to the water surface. Lower 30-day LC50 values were observed when the larvae had access to the water surface. None of the 3-year-old larvae had died after 30 days at 1.0 mg/liter, though all remained at the water surface. Two-year-old *Pteronarcys dorsata* larvae, when restricted below the water surface, had a 30-day LC50 of 4.4 mg/liter; they had a 30-day LC50 of only 1.0 mg/liter when they could get to the water surface.

The mayflies *Leptophlebia nebulosa*, *Baetisca laurentina*, and *Ephemera simulans* required higher oxygen concentrations to complete the strenuous transition from larva to adult; and survival and adult emergence decreased as percentage saturation decreased (Table 3). Adult emergence was inhibited at

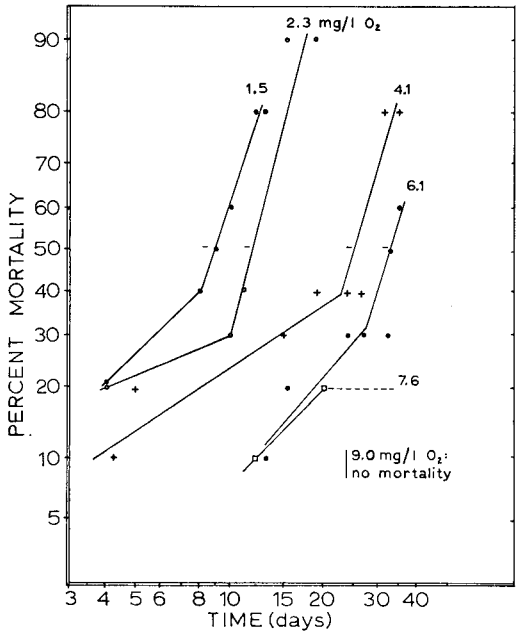

FIGURE 3.—Percentage mortality of *Ephemera simulans* at different dissolved oxygen concentrations plotted against time.

oxygen concentrations much higher than the 96-hr LC50 value. Seventy percent of *Leptophlebia nebulosa* emerged at 9.0 mg/liter, 30% at 7.6, 20% at 6.0, and only 10% emerged at 4.0. *Baetisca* completed 70% emergence at 7.0 mg/liter and 30% at 6.0, but none emerged below 4.0 mg/liter. *Ephemera simulans* (Fig. 2) had 40% emergence success at 9.0 mg/liter and 20% at 7.6. Fifteen percent emerged at 6.1 and none emerged below 4.0 mg/liter, illustrating their sensitivity to oxygen reduction.

TABLE 3.—*Percentage adult emergence in four species of aquatic insects at various dissolved oxygen concentrations at 18.5 C*

Species tested	Emergence (%)	Average dissolved oxygen concentration (mg/liter)
Leptophlebia nebulosa (mayfly)	70	9.0
	30	7.6
	20	6.0
	10	4.0
	0	2.4
Baetisca laurentina (mayfly)	70	7.0
	30	6.0
	0	4.0
Ephemera simulans (mayfly)	40	9.0
	20	7.6
	15	6.1
	0	4.1
Tanytarsus dissimilis (midge)	80+	<.6
	0	0.0

The midge *Tanytarsus dissimilis* (Table 3) was tolerant of low oxygen concentrations and emerged and reproduced successfully on several occasions at oxygen concentrations as low as 0.6 mg/liter.

Tests to determine survival in low dissolved oxygen over short periods of time, such as 24 or 96 hours, will give little information about how the animals will tolerate such conditions in nature. The 96-hr test so commonly employed (American Public Health Association, 1965) does not reflect the true effect of oxygen concentrations on aquatic insects. For example, 90% of *Ephemera simulans* survived for 96 hours at a dissolved oxygen concentration of 4.0 mg/liter, but no adults emerged successfully (Fig. 2). Studies over many weeks are necessary and should last through a complete life cycle, i.e., egg to egg. If long-term tests indicate that certain stages of the life cycle are more sensitive than others, then shorter tests covering the crucial life stage might accurately estimate the survival over greater lengths of time. Emergence tests with insects and full life cycle studies will give a more accurate description of long-term response to oxygen stress.

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