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Studies on the tolerance of aquatic insects to low oxygen concentrations

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STUDIES ON THE TOLERANCE OF AQUATIC INSECTS TO LOW OXYGEN CONCENTRATIONS[1]

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ABSTRACT.— Acute, short-term (96-hour) tests were conducted to determine the relative sensitivity of low oxygen concentrations to 20 species of aquatic insects. In addition, the longer-term effects of low oxygen levels on the survival, molting, growth, and emergence of 21 species were studied. This paper encompasses work conducted at the University of Montana Biological Station from 1968 to 1970 and at the University of Utah from 1966 to 1972.

An evaluation of the average minimum dissolved-oxygen requirements of the different groups of aquatic insects tested indicates that the mayflies are the most sensitive, that the stoneflies are next, and that the caddis flies, freshwater shrimp, true flies, and damselflies follow, in that order. While two species of mayfly could tolerate as low a dissolved-oxygen concentration as 3.3 mg/l for 10 days, a level of 4.6 mg/l was required for 50-percent survival at 30 days. Fifty percent of the true flies and damselflies tested were able to survive at levels ranging from 2.2 to 2.8 mg/l for periods ranging from 20 to 92 days.

Oxygen is a basic need of aquatic insects, yet information concerning exact oxygen requirements is known for but a very few species. Gaufin and Tarzwell (1956) pointed out that if the oxygen requirements of different species of aquatic insects were better known, it should be possible to estimate in retrospect, with considerable accuracy, what oxygen levels have existed in a given aquatic environment during the life history of the organisms. Thus, aquatic insects could be used as an excellent index of water quality.

The literature is extensive on oxygen consumption by various animals, yet such values are meaningful only for the particular conditions of measurement. The conditions under which such measurements were made are important because the rate of oxygen consumption is influenced by several internal and external variables: activity, temperature, nutrition, body size, stage in life cycle, season, time of day, and previous oxygen experience and genetic background (Prosser and Brown, 1961). The highest respiratory rates usually occur in the small, very active forms, whereas the lowest occur in the large, relatively sedentary forms.

Wigglesworth (1950) and Edwards (1946) summarized much of the work done on respiration rates of insects. The majority of the publications on immature aquatic insects has been on European species. Extensive work on individual, immature aquatic insects was done by Balke (1957) on European species of the orders Neuroptera, Odonata, Plecoptera, and Trichoptera. The difficulty in selecting a suitable and adequate method for the measurement of the respiratory rate in a particular species of aquatic insect was evaluated by Kam-

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ler (1969). An analysis of the various factors that influence the oxygen requirements and respiratory rates of benthic invertebrates is presented by Hynes (1970). The oxygen consumption of 10 of the most common species of stonefly of the western United States and the factors that modify their metabolic rate are discussed by Knight and Gaufin (1966). The oxygen requirements of immature aquatic insects in relation to their classification as index organisms are thoroughly evaluated by Olson and Rueger (1968). Their statistical analyses of oxygen-consumption rates by 12 representative species of aquatic insects of the upper Great Lakes Region constitute very valuable data for establishing water-quality criteria for the protection of aquatic life.

The principal objectives of the studies presented in this report were to determine the oxygen requirements of representative species of aquatic insects of the Intermountain Region and to determine their relative sensitivity to low oxygen concentrations. Oxygen levels necessary for survival and the long-term effects of low oxygen concentrations on molting, growth rates, times of emergence, and behavior patterns were investigated.

This report summarizes the results of acute, short-term (96-hour) tests (TLm^{96}) used in screening 20 species of aquatic insects to determine their relative sensitivity to low oxygen concentrations. In addition, the longer-term effects of low oxygen levels on the survival, molting, growth, time of emergence, and behavior patterns of 21 species are considered. The 96-hour TLm (Standard Methods, 1965) was used as a measure of survival in the tests. This report encompasses work conducted at the University of Montana Biological Station from 1968 to 1970 and at the University of Utah from 1966 to 1972.

Materials and Methods

The organisms used in the tests were all insects, except for one species of Amphipoda. All organisms were collected from streams and ponds in northwestern Montana and northern Utah. The organisms for a test were all collected from the same area at the same time. The specimens were kept in well oxygenated holding tanks for three days prior to testing. Only specimens of the same age group were utilized. These were generally of the oldest year class present. Test procedures were those outlined in Standard Methods (1965).

Deoxygenated water was obtained from degassing equipment as described by Mount (1964). Modifications included a cooling system and an oxygen "ladder." The ladder is constructed of single pane glass cemented with silicone. The ladder is 5 1/2 feet long, 7 inches wide, and 7 inches deep. It is divided into 15 compartments, each separated by a glass partition 2 inches high. The remainder of the divider is composed of fiberglass screen with a 1 mm mesh opening.

The deoxygenated water comes from the degasser through plastic tubing, passes through the cooler and then enters the elevated end of the ladder. As the water flows over the 2-inch compartment dividers toward the lower end, its oxygen content increases. Rates of increase are dependent upon rate of inflow and angle of inclination of the ladder. At an inclination of 40 degrees from the horizontal and at a flow rate of 1000 cc/min, the oxygen increase per chamber is about 0.5 mg/1 at 10 C.

Ten organisms were placed in each of seven test chambers and observed twice daily. Point of death was determined by lack of response when stimulated. Small rocks were placed in the test chambers to which the organisms could cling.

The flow rate was checked weekly and was found to vary ±25 cc/min. The temperature was taken daily with a pocket thermometer and was found to vary ±0.5 C. Oxygen concentration was taken daily using the modified Winkler method, utilizing a 50 ml sample. Variations of plus or minus 0.2 mg/l occurred.

Water used in the tests at the Biological Station was unchlorinated well water with the following chemical composition: pH 7.8; total hardness, 135 mg/l; temperature, 6.4 C; turbidity, 0-5 J. T. U.; carbon dioxide, 1-2 mg/l.

Short-term (Acute) Bioassays Conducted at University of Montana Biological Station

Results

Nineteen species of aquatic insect and one species of Amphipoda were studied to determine their 96-hour median tolerance limit (TLm). Eight species of Plecoptera were tested. The mean TLm for this group was 3.04 mg/l of oxygen. *Acroneuria pacifica* Banks had the lowest TLm, 1.6 mg/l at a flow rate of 1000 cc/min (Table 1). The highest TLm was obtained with *Pteronarcys californica* Newport (3.9 mg/l) at a rate of 500 cc/min. The TLm for this species decreased to 3.2 mg/l at a flow of 1000 cc/min. All of the specimens of *Arcynopteryx parallela* Frison survived at oxygen concentrations of from 2 to 5 mg/l at a flow of 1000 cc/min. All of the test species were stream forms.

Four species of mayfly (Ephemeroptera) were examined. Two species were lotic forms, *Hexagenia limbata* Guerin and *Callibaetis montanus* (Eaton). Their TLm's were 1.8 mg/l and 4.4 mg/l respectively. The lentic forms tested were *Ephemerella doddsi* Needham and *Ephemerella grandis* Eaton, with D. O. values of 5.2 mg/l and 3.0 mg/l respectively. The mean for the group was 3.6 mg/l.

Seven species of Trichoptera were tested, and all were from lentic environments. Several of these organisms could not be identified to the species level. Ninety percent of the specimens of *Brachycentrus occidentalis* Banks survived at oxygen concentrations of 2-4 mg/l and at a flow rate of 500 cc/min. *Neothremma alicia* Banks, a small species (5 mm), had the lowest TLm of 1.7 mg/l. *Neophylax* sp. had the highest TLm of 3.8 mg/l. The mean for the entire group was 2.86 mg/l.

One Dipteran was tested (*Simulium vittatum* Zetterstadt) and had a TLm of 3.2 mg/l. One Amphipoda was examined (*Gammarus*

TABLE 1. Test organisms, TLm in mg/l, percent saturation and water flow in cc/min.

Organisms	TLm	Saturation	Flow
PLECOPTERA			
Acroneuria pacifica Banks	1.6	14	1000
Arcynopteryx aurea Smith	3.3	29	1000
Arcynopteryx parallela Frison	100*	2-5 mg/l	1000
Diura knowltoni (Frison)	3.6	32	500
Nemoura cinctipes Banks	3.3	29	1000
Pteronarcys californica Newport	3.9	34	500
Pteronarcys californica Newport	3.2	28	1000
Pteronarcella badia (Hagen)	2.4	21	1000
EPHEMEROPTERA			
Callibaetis montanus Eaton	4.4	38	500
Ephemerella doddsi Needham	5.2	46	500
Ephemerella grandis Eaton	3.0	27	1000
Hexagenia limbata Guerin	1.8	15	1000
TRICHOPTERA			
Brachycentrus occidentalis Banks	90*	2-4 mg/l	500
Drusinus sp.	1.8	15	1000
Hydropsyche sp.	3.6	32	500
Lepidostoma sp.	80*	3-4 mg/l	1000
Limnephilus ornatus Banks	3.4	30	500
Neophylax sp.	3.8	33	500
Neothremma alicia Banks	1.7	14	500
DIPTERA			
Simulium vittatum Zetterstadt	3.2	28	500
AMPHIPODA			
Gammarus limnaeus Smith	80*	3 mg/l	500

*Percentage of survival

limnaeus Smith) with a survival of 80 percent at 3 mg/l of oxygen and at a flow rate of 500 mg/l.

The mean TLm for all organisms tested was 3.1 mg/l. The mean for all organisms tested at a flow of 1000 cc/min was 2.55 mg/l and 3.64 mg/l at a flow of 500 cc/min. The lowest TLm recorded was 1.6 mg/l for *Acroneuria pacifica*, or 14-percent oxygen saturation. The highest TLm was 5.2 mg/l for *Ephemerella doddsi*, or 46-percent oxygen saturation.

Discussion

Of the organisms tested, the group most tolerant to low dissolved-oxygen (D.O.) values was the Trichoptera (2.86 mg/l). All of the Trichoptera tested, except *Hydropsyche*, were cased forms, and all came from lentic environments. All the organisms except *Drusinus* sp. were tested at a flow rate of 500 cc/min. Higher flow rates would probably reduce the TLm of many of the forms.

Acroneuria pacifica, a predacious stonefly, was the most resistant form tested with a TLm of 1.6 mg/l (14-percent saturation). The largest organism tested, *Pteronarcys californica*, showed a decrease in TLm as the flow rate increased (3.9 mg/l to 3.2 mg/l).

The mayfly, *Ephemerella doddsi*, had the highest TLm of 5.2 mg/l (46-percent saturation) at 500 cc/min. This species is found attached to rocks in fast streams.

It has been shown by Knight and Gaufin (1963, 1964) that rate of water flow is very important in determining tolerance limits. And that conclusion is supported by the flow-rate ranges and means for *Pteronarcys californica* observed in this study. The TLm range for 11 species tested at 500 cc/min was 1.7 mg/l to 5.2 mg/l, with a mean of 3.64 mg/l. At 1000 cc/min, the range for 10 species was 1.6 mg/l to 3.3 mg/l with a mean of 2.55 mg/l, a substantially lower value.

Behavior of organisms during testing was of interest. All of the Plecoptera initiated "push-up" movements upon introduction to the test chambers. Most species ceased this motion after several hours but *Pteronarcys californica* continued these movements periodically throughout the test. *Pteronarcys californica* also assumed a position half out of the water in the low oxygen chambers. *Nemoura cinctipes* assumed a stilted position upon death.

Number of gill beats per unit time was indicative of oxygen concentration. Gill beats in *Ephemerella grandis* were counted after 12 hours in the test chambers and results are given in Table 2. Each value is the mean number of beats for the ten organisms in each chamber.

Except at the lowest D.O. concentration, the gill beat decreased as the oxygen increased. The rhythm of gill beats also became erratic as the oxygen increased.

The high TLm of the pond mayfly, *Callibaetis montanus*, was surprising. It had the second highest TLm of all species tested (4.4 mg/l). Another lotic species *Hexagenia limbata* had a low TLm of 1.8 mg/l. This could probably be explained by its acclimation to lower oxygen concentrations in its normal environment.

In response to low oxygen values, the Trichoptera undulated their abdomens in their cases. *Simulium vittatum* congregated on the chamber walls where the flow was the greatest. *Gammarus limnaeus* showed no behavioral response to the low oxygen values.

Long-term Bioassays Conducted at the University of Montana Biological Station and the University of Utah

Results

Eight species of aquatic insects from northwestern Montana were studied to determine their tolerance levels and behavior patterns when exposed to low oxygen levels over periods of time longer than 96 hours. Five of these species, and an additional 13 species from northern Utah, were also tested for periods of time ranging from 4 to 104 days to determine their long-term reactions (Tables 3, 4, 5).

The results of the longer-term bioassays clearly indicate increased sensitivity and mortality of test specimens with increased length

Table 2. Gill beats/minute for *Ephemerella grandis* Eaton.

Oxygen conc. (mg/l)	Beats	Rhythm
2.4	176	steady
3.0	192	steady
3.6	192	steady
4.6	184	erratic
5.0	160	erratic
6.0	100	erratic

Table 3. Long-term dissolved-oxygen bioassays conducted at University of Montana Biological Station.

Species	Minimum D.O. level (mg/l)	Percentage of Survival	Survival time (days)
Plecoptera			
Pteronarcella badia (Hagen)	4.4	50	69
Pteronarcys californica Newport	4.8	40	97
Arcynopteryx aurea Smith	4.8	30	12
Acroneuria pacifica Banks	5.8	50	111
Ephemeroptera			
Ephemerella grandis Eaton	4.6	30	30
Trichoptera			
Brachycentrus occidentalis Banks	3.2	50	120
Hydropsyche sp.	4.8	30	50
Diptera			
Atherix variegata Walker	2.4	90	40
Amphipoda			
Gammarus limnaeus Smith	2.8	50	20
Flow rate of 1000 cc/min			

of exposure to low oxygen levels. For example, while 50 percent of the specimens of *Acroneuria pacifica* in Montana survived an oxygen concentration of 1.6 mg/l for 4 days, the minimal dissolved-oxygen level for 50-percent survival at 111 days was 5.8 mg/l. Similarly, 50 percent of the specimens of *Arcynopteryx aurea* survived in an oxygen concentration of 3.3 mg/l for 4 days, but only 30 percent survived at a dissolved-oxygen level of 4.8 mg/l for 12 days. This increased sensitivity can be explained partly on the basis of fungus infection and debilitation caused by lack of food. For example, 60 percent of the larvae of the crane fly, *Holorusia* sp., survived for 86 days at a dissolved oxygen level of only 2.0 mg/l. Infection with fungus and shrinkage of the larvae bodies owing to starvation caused a rapid die-off after 86 days.

Of the eight species of aquatic insects tested at the Biological Station, the carnivorous stonefly, *Acroneuria pacifica*, had the highest TLm, with a 50-percent death rate at an oxygen level of 5.8 mg/l for 111 days. The most tolerant species was the Dipteran, *Atherix variegata*, with 90 percent of the specimens surviving for 40 days at

Table 4. Long-term dissolved-oxygen bioassays conducted at the University of Utah (50-percent-plus survival).

Species	Minimum D.O. level (mg/l)	Percentage of Survival	Survival time (days)
Plecoptera			
Acroneuria pacifica Banks	3.0	50	24
Brachyptera nigripennis (Banks)	2.3	60	4
Isoperla fulva Claassen	2.3	50	13
Ephemeroptera			
Ephemerella grandis Eaton	3.3	50	18
Rhithrogena robusta Dodds	3.3	50	7
	3.3	50	4
Trichoptera			
Brachycentrus occidentalis Banks	2.6	80	91
Rhyacophila sp.	1.4	50	45
Arctopsyche grandis (Banks)	3.4	50	26
Parapsyche elsis Milne	5.2	60	30
Diptera			
Atherix variegata Walker	2.4	90	97
Holorusia sp.	2.0	60	86
Odonata			
Argia vivida Hagen	3.0	50	56
Enallagma anna Williamson	1.4	50	21
Flow rate of 1000 cc/min			

Table 5. Long-term dissolved-oxygen bioassays conducted at the University of Utah (minimum D.O. with survival).

Species	Minimum D.O. level (mg/l)	Percentage of Survival	Survival time (days)
Plecoptera			
Acroneuria pacifica Banks	3.0	20	41
Arcynopteryx parallela Frison	3.4	10	8
	4.2	20	28
Brachyptera nigripennis (Banks)	3.7	20	9
Isoperla fulva Claassen	2.1	10	27
Pteronarcella badia (Hagen)	2.0	30	30
Ephemeroptera			
Baetis bicaudatus Dodds	3.8	10	3
Ephemerella grandis Eaton	3.5	50	21
Trichoptera			
Parapsyche elsis Milne	4.8	40	16
Diptera			
Atherix variegata Walker	1.7	70	90
Bibiocephala sp.	3.4	40	21
Odonata			
Argia vivida Hagen	1.7	10	100
Enallagma anna Williamson	1.1	20	35
Flow rate of 1000 cc/min			

TABLE 6. Average minimum dissolved-oxygen requirements of different groups of aquatic invertebrates*.

	Montana species	Average survival (days)	Utah species	Average survival (days)
Plecoptera	4.9 mg/l	62	2.8 mg/l	14
Ephemeroptera	4.6 mg/l	30	3.3 mg/l	10
Trichoptera	4.0 mg/l	85	3.1 mg/l	48
Diptera	2.4 mg/l	40	2.2 mg/l	92
Odonata			2.2 mg/l	39
Amphipoda	2.8 mg/l	20		

*Averages based on 50-percent-plus survival for time indicated.

an oxygen concentration of 2.4 mg/l. This species was also the most tolerant of the Utah forms listed, 90 percent of the specimens surviving at the same oxygen level for 97 days. The higher oxygen requirement of *Acroneuria pacifica* under long-term conditions may be partially owing to its food requirements. Inasmuch as this species is carnivorous, lack of a varied animal diet may have reduced its ability to tolerate low oxygen levels for extended periods of time.

A comparison of the long-term median tolerance limits of the same species of aquatic insects from Montana and Utah shows considerable variation. Fifty percent of the specimens of the stonefly, *Acroneuria pacifica*, from Montana died at a dissolved-oxygen level of 4.4 mg/l in 69 days. The same percentage of Utah specimens survived at a much lower dissolved-oxygen concentration, 3.0 mg/l, but for only 24 days. A mayfly, *Ephemerella grandis*, was tested from both Montana and Utah with similar results. Thirty percent of the Montana specimens survived at a dissolved-oxygen level of 4.6 mg/l for 30 days, while 50 percent of the Utah specimens survived at a dissolved-oxygen concentration of 3.3 mg/l, but for only 18 days. The differences in tolerance limits between the same species may have been much less if the tests had been conducted under exactly the same conditions in the two locations. Time did not permit this, so the Utah tests were run at lower oxygen levels to determine maximum survival rates at these much lower oxygen limits.

An evaluation of the average minimum dissolved-oxygen requirements of the different groups of aquatic invertebrates tested shows that the mayflies are the most sensitive, that the stoneflies are next, and that the caddis flies, freshwater shrimp, true flies, and damselflies follow, in that order. While two species of mayfly could tolerate as low a dissolved-oxygen concentration as 3.3 mg/l for 10 days, a level of 4.6 mg/l was required for 50-percent survival at 30 days. Three species of stonefly from Utah survived at a dissolved-oxygen concentration of 2.8 mg/l for 14 days with 50 percent surviving, but an average oxygen concentration of 4.9 mg/l was required for 30- to 50-percent survival for 62 days. Tests on caddis flies also indicated that higher oxygen levels were necessary with longer exposure, a minimum of 4.0 mg/l being required for 50-percent survival for 84 days.

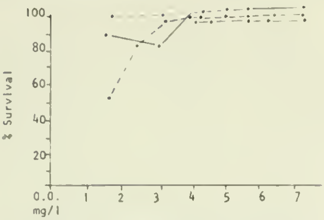

Fig. 1. *Acroneuria pacifica*: 1000 cc/min, 96-hour TLm results, oxygen.

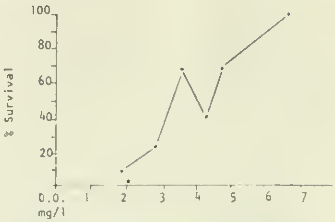

Fig. 2. *Arcynopteryx aurea*: 1000 cc/min, 96-hour TLm results, oxygen.

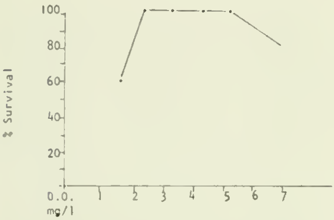

Fig. 3. *Arcynopteryx parallela*: 1000 cc/min, 96-hour TLm results, oxygen.

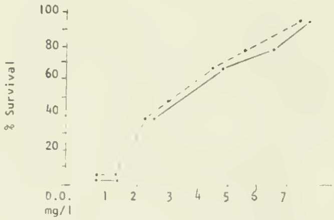

Fig. 4. *Pteronarcella badia*: 1000 cc/min, 96-hour TLm results, oxygen.

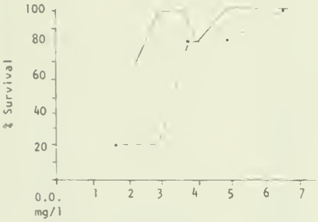

Fig. 5. *Pteronarcys californica*: 1000 cc/min, 96-hour TLm results, oxygen.

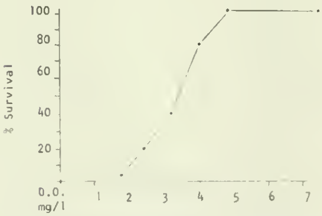

Fig. 6. *Nemoura cinctipes*: 1000 cc/min, 96-hour TLm results, oxygen.

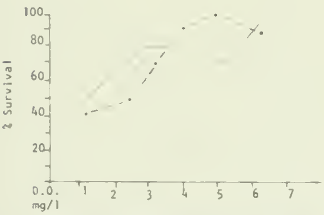

Fig. 7. *Hexagenia limbata*: 1000 cc/min, 96-hour TLm results, oxygen.

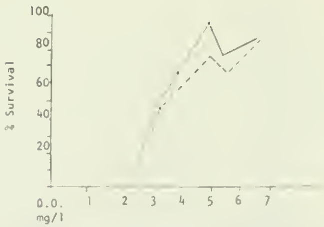

Fig. 8. *Ephemerella grandis*: 1000 cc/min, 96-hour TLm results, oxygen.

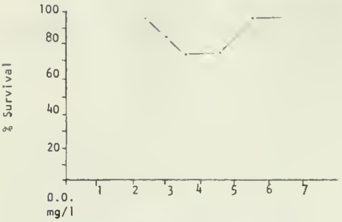

Fig. 9. *Lepidostoma* sp.: 1000 cc/min, 96-hour TLm results, oxygen.

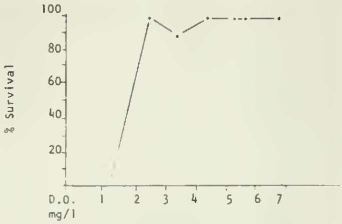

Fig. 10. *Drusinus* sp.: 1000 cc/min. 96-hour TLm results, oxygen.

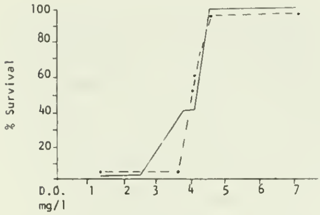

Fig. 11. *Pteronarcys californica*: 500 cc/min, 96-hour TLm results, oxygen.

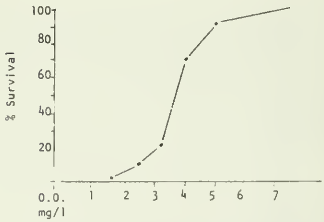

Fig. 12. *Diura knowltoni*: 500 cc/min, 96-hour TLm results, oxygen.

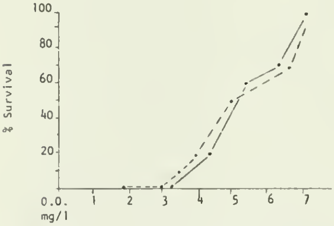

Fig. 13. *Ephemerella doddsi*: 500 cc/min, 96-hour TLm results, oxygen.

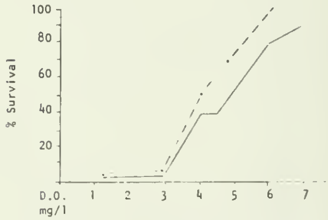

Fig. 14. *Callibaetis montanus*: 500 cc/min, 96-hour TLm results, oxygen.

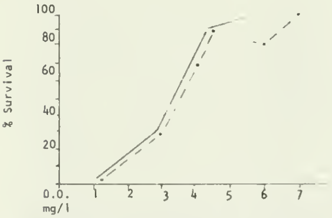

Fig. 15. *Limnephilus* sp.: 500 cc/min, 96-hour TLm results, oxygen.

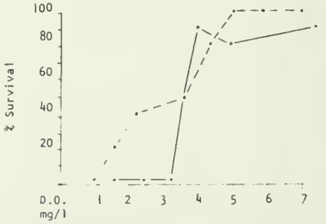

Fig. 16. *Hydropsyche* sp.: 500 cc/min, 96-hour TLm results, oxygen.

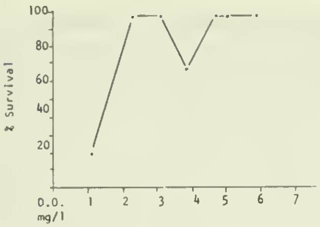

Fig. 17. *Neothremma* sp.: 500 cc/min. 96-hour TLm results, oxygen.

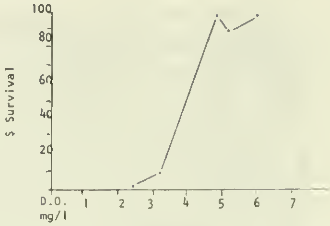

Fig. 18. *Neophylax* sp.: 500 cc/min, 96-hour TLm results, oxygen.

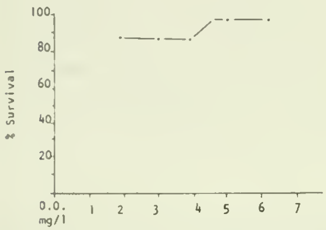

Fig. 19. *Brachycentrus occidentalis*: 500 cc/min, 96-hour TLm results, oxygen.

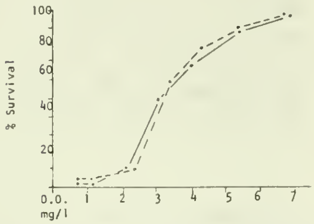

Fig. 20. *Simulium vittatum*: 500/cc min, 96-hour TLm results, oxygen.

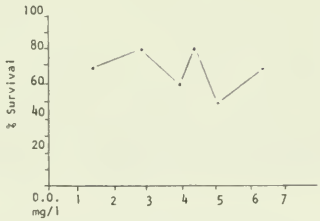

Fig. 21. *Gammarus limnaeus*: 500 cc/min, 96-hour TLm results, oxygen.

The true flies, freshwater shrimp, and damselflies displayed a much greater tolerance to low oxygen levels than did the previous three groups. Fifty percent of the specimens of these three groups were able to survive for periods ranging from 20 to 92 days at dissolved-oxygen levels ranging from 2.2 to 2.8 mg/l.

While the principal objective of this project was to determine the minimal dissolved-oxygen levels required for both short- and long-term exposure, survival without growth and metamorphosis would eventually eliminate a species of aquatic insect. While not all of the species tested molted or emerged during the study, many species did. All of the species on which bioassays were run for over 30 days molted one or more times at the oxygen levels required for 50-percent survival. Species such as the stoneflies *Brachyptera nigripennis*, *Pteronarcys californica*, and *Pteronarcella badia*, the mayfly *Ephemerella grandis*, and the damselfly *Enallagma anna* emerged during the tests at oxygen concentrations of 4.8 mg/l or less. None of the caddis flies or Dipterans emerged, inasmuch as only larvae and not pupae were used for testing purposes.

Discussion

Aquatic environments in which dissolved oxygen is available in excess at all times are rare. Many aquatic animals possess varied adaptations that facilitate the acquisition of oxygen when it becomes scarce. In stoneflies, diffusion, along with special ventilation mechanisms, provides extensive absorbing surfaces for the absorption of oxygen from the environment. An adaption utilized by the nymphs of *Pteronarcys californica* when environmental oxygen becomes reduced is undulating the body—to destroy the oxygen gradient that develops around the body and gills. Of particular interest is the variation in rate of undulation according to year class. In studies conducted at the University of Utah from 1963 to 1965, the undulations of the smaller nymphs of this species (year I, 17-18 mm long) were more rapid than those of the larger (year II, 30 mm long).

The respiratory mechanism possessed by different species of aquatic insects greatly influences their ability to withstand low oxygen concentrations. In work conducted by Knight and Gaufin (1966) at the University of Utah, the value of gills in enabling some species to better withstand low dissolved-oxygen levels was demonstrated clearly. The nymphs of *Pteronarcella badia*, *Isoperla fulva*, and *Acroneuria pacifica* were all exposed to an environment of reduced dissolved oxygen of 1.0 cc/l and water flow of 0.004 feet/second, at 10 C. The forms possessing gills exhibited quite similar mortalities during the exposure period. *Pteronarcella badia* nymphs exhibited a 13-percent mortality after 24 hours and 48 hours of exposure, and 29-percent at the end of 72 hours, with no further mortality for the remainder of the exposure period. *Acroneuria pacifica* showed the same mortality as did *Pteronarcella badia* after 72 hours of exposure. After 96 hours exposure, *Acroneuria pacifica* displayed a 25-percent mortality. No further mortality was noted for the remainder of the experimental period. Eighty percent of the *Isoperla fulva* nymphs, a species without gills, died within 24 hours. After 144 hours of exposure, all had succumbed. The increased mortality shown by the *Isoperla fulva* nymphs may have been owing to their smaller size and the fact that they were year class I, as opposed to year class II in the gilled forms. *Isoperla fulva* has only a one-year life cycle, so it was impossible to compare nymphs of similar size.

In view of the above, a second evaluation was carried out comparing nymphs of *Acroneuria pacifica* (gills) to those of *Arcynopteryx parallela* (no thoracic gills). The nymphs were tested at a temperature of 15.6 C with a water flow of 0.25 feet/second and a dissolved-oxygen concentration of 1.0 cc/l. The nymphs of both species were between 25 and 30 mm in length. In general, the results of this test—like those of the previous one—indicated that forms lacking gills are more sensitive to reduced dissolved oxygen than are forms possessing gills. No mortality of *Acroneuria pacifica* nymphs occurred during the experimental period, while nymphs of *Arcynopteryx parallela* showed 82-percent mortality after 10 hours of exposure, 88.5-percent mortality at the end of 24 hours, and 100-percent mortality after 34 hours.

The metabolism of poikilotherms rises with temperature about two and one-half times per 10 C change in temperature (Prosser and Brown, 1961). This metabolic increase in response to increased environmental temperature produces increased oxygen consumption. The increase in oxygen consumption with increased water temperature would cause an aquatic insect subjected to the higher temperature (15.6 C) to incur an oxygen debt at a higher dissolved-oxygen concentration than one subjected to a situation similar except for a reduced temperature (10 C). Stoneflies, mayflies, and caddis flies do not have an apparent ability to get along without oxygen for an extended period. They do survive for a short period in greatly reduced oxygen by greatly reducing their activity, and they use energy apparently produced by the anaerobic phase of glycolysis. If the oxygen supply is not restored within a certain time, the specimens die from asphyxiation.

In the work conducted to date by the author and his colleagues, there has been a great difference in the dissolved-oxygen concentration at which initial mortality of test organisms was recorded. This difference was greatly influenced by the temperature difference in the experimental environment. In a natural situation in which the dissolved oxygen is gradually reduced for short periods of time (for example, by intermittent discharges of organic oxygen-demanding wastes), the onset of stonefly mortality would be influenced by the existing water temperature. Provided that the water flow and other variables remained constant, one could expect the aquatic insects subjected to an environmental temperature of 10 C to withstand reduced oxygen concentrations about 2.4 times lower than would the same specimens exposed to similarly reduced oxygen concentrations at a water temperature of 15.6 C. In a hypothetical situation based on the work of Knight and Gaufin (1966), a stream possessing a temperature of 15.6 C and a dissolved-oxygen concentration of 0.6 cc/l would have a stonefly mortality of 18 percent while a stream similar in all respects except that its water temperature was 10 C would exhibit 100-percent survival. Thus, the water temperature of a stream is a very important factor in the survival of aquatic insects when they are subjected to a reduction in dissolved oxygen over a short period of time.

The rate of water flow in a stream also is a very important factor in the survival of aquatic insects exposed to low oxygen concentrations. Knight and Gaufin (1966) showed that a gradual reduction of dissolved oxygen with water flow of 0.06 ft/sec produced approximately 50-percent stonefly mortality and that a similar situation provided with a water flow of 0.25 ft/sec resulted in 100-percent survival.

In the present study the mean oxygen concentration required for 50-percent survival by 11 species of aquatic insects at a flow rate of 500 cc/min was 3.64 mg/l. The mean for 10 species at a flow rate of 1000 cc/min was considerably lower or 2.55 mg/l.

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