1972

Dynamics and Productivity of Aquatic Invertebrates in a Desert Environment

A. R. Gaufin

J. Stanford

E. Clubb

E. Nisonger

IBP

RESEARCH MEMORANDUM

RM 72-45

DYNAMICS AND PRODUCTIVITY OF AQUATIC INVERTEBRATES IN A DESERT ENVIRONMENT

A.R. Gaufin, J. Stanford,
R. Clubb, & E. Nisonger

DESERT BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

1971 PROGRESS REPORT

DYNAMICS AND PRODUCTIVITY OF AQUATIC INVERTEBRATES IN A DESERT ENVIRONMENT

A.R. Gaufin - Project Leader

J. Stanford, R. Clubb, E. Nisonger
Other Authors

University of Utah
Salt Lake City, Utah

JUNE 1972

ABSTRACT

This report presents progress and results of research conducted during the first year (1971-72) of the Deep Creek, Locomotive Springs, studies. The principal accomplishments are as follows:

1. Determination of the effects of different temperature levels on the survival, growth and emergence of four representative species of aquatic invertebrates.
2. Measurement of the respiratory rates of the test specimens in relation to the size and activity of the species and water temperature.
3. Determination of the effects of low dissolved oxygen concentrations on the survival, growth, behavior, and emergence of representative species of aquatic invertebrates.

The organisms that were studied intensively included *Hydropsyche occidentalis* (Insecta: Trichoptera), *Argia vivida* and *Enallagma anna* (Insecta: Odonata), *Gammarus limnaeus* and *Hyalella azteca* (Crustacea: Amphipoda). Species from other habitats were also studied for comparative purposes.

OBJECTIVES

The objectives of these process studies were as follows:

1. To determine the effects of elevated temperatures on the survival, growth, emergence, and respiratory rates of aquatic invertebrates.
2. To determine the respiratory rates of selected aquatic invertebrates in relation to their size, activity, and varying water temperatures.
3. To determine the effects of low dissolved oxygen concentrations on the survival, growth, behavior, and emergence of aquatic invertebrates.

METHODS

The following project list enumerates the specific methods used for each section of the study:

Temperature Effects on Aquatic Invertebrates (DSCODE A3UG101)

Data recorded: Species, date, hour, temperature °C, number of specimens, behavior.

Species: *Hydropsyche occidentalis, Argia vivida, Enallagma anna, Gammarus limnaeus.*

Experimental Methods: Nine stainless steel tanks measuring 90 cm by 17 cm by 17 cm in size, immersed in two refrigerated water baths, were used to conduct the thermal bioassays. The temperature in each test chamber was controlled by a thermostatically regulated heating element. The water in each chamber was circulated by a paddle wheel. Ten specimens of each test species were exposed to a range of temperatures from 5°C to 30°C.

Citations: Gaufin, A.R., and S. Hern. 1971. Laboratory studies on tolerance of aquatic insects to heated waters. J. Kansas Ent. Soc. 44:240-245.

Parker, F.L., and P.A. Krenkel. 1969. Thermal Pollution: Status of the Art. Vanderbilt Univ., Dept. of Env. and Water Resources Eng. Report No. 3, Chapt. 3, pp. 1-65.

Schraer, W.D. 1972. The effect of heated water on homeostasis, survival, and emergence of selected aquatic insect fauna. Unpublished. Doctoral Dissertation. Univ. of Utah.

Oxygen Consumption Over Period of Time (DSCODE A3UG102)

Data recorded: Species, date, hour, temperautre °C, ml O_2 per hour, grams dry weight of specimens.

Species: *Hydropsyche occidentalis, Argia vivida, Enallagma carunculatum, Gammarus limnaeus, Hyalella azteca.*

Experimental Methods: A Gilson respirometer was used for determining the respiratory rates of each test organism. From one to six specimens, depending on the size of the species and specimens, were placed in each of 20 manometer vessels and the oxygen utilized each hour over a 24-hour period was recorded. The dry weight of each specimen (in grams) was determined at the end of each test run.

Citations: Knight, A.W., and A.R. Gaufin. 1966. Oxygen consumption of several species of stoneflies (Plecoptera). J. Insect Physiol. 12:347-355.

Schraer, W.D. 1972. The effect of heated water on homeostasis, survival, and emergence of selected aquatic insect fauna. Unpublished Doctoral Dissertation. Univ. of Utah.

Effects of Minimal Dissolved Oxygen (DSCODE A3UG103)

Data recorded: Species, date, hour, temperature °C, D.O. concentration, number of specimens, flow rate.

Species: *Hydropsyche occidentalis, Argia vivida, Enallagma anna, Gammarus limnaeus, Hyalella azteca.*

Experimental Methods: A Mount oxygen degasser and an oxygen ladder were used in exposing specimens to low D.O. concentrations. Ten specimens of each test species were exposed to oxygen levels ranging from 1.0 to 6.0 mg/l for periods ranging from 24 hours to 30 days. Median tolerance levels, survival rates, and behavioral reactions were determined for each species.

Citations: Gaufin, A.R. 1972. Water quality requirements of aquatic insects. Report to the Water Quality Office. E.P.A. Pages 1-89.

Gaufin, A.R., and A.W. Knight. 1965. Function of stonefly gills under reduced dissolved oxygen concentration. Proc. Utah Acad. Sciences 42(2):186-190.

RESULTS

Temperature Effects on Aquatic Invertebrates (DSCODE A3UG101)

The rate of development and the time of emergence of aquatic insects is directly influenced by the temperature. An increase in water temperature in the winter above 5°C might completely eliminate winter stoneflies belonging to the family Capniidae.

Many species of stoneflies, mayflies, and caddis flies emerge in late spring before stream temperatures reach high summer levels. An increase in stream temperatures during the winter would very likely cause these species to develop more rapidly, emerge earlier, and be killed by cold air temperatures, and may substantially reduce the population or eliminate the species.

The stonefly, *Isogenus aestivalis,* and mayfly, *Cinygmula par,* are largely restricted to clear, cold-water streams in the Rocky Mountain region, and even a slight increase in water temperature may have an adverse effect on their survival. By comparison, the snipe fly (*Atherix variegata*) is often found in open sections of streams which warm up during the summer months, and this species is decidedly temperature tolerant.

Higher temperatures and a more uniform environment may greatly increase the rate of development of aquatic insects and thereby alter their life cycles. Knight and Gaufin (1966) showed that the rate of metabolism in stoneflies is decidedly temperature dependent. The distribution of aquatic invertebrates is also greatly influenced by maximal and minimal seasonal temperatures.

In this study the scud, *Gammarus limnaeus* Smith, proved to be surprisingly sensitive to temperature changes, exhibiting a 24-hour TLm of only 14.5°C. This species is most abundant in cold water streams in the intermountain region associated with beds of higher aquatic plants such as pondweeds and watercress. Its sensitivity to temperature changes, low dissolved oxygen and high pH when tested under laboratory conditions, might very well reflect a need for a more natural environment than was provided in the laboratory.

The damselfly, *Argia vivida,* was the least sensitive species from Deep Creek that was tested, with 50% of the test organisms in one test tank surviving for 96 hours at a temperature of 37°C. At a temperature of 25.5°C, 50% of the specimens of this species survived for 17 days. These latter temperatures are well above the maximum summer temperatures in Deep Creek. Another species of damselfly, *Enallagma anna,* was also very tolerant of high temperatures with 50% of the test specimens surviving for 12 days at 30°C and for 14 days at 28°C. Both of these species emerge during late spring or early summer but the acclimation to comparatively high temperatures in the laboratory coincides with their distribution in nature in ponds and streams which exhibit high summer temperatures.

The caddis fly, *Hydropsyche occidentalis,* also proved to be quite tolerant of fairly high temperatures with a 96-hour TLm of 31°C and with 50% of the test specimens surviving for 9 days at a temperature of 28.0°C. These temperatures are considerably higher than the 96-hour TLm of 21.8°C displayed by a closely-related species, *Parapsyche elsis,* which is restricted to fast-flowing cold water streams in the western United States.

The adaptation of *Hydropsyche occidentalis* to warmer waters was also displayed by its altered life cycle under both stream and laboratory conditions. Adults of this species were induced to emerge in the laboratory in February when the pupae were exposed to temperatures of 20°C. Similarly, larvae collected from Deep Creek in February when stream temperatures were 16°C began to pupate in the laboratory at 19 - 20°C.

Hydropsyche occidentalis, resembling the two species of damselflies, was much more difficult to use as an experimental animal than most of the mayflies and stoneflies tested, because of cannibalism. Specimens in all tests had to be kept in separate containers, thus preventing many replications of tests over a long-term period.

Short and long-term temperature tolerance limits for *Hydropsyche occidentalis* are summarized in Tables 1 and 2 and Figures 1 and 2. Long-term tolerance limits are given for *Argia vivida* in Table 3 and Figure 3. Long-term tolerance limits are given for *Enallagma anna* in Table 4 and Figure 4. Comparative tolerance limits are presented in Tables 5 and 6.

Table 1. *Hydropsyche occidentalis* - Short-term temperature tolerance.

Temperature (°C)	Number Surviving 24 hours	Number Surviving 140 hours
11	10	10
14	10	10
16	10	10
18	10	10
22	10	9
24	10	8
26	10	9
28	9	6
29	10	8

Table 2. *Hydropsyche occidentalis* - Long-term temperature tolerance.

Temperature (°C)	Hours to 50% mortality larvae	% Larvae surviving 210 hours	% Pupae surviving 210 hours
18.8		100	0
20.4		100	40
22.1		100	30
24.0		90	20
28.0		60	30
29.2		79	33
31.1	72 hours	45	0
32.7	24 "	0	0
36.5	6 "	0	0

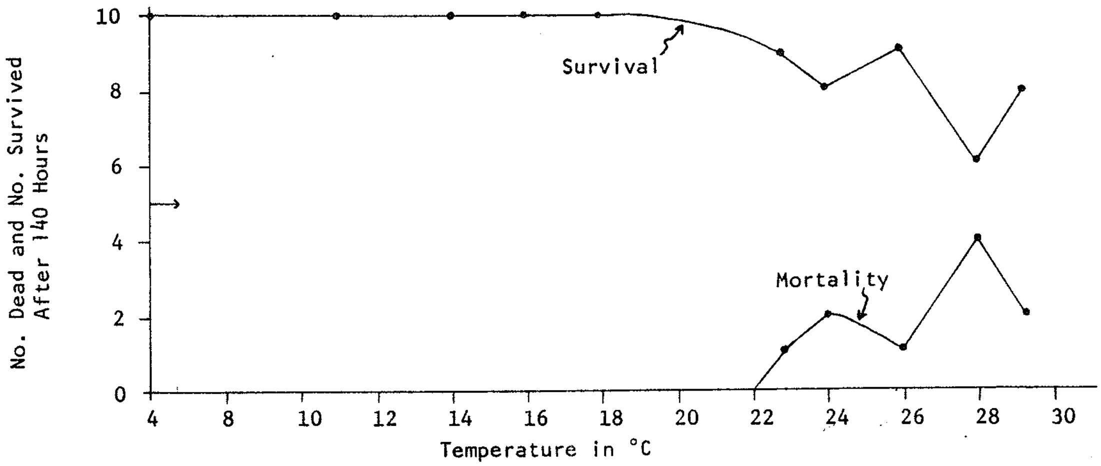

Figure 1. Survival and death of *Hydropsyche occidentalis* under differing temperature regimes.*

*Conclusions:

1) Apparently very resistant to thermal shock as only 2 deaths observed in 24 hours.
2) Larger organisms more prone to heavy net spinning.
3) Some net spinning evident at all temperatures but
   a) heavy net spinning at 4° and 24° - 26°,
   b) net spinning at 24° - 26°, appeared to be a formation of a pupal case in several instances.

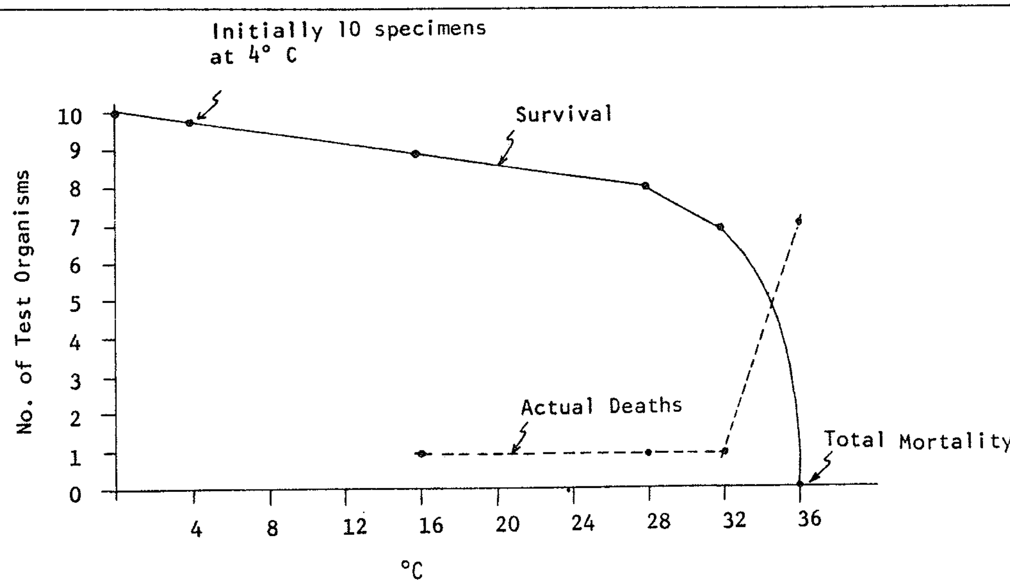

Figure 2. Survival and mortality of *Hydropsyche occidentalis* under differing temperature regimes.* Each 4°C increment covers 24 hours. Total time to total mortality equals 216 hours.

* Conclusions:

1) Temperatures above 32°C very detrimental.
2) Test organisms under stress from 24 + °C.

Table 3. Long-term temperature tolerance of *Argia vivida*.

Date Semi-Weekly	Days of Exposure	Aquarium Number: Control		2		3		4		5		6	
		Temp °C	Number Alive	Temp °C	Number Alive	Temp °C	Number Alive	Temp °C	Number Alive	Temp °C	Number Alive	Temp °C	Number Alive
3-25-71	0	13.0	10	15.0	10	18.0	10	20.0	10	24.0	10	26.0	10
3-26-71	1	12.5	10	14.5	9	18.0	10	19.0	10	24.0	10	25.5	8
3-30-71	5	13.0	10	15.0	8	18.0	10	19.5	8	24.0	10	25.5	8
4-02-71	8	13.0	10	15.0	5	18.0	10	20.0	8	24.0	10	25.5	8
4-06-71	12	13.0	10	15.0	5	18.0	10	20.0	6	24.0	8	25.5	8
4-09-71	15	13.0	10	15.0	4	18.0	10	20.0	6	24.0	8	25.5	7
4-13-71	19	13.5	10	15.0	4	18.0	10	19.0	6	24.0	5	25.5	3
4-16-71	22	12.5	10	15.0	2	18.0	10	18.0	6	24.0	4	25.0	2
4-20-71	26	12.5	10	14.5	2	18.0	10	18.0	5	24.0	4	25.0	2
4-23-71	29	12.5	10	15.0	1	18.0	5	18.0	2	24.0	2	25.0	1
4-27-71	33	12.0	10	15.0	1	18.0	5	18.0	1	23.5	1	25.0	1
4-30-71	36	12.0	10	15.0	1	18.0	3	19.5	0	24.0	0	26.0	0
5-04-71	40	11.5	10	15.0	1	18.0	2						
5-07-71	43	12.0	10	15.0	1	18.0	1						
5-11-71	47	12.0	10	15.0	0	18.0	1						
5-14-71	50	13.0	10			18.0	0						

Table 4. Long-term temperature tolerance of *Enallagma anna*.

Tank No.	°C	Day 1		Days 5		Days 6		Days 7		Days 9		Days 10		Days 11		Days 12		Days 13		Days 14		Days 15		Days to 50% mortality	
		S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D		
4	12	100																							
3	14	100	0	90	10																				
1	16	100	0																						
2	18	100	0																						
8	23	100	0	90	10	90	10	90	10	90	10	80	70	70	30	70	30	60	40	40	60	20	80	14	
9	24	100	0													80	20	70	30	50	50	50	50	14	
7	26	100	0							80	20	70	30	70	30	60	40	50	50	20	80	10	90	13	
6	28	100	0									80	20	70	30	40	60	30	70	20	80	0	100	12	15
5	30	100	0			90	10	90	10	90	10	60	40	60	40	40	60	30	70	30	70	0	100	12	15

S: % surviving

D: % dead

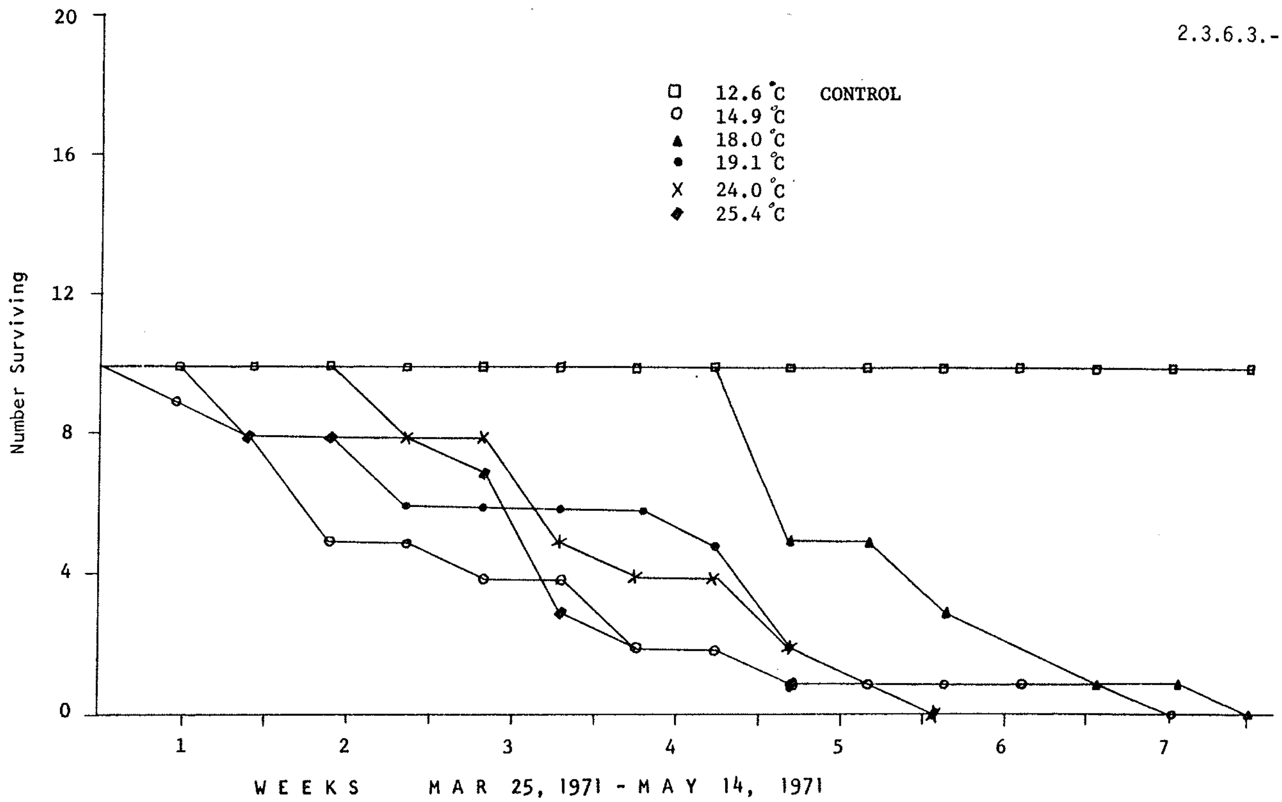

Figure 3. Long-term temperature tolerance of *Argia vivida*.

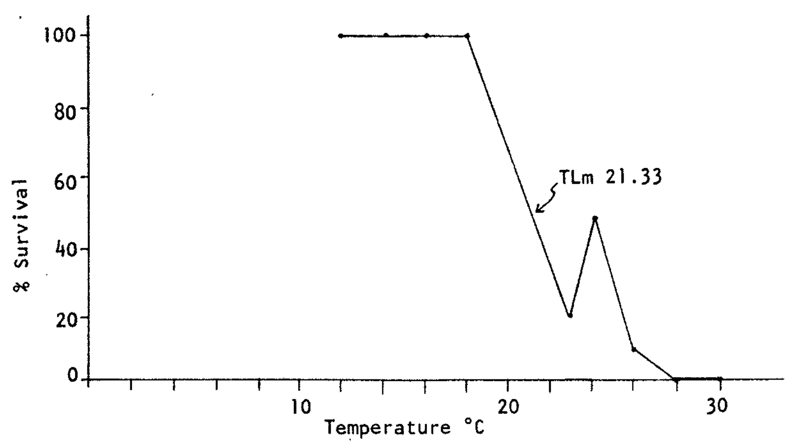

Figure 4. Long-term temperature tolerance of *Enallagma anna*.* [15 days to 100% mortality at 30°C.]

---

* Conclusions:

1) 15 days TLm - 21.33°C; below any recorded mortality because of 80% mortality at 23° and only 50% at 24°.

2) Replicate runs needed to determine mortality in 20° - 25° range more accurately. Also need test of precision to give reliability of calculated TLm.

3) Estimated TLm is too low. The test specimens were under stress (?); i.e., shock had some latent effect, or weakened from lack of food.

Table 5. Comparative temperature tolerance of Deep Creek species.

Species	Temperature Tolerance Limits 0°C 96 hr TLm	9 days	12 days	14 days	17 days
Gammarus limnaeus	14.5				
Hydropsyche occidentalis	31.0	28.0			
Argia vivida	37.0				25.5
Enallagma anna			30.0	28.0	

Table 6. Temperatures at which 50% of the specimens died after 96 hours exposure.

Species Tested	TLm^{96} (°C)
Cinygmula par (mayfly)	11.7°C
Gammarus limnaeus (amphipod)	14.5°C
Ephemerella doddsi (mayfly)	15.4°C
Isogenus aestivalis (stonefly)	16 °C
Parapsyche elsis (caddis fly)	21.8°C
Pteronarcys californica (stonefly)	26.4°C
Hydropsyche occidentalis (caddis fly)	31 °C
Atherix variegata (snipe fly)	32.6°C

Oxygen Consumption Over Periods of Time (DSCODE A3UG102)

The rate of oxygen consumption of the various species tested varied greatly between species -- with the size, activity, and behavior of the test specimens, and with the temperature of the water in the Gilson water bath. Specimens of all species tested displayed periods of inactivity when oxygen consumption was too low to measure with the instrumentation being used.

In these respiration tests a Gilson Model GR20 respirometer of the differential type was utilized. The respirometer has a precise, constant-temperature water bath regulated by a heater and refrigeration unit. Water is constantly circulated by a stirring motor and propeller. Reaction vessels were gently oscillated at a rate of 84 cycles per minute.

Twenty-four hour oxygen consumption tests were conducted with *Argia vivida, Enallagma carunculatum* and *Hydropsyche occidentalis* to determine fluctuations in respiration. Similar tests were conducted with ten other species from the Weber and Provo Rivers and from Timpie Springs to determine the effects of heated water on the homeostasis, survival, and emergence of aquatic insects from the intermountain region.

Oxygen consumption was recorded over a one- to four-hour period for a given temperature. This was to determine the QO_2 of a specimen at an acclimatized temperature. The effect of prior exposure to temperatures higher and lower than that in the water bath was investigated for a number of species, including *Argia vivida*. The effects of such acclimation are summarized in the following discussion and in Table 7 and Figures 5, 6 and 7.

Argia Vivida:

Diurnal respiration at an acclimatized temperature.

A gradual increase occurred from 4.6 to 7 μl O_2/min/g, which then leveled off during the last seven hours of the test. This indicated a cyclic pattern that potentially may exceed the 24-hour period. The diurnal test displayed no association with periods of daylight and darkness.

Temperature acclimation of respiration.

An overshoot transpired at 13.5°C to 18.5°C and was followed by an erratic QO_2. Oxygen consumption settled close to the original respiratory rate at 18.5°C. This demonstrated a very slight partial acclimatization. An undershoot occurred at a temperature decrease of 18.5°C to 13.5°C with a gradual lowering in QO_2. A partial acclimatization developed as illustrated by the final QO_2 stabilizing between the undershoot maximum and the acclimitized rate at 18.5°C.

Twenty-four hour respiratory runs were also conducted with *Enallagma carunculatum* and *Hydropsyche occidentalis* with oxygen consumption being measured at hourly intervals. The temperature of the water bath for the tests with *E. carunculatum* was held at 15°C, similar to the temperatures in Deep Creek in February at the time of the run. The temperature of the water bath for the tests with *H. occidentalis* was held at 18°C, corresponding with temperatures in Deep Creek in November.

A variation in oxygen consumption from 0.0 to 0.965 ml O_2/gram of dry weight was recorded for the different specimens of *H. occidentalis*. The uptake varied with the size and activity of the specimens, with all of the specimens attempting to build nests during their confinement accounting for erratic activity.

The oxygen consumption of *Enallagma carunculatum* varied from 0.0 to 0.816 ml O_2/gram of dry weight for all except one specimen. A late instar larva of this species showed the highest respiratory rate of the organisms tested with 5.4 ml O_2 per gram of dry weight being consumed during a one-hour interval. The complete data for the runs for these two species have been deposited in the data bank.

Table 7. Effects of temperature acclimation of *Argia vivida* on oxygen consumption.

DIURNAL RESPIRATION

Time Hours	Time (Minutes at Measurement)	Time of Day	Temperature	QO_2 µl O_2/min/g
0 - 4:00	150	1:55 P.M.- 5:55 P.M.	13.5°C acclimatized	4.67
4:15- 8:15	390	6:15 P.M.-10:15 P.M.		5.62
8:40-12:40	670	10:40 P.M.- 2:40 A.M.		6.47
13:30-17:30	960	3:30 A.M.- 7:30 A.M.		7.07
18:45-21:45	1240	8:10 A.M.-12:45 P.M.		7.00

TEMPERATURE ACCLIMATION OF RESPIRATION

Time Hours	Time (Minutes at Measurement)	Temperature	QO_2
0 - 2:30	90	13.5°C acclimatized	3.93
6:45- 8:45	465	18.5°C thermal stress	10.21
8:45-10:45	615		11.00
11:25-14:25	805		10.17
16:35-18:35	1070		9.43
19:55-21:55	1270		7.46
22:30-25:30	1485		10.22
28:15-29:45	1720		8.94
31:05-33:05	1930		10.60
33:45-35:45	2025		8.04
36:55-39:55	2275		7.65
40:45-43:45	2565		8.83
46:10-48:40	2875		10.41
50:05-53:05	3095		10.00

TEMPERATURE ACCLIMATION OF RESPIRATION

Time Hours	Time (Minutes at Measurement)	Temperature	QO_2
0 - 2:00	60	18.5°C acclimatized	14.63
7:00- 7:30	430	13.5°C thermal stress	8.13
7:30- 8:00	463		5.45
9:35-13:35	665		8.02
14:35-18:35	995		7.44
20:45-24:45	1395		6.95
25:00-27:00	1575		6.11

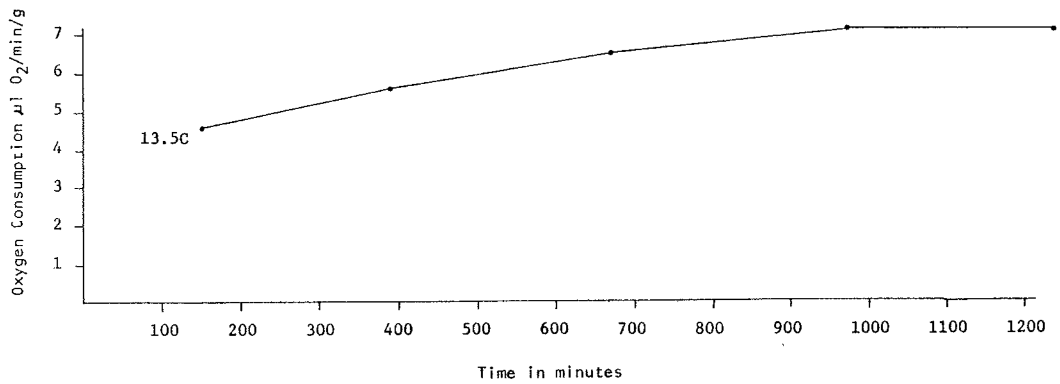

Figure 5. Dirunal respiration of *Argia vivida* during acclimation at 13.5°C.

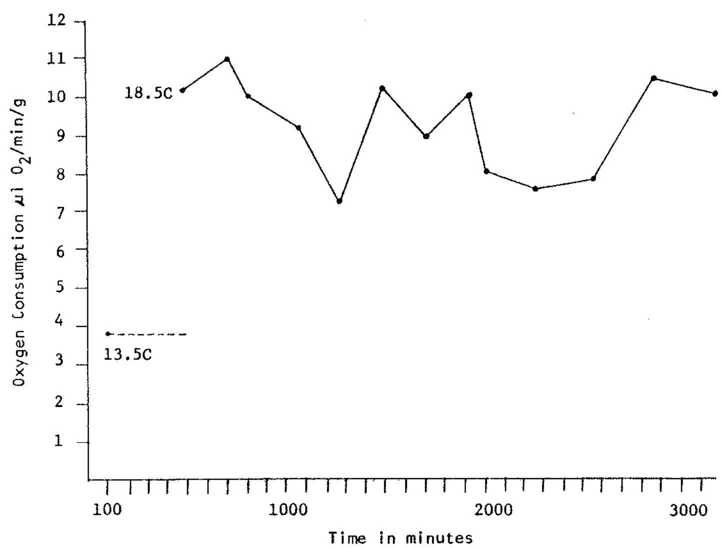

Figure 6. Oxygen consumption of *Argia vivida* under stress following temperature acclimation at 13.5 °C. - - - Oxygen consumption at 13.5 °C; —— Oxygen consumption during thermal stress at 18.5°C.

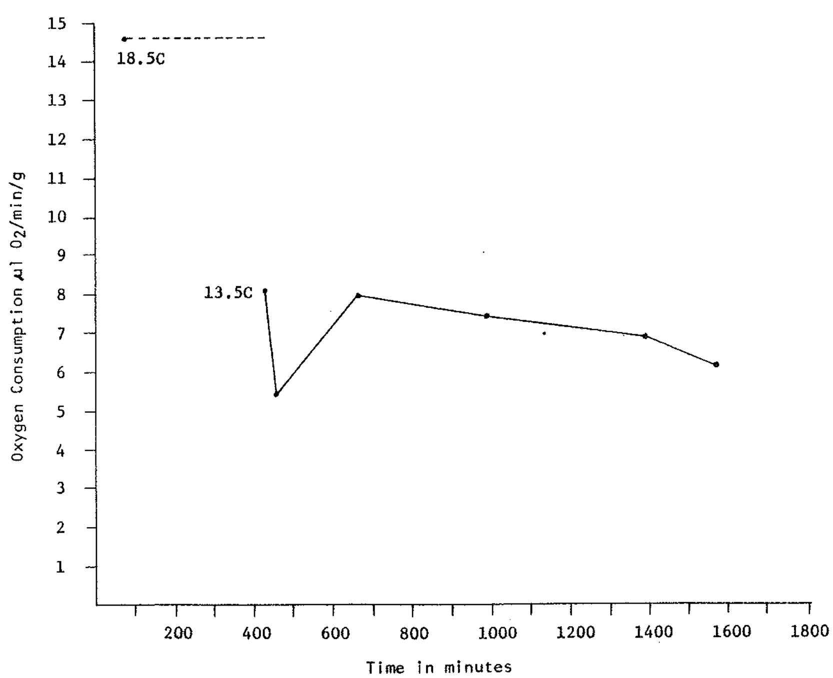

Figure 7. Oxygen consumption of *Argia vivida* at 13.5°C following acclimation at 18.5°C. ---- oxygen consumption at 18.5 °C; —— oxygen consumption at 13.5 °C.

Table 10. Oxygen consumption of *Hydropsyche occidentalis* (from Deep Creek) pupae. Replicate 1, May 16, 1972. Total time = 5 hours; water temperature = 15°C.

Manometer		ml O_2 used* per hour	Dry weight specimen (mg)
7		25.3	3.1
8		44.6	7.1
10		38.0	7.4
12		34.5	7.4
16		34.1	9.8
17		28.5	4.6
20		21.3	3.0
	TOTALS	226.3	42.4

$$Av = \frac{226.3}{42.4} = 5.2 \text{ Microliters } O_2/mg$$

*Values uncorrected

Factor = .04

Table 11. Oxygen consumption of *Hydropsyche occidentalis* (from Deep Creek) pupae. Replicate 2, May 17, 1972. Total time = 5 hours; water temperature = 15°C.

Manometer		ml O_2 used per hour	Dry weight specimen (mg)
6		23.7	8.4
7		51.6	3.1
10		59.2	7.4
12		30.0	7.4
14		55.2	7.4
16		47.0	9.8
17		29.2	4.6
20		20.4	3.0
	TOTALS	316.3	51.1

$$Av = \frac{316.3}{51.1} = 6.2 \text{ Microliters } O_2/mg$$

Table 12. Oxygen consumption of *Gammarus limnaeus* (Fairmont Park) adults. Replicate 1, May 18, 1972. Total time = 5 hours; water temperature = 15°C.

Manometer		ml O_2 used per hour	Dry weight specimen (mg)
7		50.2	11.3
8		30.2	10.0
10		40.0	9.3
12		47.8	11.8
14		39.0	8.5
15		65.6	7.0
16		65.0	13.2
17		89.4	10.0
18		72.3	7.2
20		65.9	6.7
	TOTALS	565.4	95.0

$$Av = \frac{565.4}{95.0} = 5.9 \text{ Microliters } O_2/mg$$

Table 13. Oxygen consumption of *Gammarus limnaeus* (Fairmont Park) adults. Replicate 2, May 19, 1972. Total time = 5 hours; water temperature = 15°C.

Manometer	ml O_2 used per hour	Dry weight specimen (mg)
7	51.2	11.3
8	30.0	10.0
10	41.6	9.3
12	36.2	11.8
15	40.0	7.0
16	87.2	6.6
17	74.5	6.5
19	73.0	7.2
20	42.8	6.7
TOTALS	476.5	76.4

$$Av = \frac{476.5}{76.4} = 6.2 \text{ Microliters } O_2/mg$$

Note: #16 and #17 died after Replicate 1 was completed: replaced with new specimens.

Table 14. Oxygen consumption of *Hyalella azteca* (Clover Creek). First run, May 22, 1972. Total time = 5 hours; water temperature = 15°C. Six specimens per manometer flask.

Manometer	ml O_2 used per hour	Dry weight of 6 specimen (mg)
7	30.2	3.3
10	11.0	3.1
11	14.6	3.9
13	14.2	2.8
14	15.2	3.1
15	7.2	3.7
16	5.8	3.2
17	11.2	3.4
20	5.7	3.6
TOTALS	115.1	30.1

$$Av = \frac{115.1}{30.1} = 3.8 \text{ Microliters } O_2/mg$$

Table 15. Oxygen consumption of *Hyalella azteca* (Clover Creek). Second run (new specimens), May 23, 1972. Total time = 5 hours; water temperature = 15°C. Six specimens per manometer flask.

Manometer	ml O_2 used per hour	Dry weight of 6 specimen (mg)
8	21.2	3.8
10	15.0	4.0
11	27.0	3.2
12	19.8	3.2
14	23.2	3.8
15	24.0	4.2
17	16.7	3.5
TOTALS	146.9	25.7

$$Av = \frac{146.9}{25.7} = 5.7 \text{ Microliters } O_2/mg$$

Effects of Minimal Dissolved Oxygen on Aquatic Invertebrates (DSCODE A3UG103)

Introduction

Oxygen is a basic need of aquatic insects, yet information concerning their 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. The rate of oxygen consumption is influenced by activity, temperature, nutrition, body size, stage in life cycle, season and time of day, as well as by 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 that has been 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 Kamler in 1969. An analysis of the various factors which influence the oxygen requirements and respiratory rates of benthic invertebrates is presented in "The Ecology of Running Waters" by Hynes (1970). The oxygen consumption of ten of the most common species of stoneflies of the western United States and the factors which modify their metabolic rates 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 twelve 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 this project were to determine the oxygen requirements of representative species of aquatic invertebrates from Deep Creek and to determine their relative sensitivity to low oxygen concentration. Oxygen levels necessary for survival and the long-term effects of low oxygen concentrations on molting, growth rates, time of emergence, and behavior patterns were investigated. While this project involved only four species from Deep Creek, 16 additional species of aquatic insects were also studied in connection with a water quality requirements research project being conducted at the University of Utah.

Methods

De-oxygenated water for the tests was obtained from degassing equipment as described by Mount (1965). Modifications included a cooling system and an oxygen "ladder." The ladder is constructed of single pane glass and cemented with silicone sealant. The ladder is 165 cm long, 17.5 cm wide and 17.5 cm deep. It is divided into 15 compartments each separated by a glass partition 5 cm high. The remainder of the divider is composed of fiberglass screen with a 1 mm mesh opening.

The de-oxygenated water comes from the degasser through plastic tubing, passes through the cooler and then enters one end of the ladder, which is elevated above the outlet end. As the water flows over the 5 cm compartment dividers its oxygen content increases. Rates of increase are dependent upon rate of inflow and the angle of inclination of the ladder. At an inclination of 40° from the horizontal and a flow rate of 1000 cc/min the oxygen increase per chamber is about 0.5 mg/l at 10°C.

Ten organisms were placed in each of seven test chambers and observed tw ce 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 varied plus or minus 25 cc/min. The temperature was taken daily with a pocket thermometer. A variation of plus or minus 0.5°C occurred. 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.

Results

Of the four species of aquatic invertebrates from Deep Creek that were tested, the scud (*Gammarus limnaeus*) was most sensitive to low dissolved oxygen concentrations with 60% of the test organisms dying within 24 hours at a dissolved oxygen concentration of 2.7 mg/l. The 96-hour TLm for this species was 3.2 mg/l. The caddis fly, *Hydropsyche occidentalis*, was nearly as sensitive with a 96-hour TLm of 2.4 mg/l, but 50% of the test specimens lived for 25 days at a dissolved oxygen concentration of only 3.3 mg/l.

By comparison, the damselfly (*Enallagma carunculatum*) was much less sensitive, with 50% of the test organisms surviving for 5 days at a dissolved oxygen concentration of 1.7 mg/l and for 14 days at a D.O. concentration of 1.9 mg/l. *Argia vivida*, another species of damselfly, was also very tolerant with a 96-hour TLm of 1.2 ml of oxygen per liter and 50% of the test specimens surviving for 15 days at a D.O. concentration of 4.2 mg/l.

In order to compare the oxygen requirements of these species from different habitats, specimens from Fairmont Park in Salt Lake County and Timpie Springs near Grantsville, Utah, were also tested, and the results were surprisingly close. The results of these tests are summarized in Tables 16 and 17. The complete data for the tests are on file in the data bank.

Table 16. Minimal dissolved oxygen requirements (mg/l) of four aquatic invertebrates.

	DEEP CREEK SPECIMENS				
Species	96-hour TLm	5-day TLm	14-day TLm	25-day TLm	
Gammarus limnaeus	3.2				
Hydropsyche occidentalis	2.3			3.3	
Enallagma carunculatum		1.7	1.9		
	FAIRMONT PARK - TIMPIE SPRINGS SPECIMENS				
	24-hour TLm	96-hour TLm	11-day TLm	15-day TLm	19-day TLm
Gammarus limnaeus	3.6	3.3			
Hydropsyche occidentalis		2.4	2.5	3.0	3.2
Enallagma anna			1.1	1.1	3.2
Argia vivida		1.2	2.9	4.2	

Table 17. Long-term dissolved-oxygen bioassays conducted at the University of Utah (Minimum dissolved oxygen with survival). Flow rate of 1000 cc/min.

Species	Minimum D.O. level (mg/l)	% 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

None of the specimens molted or emerged during these tests and any growth was undetectable. While the specimens were fed algae, decayed leaves, or fish food pellets during the runs, the comparatively short duration of each test did not allow for growth measurements to be taken.

While the principal objective of this project was to determine the minimal dissolved oxygen levels required for both short and long-term exposure, mere survival without growth and metamorphosis occurring would eliminate a species of aquatic insect eventually. While not all of the species tested molted or emerged during the long-term studies that have been conducted, 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% survival. Species such as 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 above.

The behavior of the organisms during testing was of interest. Stonefly (Plecoptera) nymphs subjected to low dissolved oxygen concentrations displayed "push up" movements periodically to increase the flow of water around their bodies and thereby increase diffusion of oxygen into their gills or trachea. Mayfly nymphs of several species displayed "gill beats" in order to increase oxygen transfer. The *H. occidentalis* nymphs tested undulated their abdomens, while the damselfly nymphs on occasion moved their gills slowly but erratically. *Gammarus limnaeus* showed no evident behavioral response to low oxygen values.

An evaluation of the average minimum dissolved oxygen requirements of the different groups of aquatic invertebrates that have been tested at the University of Utah shows the mayflies to be most sensitive, stoneflies next, and the caddis flies, fresh water shrimp, true flies, and damselfly, following in that order. While two species of mayflies 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% survival at 30 days. Three species of stoneflies from Utah survived at a dissolved oxygen concentration of 2.8 mg/l for 14 days with 50% surviving, but an average oxygen concentration of 4.9 mg/l was required for 30-50% survival for 62 days. The caddis flies tested also indicated higher oxygen levels were necessary with longer exposure, with a minimum of 4.0 mg/l being required for 50% survival for 84 days.

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

A comparison of the dissolved oxygen requirements for survival over longer periods of time than during conventional 96-hour bioassays is presented in Table 17. It should be noted that two of the species of damselflies from Deep Creek that were tested were among the most tolerant forms evaluated. Adaptation of these species to the relatively high temperatures and slow flow of the habitats in which they occur may be responsible for this increased tolerance.

LITERATURE CITED

American Public Health Association. 1965. Standard methods for the examination of water and wastewater. Twelfth Edition New York, N.Y. pp. 769.

Balke, E. 1957. Der O_2 Konsum und die Tracheen-Innenflache bei durch Tracheen Kiemen atmenden Insekten larven in Abhangig Keit von der Korpergrosse. Z. Vergl. Physiol. 40:415-439.

Brinck, Per. 1949. Studies on Swedish Stoneflies (Plecoptera). Lund Berlingeka Boktryckeiret, Sweden.

Dodds, G.S., and F.L. Hisaw. 1924. Ecological studies of aquatic insects. II. Size of respiratory organs in relation to environmental conditions. Ecology. 5:262-271.

Edwards, G.A. 1946. The influence of temperature upon the oxygen consumption of several arthropods. Jour. Cell. Comp. Physiol. 27:53-60.

Fair, G.M., and M.C. Whipple. 1948. Revision of the Microscopy of Drinking Water. 4th ed. John Wiley, New York.

Fox, H.M. 1936. Oxygen consumption of mayfly numphs in relation to available oxygen. Nature (London) Dec. p. 1015.

Gaufin, A.R., and C.M. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. Pub. Health Reprt., 67:57-64.

Gaufin, A.R., and C.M. Tarzwell. 1956. Aquatic macroinvertebrate communities as indicators of organic pollution in Lytle Creek. Sewage and Wastes. 28:906-924.

Gaufin, R.F., and A.R. Gaufin. 1961. The effects of low oxygen concentrations on stoneflies. Proc. Utah Acad. Sci., Arts and Let. 38:57-64

Knight, A.W., and A.R. Gaufin. 1963. The effect of water flow, temperature, and oxygen concentration on the Plecoptera nymph *Acroneuria pacifica* Banks. Proc. Utah Acad. Sci., Arts and Let. 40(2):175-184.

Knight, A.W., and A.R. Gaufin. 1964. Relative importance of varying oxygen concentration, temperature, and water flow on the mechanical activity and survival of the Plecoptera nymph *Pteronarcys californica* Newport. Ibid 41:14-28.

Knight, A.W., and A.R. Gaufin. 1965. Function of stonefly gills under reduced dissolved oxygen concentration. Ibid. 42(2):186-190.

Knight, A.W., and A.R. Gaufin. 1966. Oxygen consumption of several species of stoneflies (Plecoptera). J. Insect Physiol. 12:347-355.

Hynes, H.B.N. 1960. The Biology of Polluted Waters. Liverpool University Press.

Hynes, H.B.N. 1970. The Ecology of Running Waters. Univ. of Toronto Press. pp. 555.

Kamler, E. 1969. A comparison of the closed bottle and flowing water methods for measurement of respiration in aquatic invertebrates. Pol. Arch. Hydrobiol. 16:31-49.

Kolkwitz, R., and M. Marsson. 1909. Ockologie der tierschen Saprobien. Int. Revue ges. Hydrobiol. Hydrogr. 2:126-152.

Liebmann, H. 1951. Handbuch der Frischwasser und Abwasserbiologie. Vol. 1. R. Oldenbaurg. Munchen.

Madsen, B.L. 1968. The distribution of nymphs of *Brachyptera risi* Mort. and *Nemoura flexuosa* Aub. (Plecoptera) in relation to oxygen. Oikos 19:304-310.

Mount, D.I. 1964. Additional information on a system for controlling the dissolved oxygen content of water. Trans. Am. Fish. Soc., 93:100-103.

Olson, T.E., and M.E. Rueger. 1968. Relationship of oxygen requirements to index organism classification of immature aquatic insects. Jr. Water Poll. Control Fed. 40:R188-R202.

Petty, W.C. 1967. Studies on the oxygen requirements of two species of stoneflies (Plecoptera). Unpubl. Master's thesis, Dept. of Zoology and Entomology, Univ. of Utah, Salt Lake City, pp. 73.

Philipson, G.N. 1954. The effect of water flow and oxygen concentration on six species of caddis fly (Trichoptera) larvae. Proc. Zool. Soc. London (Brit.) 124:547-564.

Prosser, C.L., and Brown, F.A., Jr. 1961. Comparative Animal Physiology (2nd Ed.). W.B. Saunders, Philadelphia.

Reish, D.J., and T.L. Richards. 1966. A technique for studying the effect of varying concentrations of dissolved oxygen on aquatic organisms. Int. J. Air. Wat. Pollut. 10:68-71.

Roeder, K.D. 1953. Insect Physiology. John Wiley and Sons, Inc., New York, 1100 pp.

Wigglesworth, V.B. 1950. The Principles of Insect Physiology. E.P. Dutton and Co. New York, N.Y.