EFFECT OF PHENOL ON OXYGEN UPTAKE RATE OF A LABORATORY POPULATION OF *CHIRONOMUS ATTENUATUS*

By

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PREFACE

The objectives of this study were to measure respiration rates and energy lost to entropy through respiration in fourth-instar chironomid larvae raised in six different concentrations of phenol.

Dr. Jerry Wilhm served as major adviser. Drs. Bryan P. Glass and Roy Jones served as members of the advisory committee and criticized the manuscript. This study was part of a team research project in cooperation with Voyle D. Graham and Marlys P. Easton. The assistance and suggestions of these people are greatly appreciated.

I thank Dr. Selwyn S. Roback for identifying the larvae, Mr. Randy Overholt and the Bethany Nazarene College Data Processing Center for the computer and programs used in making calculations, and Mr. Gary Lance for assisting with the statistical design.

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CHAPTER I

INTRODUCTION

Benthic macroinvertebrates are particularly useful when investigating the effects of organic pollution on the energy flow of aquatic systems because their low motility causes them to be directly affected by substances in their environment (Gaufin and Tarzwell, 1952). These organisms indicate the history of past environments as well as present conditions (Paine and Gaufin, 1956). Although microinvertebrates share some of these advantages, macroinvertebrates are often preferred for study because of their larger size, ease of field identification, and longer life histories (Gaufin, 1958).

Since the development of the "Saprobiensystem" by Kolkwitz and Marsson (1908, 1909), the organisms of polluted waters have been used to establish biological measurements of enrichment. Analyses of structural relationships of aquatic communities have been made in an attempt to establish reliable criteria of water quality (Richardson, 1928; Gaufin and Tarzwell, 1952; Schiffman, 1953; Surber, 1953; Mackenthun, et. al., 1956; Paine and Gaufin, 1956; Gaufin, 1958). Some workers have used diversity indices to summarize community structure (Margalef, 1956; Patten, 1962; Wilhm and Dorris, 1966).

In addition to the structural approach, energy relationships in communities have been studied. The importance of evaluating aquatic

ecosystems in terms of energy flow was recognized with the introduction of the trophic-dynamic concept by Lindeman (1942). Although several studies of energy flow have been conducted in aquatic ecosystems (Odum and Odum, 1955; Patten, 1959), only a few studies have been made in systems receiving organic enrichment (Tubb and Dorris, 1965; King and Ball, 1967). Tubb and Dorris (1965) studied energy flow through three species of herbivorous chironomid larvae in a series of holding ponds receiving oil refinery effluent. The energy content of the effluent was lowered by losses to entropy as the energy passed between trophic levels of the system. They suggested that quantitative measure of energy flow through each trophic level would give an understanding of the waste treatment process. Only the more important parameters of energy flow suggested by Clarke, Edmundson, and Ricker (1946) were measured in the population. Estimates were made of standing crop in terms of caloric content and energy lost to the system through emergence. No measure of respiration was made for the community. It was suggested that additional quantitative studies on midge populations receiving organic enrichment would yield better energy estimates.

Tubb and Dorris (1965) considered that the concentration of the cyclic organic compound, phenol, was a reliable index of effluent toxicity. Phenol is a common constituent of oil refining, coal-tar processing, and other chemical manufacturing waste effluents (Klein, 1957). Phenol has strong bactericidal action (Klein, 1957) and is directly poisonous to aquatic animals (Sorenson, 1948). It has an irritating action on mucous membranes and has a direct effect on the nervous systems of all higher animals (Klein, 1957). Phenol acts on

the nervous system by solubilizing lipids and acts on the circulatory system by dissolving erythrocytes (Linhardt, 1951). Some phenolic derivatives such as 2, 4 dinitrophenol act as uncouplers inhibiting normal sequence of reactions in the cytochrome chain. Concentrations of 8 - 10 ppm phenol in water have been found lethal for Daphnia and Cyclops (Ellis, 1937). The toxic threshold for fishes is about 1.0 ppm (Wilber, 1969). Jenkins (1964) found the concentration of phenol to vary between 0 and 271 ppm in effluents of 11 Oklahoma oil refineries. Typical pollution control laws limit phenol in waste streams discharging into natural waterways to 0.02 to 2 ppm (Beychok, 1967).

The effects produced by the introduction of oil refinery effluent into an aquatic system are due to the combined action of large numbers of toxicants and environmental conditions. Therefore, a single toxicant, phenol, was chosen for the present study because the effects it produced could be interpreted without interference from other toxic components. A laboratory investigation was conducted so that important water quality parameters such as hydrogen-ion concentration, dissolved organics, temperature, and level of oxygen could be controlled or measured.

A number of studies have been made on the oxygen uptake rate of chironomid larvae. The rate of oxygen used has been shown to be dependent on the water content of the larvae (Buck, 1964). Walshe (1948) demonstrated that chironomid larvae taken from streams consume more oxygen than do those taken from still water habitats. It has been shown that oxygen uptake by unit weight is inversely related to body size (Edwards, 1958). The relationship of haemoglobin to oxygen uptake

in chironomids has been studied in some detail (Ewer, 1942; Walshe, 1947a and 1947b; Platzer-Schultz, 1968). No investigations of the effects of phenol on the oxygen uptake rate of Chironomus larvae has been conducted.

The present study is part of a team research effort to study the effect of phenol on energy flow parameters in a laboratory population of Chironomus attenuatus (Walk.). The parameters measured were feeding rates, assimilation rates, assimilation efficiency, energy content, and oxygen uptake. In this investigation the effect of life-long exposure to various levels of phenol on oxygen uptake rate of fourth instar larvae was examined.

CHAPTER II

METHODS AND MATERIALS

Laboratory Population

The Chironomus attenuatus larvae used in this study were taken from a laboratory population collected originally from an enriched area of Skeleton Creek, Oklahoma by Wilhm and Dorris (1966). The organisms were reared in six metal trays, 88 x 56 x 18 cm, painted with white non-toxic marine enamel. Each tray was covered with a plastic screen cage, 90 x 57 x 42 cm, to provide space for emergence and mating of adults. The bottom of each tray was covered with 2.5 cm of washed sand. The larvae were fed Hartz Mountain Dog Kisses[1] in solution daily (Beiver, 1965).

Phenol

Phenol in solution is subject to bacterial decomposition (Ettinger and Ruchhoft, 1949). In pilot experiments it was found that the presence of either 40 living or dead midge larvae in a 40 ppm solution of phenol caused the concentration of phenol to drop to 8 ppm in 89.5 hr. Controls without larvae remained near 25 ppm throughout

[1]Registered trademark, Hartz Mountain Products Corp., New York, New York.

the experiment (Table I). It is assumed that reduction in the concentration of phenol was due to some factor associated with the presence of living and dead larvae, such as bacteria.

Continuous-flow System

Two continuous-flow systems were constructed to provide a continuously renewed supply of phenol solution to the units in which the larvae to be tested were placed (Fig 1). This system provided a constant concentration of phenol to the units. In each system phenol solution of the proper concentration was mixed and stored in a 200-liter plastic storage tank. Solution flowed from the storage tank to a 10-liter head tank. A constant level of solution was maintained in the head tank by a float valve. The flow of solution from the head tank to the individual experimental units was adjusted by means of a medical dropping chamber and a clamp valve.

Individual experimental units consisted of plastic buckets of 10 liters capacity. Phenol solution entered the units 5 cm above the bottom. Solution left the buckets through overflow outlets, which were set at a level that would maintain exactly 5 liters of solution in the units. Proper flow adjustment maintained the concentration of phenol at the level desired. Each unit was covered with plastic and provided with air. Since pilot studies indicated that larval survival was increased if a sand substrate was provided, 500 ml of ashed sand was placed in the bottom of each unit.

TABLE I

TEMPORAL DECREASE OF PHENOL (ppm) IN UNITS CONTAINING 0, 40 DEAD, AND 40 LIVING CHIRONOMUS ATTENUATUS FOURTH-INSTAR LARVAE

Phenol	No. of	Replicate	Time (hr)			
			18.0	41.5	65.5	89.5
40	None	1	38.0	36.7	30.5	21.4
		2	38.5	38.0	34.3	26.3
		3	37.7	38.0	36.0	27.1
		$\bar{x}$	38.1	27.5	33.6	24.9
40	40 Dead	1	32.8	35.3	17.7	0.4
		2	38.3	28.5	8.8	0.2
		3	38.8	24.4	0.0	0.0
		$\bar{x}$	36.6	29.4	8.8	0.2
40	40 Live	1	38.1	32.4	21.4	7.1
		2	38.8	26.5	4.9	0.2
		3	38.3	27.6	13.7	0.1
		$\bar{x}$	38.4	28.8	13.3	2.5

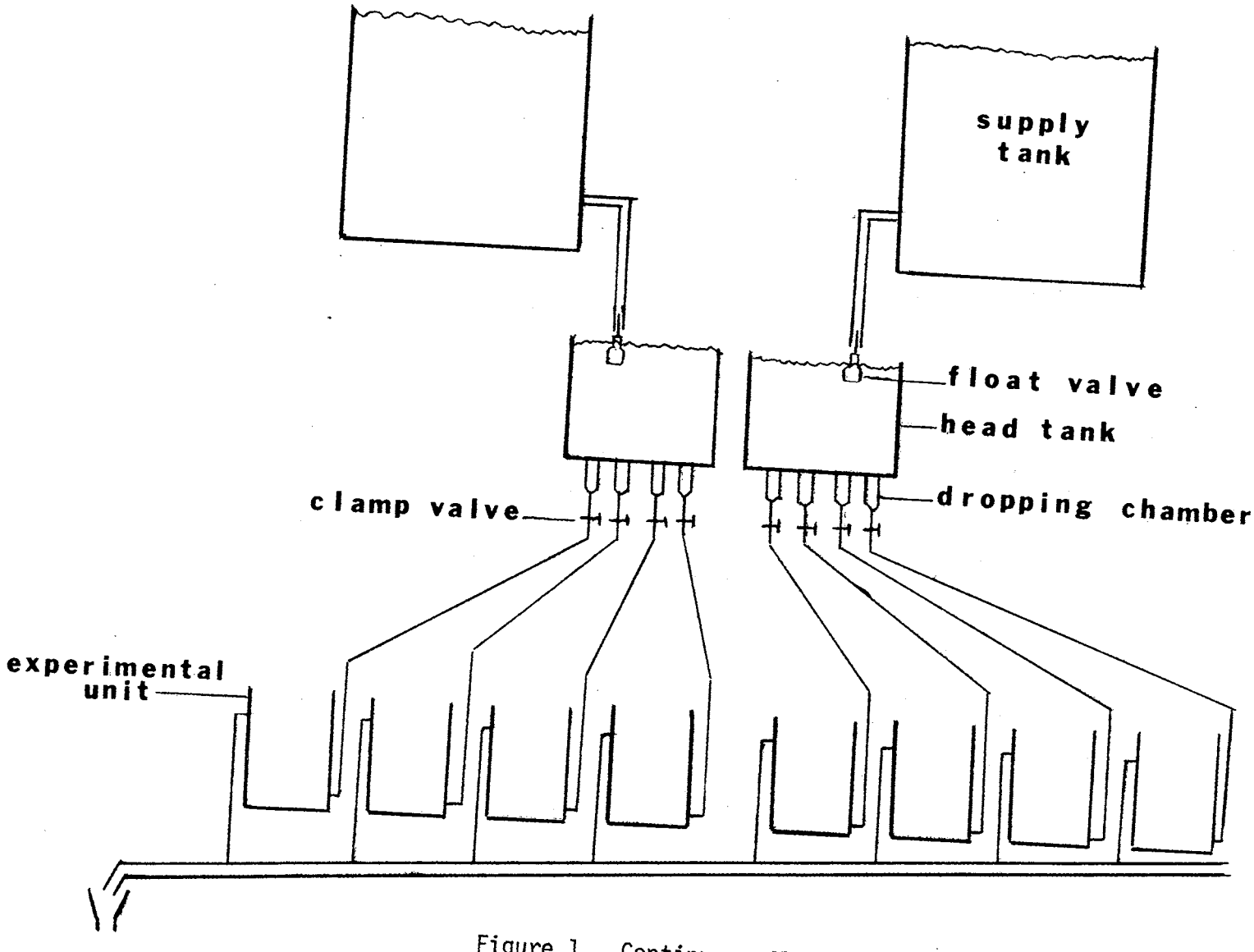

Figure 1. Continuous-flow systems.

Operation of System

Pilot studies indicated that Chironomus attenuatus will not survive to the fourth-instar if raised in a phenol solution exceeding 25 ppm. Therefore, the levels of phenol solution in the treatments selected for study were 0, 5, 10, 15, 20, and 25 ppm. All phenol solutions were made using reagent grade, crystalline phenol and deionized water.

Since only two treatments could be run simultaneously, two experimental units were started for each treatment with two more being started two days later. This was done so that differences due to time of starting could be tested and separated from differences among units due to the level of phenol.

For each level of phenol, the flow of solution was started and one egg mass from each of the six rearing trays was placed in each of the four experimental units. 100 ml of feeding solution (one Hartz Mountain Dog Kiss in 500 ml of deionized water) was placed in each unit daily. The level of phenol in each unit was determined every other day using the 4-amino-antipyrine method determination outlined by the American Public Health Association (1960). A Bausch and Lomb Spectronic 20 spectrophotometer was used for the determinations of phenol level. The concentration of oxygen, pH, and temperature of the water was measured weekly in each unit. Oxygen determinations were made using the modified Winkler method. Hydrogen-ion concentration was measured using a Beckman Zeromatic pH-meter. Temperature was determined with a mercury thermometer.

Oxygen Uptake Tests

Larvae reached fourth instar in 21 days at a laboratory temperature of 21 - 25° C. Therefore, the larvae were removed for oxygen uptake tests 21 days after the egg masses were added to the experimental units. The contents of the unit to be tested were placed in a plastic pan. The bottom of the pan was divided into one-hundred grid squares. A table of random numbers was used to designate a square to be sampled. Fifteen fourth-instar larvae from the square selected were added to a manometer flask of 15 ml volume. Volume of flask contents including the larvae was adjusted to 5.0 ml with phenol solution from the unit being tested. Carbon dioxide was absorbed by 0.3 ml of KOH in the centerwell of the flask. Five flasks were prepared for each unit tested. The flasks were attached to manometers and placed in a waterbath at 21° C. After 30 to 60 min equilibration, stopcocks were closed and readings taken every 15 min for 1.5 hr. A black plastic hood was placed over the manometers to exclude light, which causes the larvae to be hyperactive (Walshe, 1949). The direct method of Warburg was used to make all determinations (Umbreit, 1957). A 14-station Warburg manometer apparatus with controlled temperature water bath was used throughout the study. A shaking rate of 106/min with flasks describing archs of 4.8 cm was used.

After oxygen uptake measurements were made, larvae from each flask were removed, placed in tared crucibles, and dried at 103° C to determine the oven-dry weight of the group. The larvae were then ashed at 530° C to determine the ash-free weight.

Corrections for oxygen uptake in the water due to factors other than the larvae were made. Five ml of solution from the unit being tested was placed in each of two flasks. Average oxygen uptake values obtained were subtracted from those of the larvae in solution to correct for the nonlarval oxygen uptake in the flasks that contained larvae.

Statistical Design

Statistical design for this segment of the study was a completely randomized block design with subsamples. Times of starting were considered blocks. Phenol levels were the treatments. Experimental units were treated as samples and actual respiration measurements were made on the subsamples taken from the experimental units. An IBM 1130 computer was used in regression and correlation analyses. "REGRE" and "CORRE" programs from the IBM Scientific Subroutine package were used for regression and correlation analyses respectively. Other statistical proceedures were taken from Steele and Torrie (1960).

CHAPTER III

RESULTS AND DISCUSSION

Physical and Chemical Conditions

Mean values of oxygen concentration of replicate units ranged from 5.57 to 7.20 ppm with an overall mean of 6.58 and a standard deviation of 0.44 for all units (Table II). This was due to variation in air line pressure in the aeration system, in room temperature, and atmospheric pressure. Average hydrogen-ion concentration for units varied from a pH of 6.00 to 7.94 (Table III). Mean pH for all units was 7.32 with a standard deviation of 0.66. Adjustments with sodium hydroxide solution were made to retain a neutral pH if readings taken were below 7.0.

Average water temperature in replicate units varied from 20.5 to 24.3^{o} C (Table IV). Average temperature for all units was 22.5^{o} C with a standard deiation of 0.98. Higher temperatures reduce the maturation time of midge larvae (Hilsenhoff, 1966). There were no emergences from water with an average temperatue of 20.5^{o} C, whereas in water averaging 24.3^{o} C emergences occurred on the 16th day of the test (Table IV). The variation in the maturity of the populations caused by temperature difference did not affect the results of the tests since oxygen uptake was measured only in fourth instar larvae.

TABLE II

MEAN DISSOLVED OXYGEN CONCENTRATION (ppm)
IN REPLICATED UNITS BY LEVEL OF PHENOL

REPLICATE NUMBER	Level of Phenol (ppm)					
	0	5	10	15	20	25
1	6.20	7.20	7.16	7.17	5.57	5.84
2	6.70	7.10	6.83	6.58	6.73	5.83
3	6.50	6.70	6.35	6.72	6.63	6.56
4	6.00	6.90	6.23	6.99	6.80	6.65
$\bar{x}$	6.35	6.98	6.64	6.87	6.43	6.22

TABLE III

MEAN HYDROGEN-ION CONCENTRATION (pH) IN REPLICATE UNITS BY LEVEL OF PHENOL

REPLICATE NUMBER	Level of Phenol (ppm)					
	0	5	10	15	20	25
1	6.00	7.70	7.37	6.94	7.78	7.68
2	6.00	7.87	7.39	6.99	7.90	7.66
3	6.00	7.80	7.75	6.96	7.90	7.83
4	6.00	7.87	7.70	7.19	7.94	7.46
$\bar{x}$	6.00	7.81	7.55	7.02	7.88	7.66

TABLE IV

MEAN WATER TEMPERATURE AND DAY OF FIRST EMMERGENCE OF ADULT CHIRONOMIDS IN REPLICATE UNITS BY LEVEL OF PHENOL

REPLICATE NUMBER	Level of Phenol (ppm)											
	0		5		10		15		20		25	
	Temp (°C)	Day	Temp (°C)	Day	Temp (°C)	Day	Temp (°C)	Day	Temp (°C)	Day	Temp (°C)	Day
1	20.7	*	22.8	14	22.2	*	21.8	*	23.0	20	24.3	16
2	20.5	*	22.8	14	22.2	*	21.8	*	22.8	21	24.0	18
3	21.2	*	22.8	14	22.5	21	22.2	*	23.2	21	23.8	17
4	21.0	*	22.8	14	22.7	*	22.3	*	22.3	21	23.8	17

* No emmergence during study

Larval Populations

In general, fourth-instar larvae in all treatment units were active. Some larvae constructed feeding tubes in the sand covering the bottom of the experimental units. Other larvae built tubes in the bacterial slime which had formed on the sides of the containers. Larvae observed performed normal feeding movements as described by Walshe (1947c).

Mean oven-dry weight per individual varied from 0.500 to 1.085 mg (Table V). Overall average oven-dry weight per individual was 0.724 mg with a standard deviation of 0.175. Average ash-free weight per individual ranged from 0.441 to 0.979 mg (Table VI). Average ash-free weight for all units was 0.659 mg with a standard deviation of 0.156. Oven-dry weight per individual was directly related to ash-free weight per individual. Ash-free weight (Y) was 86% of oven-dry weight (X). Fig 2 shows this relationship. The regression equation was

$$Y = 0.033 + 0.865\ X.$$

Since some larvae were found to have ingested sand particles which could contribute to the oven-dry weight, but not to the ash-free weight, the latter was used in all calculations.

Phenol Level

Bacterial action reduced phenol concentration in the experimental units below the specified treatment levels despite the constant flow of phenol solution at the level desired to each unit (Tables VII and

TABLE V

MEAN DRY WEIGHT (mg) PER INDIVIDUAL OF EACH SUBSAMPLE
IN REPLICATE UNITS BY LEVEL OF PHENOL

REPLICATE NUMBER	SUBSAMPLE NUMBER	Level of Phenol (ppm)					
		0	5	10	15	20	25
1	1	0.827	1.022	0.513	0.600	0.659	1.053
	2	0.888	1.057	0.698	0.541	0.769	0.979
	3	0.767	0.943	0.600	0.547	0.729	1.077
	4	0.789	1.115	0.716	0.533	0.706	0.913
	5	0.809	1.096	0.687	0.447	1.050	0.879
	$\bar{x}$	0.816	1.047	0.643	0.534	0.783	0.980
2	1	0.739	0.786	0.619	0.607	0.574	0.755
	2	0.663	0.535	0.596	0.601	0.596	0.814
	3	0.765	0.733	0.579	0.577	0.609	0.639
	4	0.754	0.750	0.571	0.731	0.624	0.773
	5	0.743	0.849	0.541	0.761	0.466	0.599
	$\bar{x}$	0.733	0.731	0.581	0.655	0.574	0.716

TABLE V (continued)

REPLICATE NUMBER	SUBSAMPLE NUMBER	Level of Phenol (ppm)					
		0	5	10	15	20	25
3	1	0.773	0.641	0.366	0.464	0.691	0.798
	2	0.901	0.613	0.516	0.548	0.694	0.625
	3	0.931	0.714	0.611	0.534	0.691	0.816
	4	0.888	0.687	0.556	0.565	0.795	0.856
	5	0.942	0.714	0.453	0.521	0.702	0.823
	$\bar{x}$	0.887	0.674	0.500	0.526	0.715	0.784
4	1	1.205	0.887	0.620	0.613	0.592	0.788
	2	1.165	0.741	0.494	0.656	0.803	0.818
	3	0.834	0.803	0.490	0.491	0.712	0.685
	4	1.028	0.764	0.417	0.555	0.643	0.859
	5	1.200	0.800	0.551	0.725	0.775	0.791
	$\bar{x}$	1.085	0.799	0.514	0.608	0.705	0.788

TABLE VI

MEAN ASH-FREE WEIGHT (mg) PER INDIVIDUAL OF EACH SUBSAMPLE IN REPLICATE UNITS BY LEVEL OF PHENOL

REPLICATE NUMBER	SUBSAMPLE NUMBER	Level of Phenol (ppm)					
		0	5	10	15	20	25
1	1	0.765	0.934	0.486	0.524	0.609	0.977
	2	0.763	0.964	0.658	0.519	0.717	0.901
	3	0.691	0.853	0.563	0.521	0.648	0.992
	4	0.718	1.020	0.693	0.505	0.647	0.850
	5	0.740	0.999	0.647	0.420	0.629	0.808
	$\bar{x}$	0.735	0.954	0.610	0.498	0.650	0.906
2	1	0.683	0.694	0.574	0.576	0.533	0.692
	2	0.601	0.482	0.559	0.574	0.553	0.728
	3	0.711	0.621	0.529	0.553	0.561	0.597
	4	0.699	0.581	0.538	0.561	0.589	0.714
	5	0.680	0.751	0.493	0.713	0.429	0.551
	$\bar{x}$	0.677	0.626	0.539	0.595	0.533	0.656

TABLE VI (continued)

REPLICATE NUMBER	SUBSAMPLE NUMBER	Level of Phenol (ppm)					
		0	5	10	15	20	25
3	1	0.708	0.594	0.341	0.459	0.639	0.734
	2	0.827	0.558	0.482	0.516	0.650	0.569
	3	0.855	0.643	0.548	0.507	0.631	0.752
	4	0.811	0.625	0.524	0.537	0.740	0.786
	5	0.878	0.636	0.427	0.488	0.646	0.754
	$\bar{x}$	0.816	0.611	0.464	0.501	0.661	0.719
4	1	1.108	0.800	0.442	0.565	0.541	0.740
	2	1.025	0.677	0.449	0.609	0.691	0.756
	3	0.761	0.731	0.444	0.442	0.633	0.642
	4	0.895	0.699	0.369	0.511	0.586	0.810
	5	1.107	0.735	0.500	0.628	0.701	0.752
	$\bar{x}$	0.979	0.728	0.441	0.551	0.630	0.740

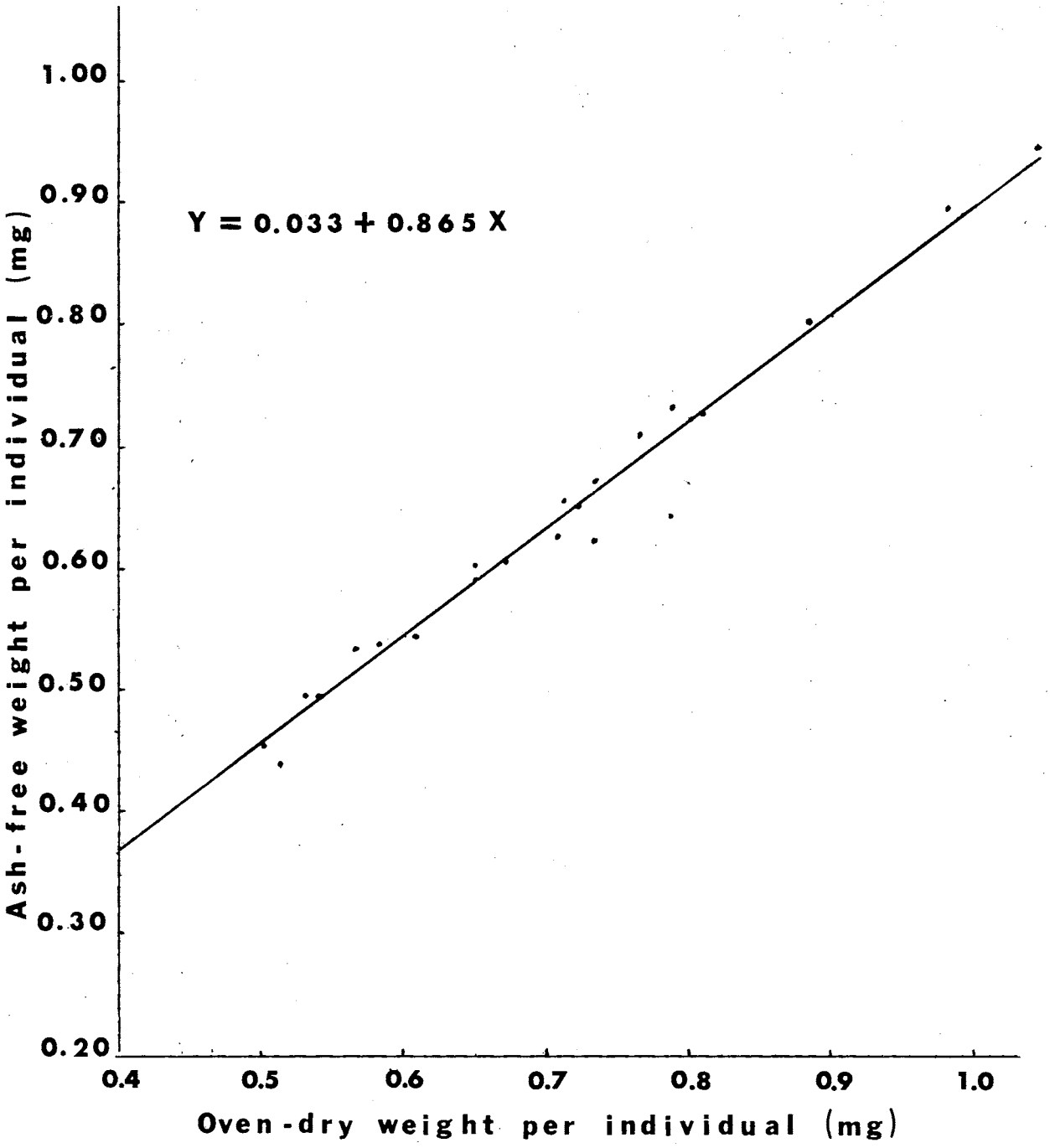

Figure 2. Regression of ash-free weight per individual on oven-dry weight per individual of Chironomus attenuatus fourth-instar larvae

VIII). Average reduction of phenol per unit was 2.83 ppm. Although the amount of phenol lost increased as treatment level increased, the two did not increase proportionately.

Oxygen Uptake

Average value of oxygen uptake for larvae raised at the 0 ppm treatment level, no phenol, was 1.65 µl/mg per hr (Table IX). This falls within the range of values measured for chironomid larvae by other investigators (Table X). Values for oxygen uptake rate were converted from those given by the authors considering that ash-free weight was 16% of wet weight and 86% of oven-dry weight. The relationship of ash-free weight and wet weight was determined in pilot studies using Chironomus larvae. That of ash-free weight and oven-dry weight was determined in this study.

Multiple regression analysis revealed that mean oxygen concentration, mean hydrogen-ion concentration, mean ash-free weight per individual, and mean phenol level were contributing to the regression of oxygen uptake by the larvae (Table XI). The regression equation determined was

$$Y = 2.985 + 0.151\ X_1 - 0.195\ X_2 - 1.284\ X_3 + 0.017\ X_4$$

where: Y = oxygen consumed (µl),

X_1 = mean oxygen concentration (ppm),

X_2 = mean hydrogen-ion concentration (pH),

X_3 = mean ash-free weight per individual (mg),

X_4 = mean level of phenol (ppm).

TABLE VII

MEAN PHENOL LEVEL (ppm) IN REPLICATE UNITS
BY LEVEL OF PHENOL

REPLICATE NUMBER	Level of Phenol (ppm)					
	0	5	10	15	20	25
1	0.00	3.04	7.40	11.79	16.49	21.47
2	0.00	3.05	7.65	10.71	16.41	22.70
3	0.00	2.24	8.50	11.23	15.92	23.01
4	0.00	2.94	9.16	11.08	16.44	22.26
$\bar{x}$	0.00	2.82	8.18	11.20	16.32	22.36

TABLE VIII

AMOUNT OF PHENOL REDUCTION (ppm) BY BACTERIAL ACTION IN REPLICATE UNITS BY LEVEL OF PHENOL

REPLICATE NUMBER	Level of Phenol					
	0	5	10	15	20	25
1	0.00	1.96	2.60	3.21	3.51	3.53
2	0.00	1.95	2.35	4.29	3.59	2.30
3	0.00	2.76	1.50	3.77	4.08	1.99
4	0.00	2.06	0.84	3.92	3.56	2.74
$\bar{x}$	0.00	2.18	1.82	3.80	3.67	2.64

TABLE IX

UPTAKE OF OXYGEN (µl/hr) PER MG ASH-FREE WEIGHT IN A LABORATORY POPULATION OF CHIRONUMUS ATTENUATUS NOT EXPOSED TO PHENOL

SUBSAMPLE	Sample			
	1	2	3	4
1	1.47	1.86	1.75	1.34
2	1.42	1.82	1.94	1.46
3	1.38	1.16	1.77	1.88
4	1.42	1.91	1.91	1.59
5	1.67	1.73	2.15	1.27

TABLE X

UPTAKE OF OXYGEN (μl/hr) PER MG ASH-FREE WEIGHT BY CHIRONOMID LARVAE AS REPORTED BY OTHER INVESTIGATORS

REFERENCE	SPECIES	SEASON	TECHNIQUE	TEMP	WEIGHT	QO_2
Edwards, 1957	C. riparius	August	Manometer	20°C	1.45	2.51
Ransom, 1969	C. plumosus	*	Manometer	*	*	3.77
Ransom et. al., 1969	C. plumosus	*	Manometer	30°C	0.91	4.78
Walshe, 1947a	C. plumosus	May-June	Micro-Winkler	17°C	*	1.16
Walshe, 1947a	C. plumosus	September	Micro-Winkler	17°C	*	1.02
Walshe, 1947b	Tanytarsus	*	Micro-Winkler	17°C	*	3.19

* Not given

TABLE XI

MULTIPLE REGRESSION ANALYSIS OF FOUR INDEPENDENT, ADJUSTED VARIABLES ON THE DEPENDENT VARIABLE, OXYGEN UPTAKE

VARIABLE TESTED	VARIABLES USED IN ADJUSTMENT	SUM OF SQUARES	MEAN SQUARES	F VALUE	SIGNIFICANCE LEVEL
Mean Oxygen Level	Mean Phenol Level Mean pH Value Mean A-F Wt/Indiv	0.40881	0.40881	3.02531	0.100
Mean pH Level	Mean Phenol Level Mean Oxygen Level Mean A-F Wt/Indiv	1.20282	1.20282	8.90121	0.100
Mean A-F Wt/Indiv	Mean Phenol Level Mean Oxygen Level Mean pH Level	3.52096	3.52096	25.70400	0.005
Mean Phenol Level	Mean A-F Wt/Indiv Mean Oxygen Level Mean pH Level	1.15550	1.15550	8.55102	0.005

When mean water temperature (^{o}C) was included, it was found to be insignificant in the regression (Table XII). The effect of the mean water temperature in the treatment units during the time of exposure to phenol was minimized because oxygen uptake rates of all larvae were determined at 21^{o} C. The correlation coefficient between mean water temperature and mean hydrogen-ion concentration was 0.841 and was significant at the 0.01 level. Since mean water temperature was not significant to the regression and since the mean hydrogen-ion concentration and mean water temperature were not independent from one another, mean water temperature was not considered as an independent variable in further regressions.

Mean concentration of oxygen in the water of the experimental units contributed significantly to the regression of oxygen uptake rate at the 0.10 level when water temperature was not included as an independent variable (Table XI). Walshe (1947b) demonstrated that oxygen consumption in Tanytarsus (Chironomidae) was dependent on oxygen pressure at all concentrations below air saturation. Therefore, mean oxygen concentration was included as an independent variable in further regressions.

Mean hydrogen-ion concentration (pH) was included as an independent variable. It was significant at the 0.005 level when mean water temperature was included or excluded from the regression (Tables XI and XII). The pH level is known to have an effect on the action of phenol compounds affecting oxygen uptake. Whitley and Sikora (1970) suggested that the hydrogen-ion affected the toxicity of pentachloraphenol (PCP) by alteration of certain protein molecules and the activation and

TABLE XII

MULTIPLE REGRESSION ANALYSIS OF FIVE INDEPENDENT, ADJUSTED VARIABLES ON THE DEPENDENT VARIABLE OXYGEN UPTAKE

VARIABLE TESTED	VARIABLES USED IN ADJUSTMENT	SUM OF SQUARES	MEAN SQUARE	F VALUE	SIGNIFICANCE LEVEL
Mean Oxygen Level	Mean Phenol Level Mean Water Temp Mean pH Level Mean A-F Wt/Indiv	0.58047	0.58047	4.38289	0.050
Mean Water Temp	Mean Phenol Level Mean Oxygen Level Mean pH Level Mean A-F Wt/Indiv	0.44208	0.44208	3.33796	0.100
Mean pH Level	Mean Phenol Level Mean Oxygen Level Mean Water Temp Mean A-F Wt/Indiv	1.24286	1.24286	9.38432	0.005
Mean A-F Wt/Indiv	Mean Phenol Level Mean Oxygen Level Mean Water Temp Mean pH Level	4.22412	4.22412	31.89459	0.005
Mean Phenol Level	Mean Oxygen Level Mean Water Temp Mean pH Level Mean A-F Wt/Indiv	0.45655	0.45655	3.5424	0.100

suspension of enzyme systems. Variation in hydrogen-ion concentration may also affect the acidic properties of phenol compounds which are responsible for their toxicity.

Mean ash-free weight was significant to the regression of oxygen uptake rate at the 0.005 level both with and without mean water temperature included as an independent variable (Tables XI and XII). It has been demonstrated in aquatic insects that the rate of oxygen uptake per unit weight decreases as total body weight increases (Balke, 1957; Edwards, 1958). Such a relationship was found in the Chironomus attenuatus fourth-instar larvae examined in this study. The oxygen uptake rates recorded were adjusted for mean oxygen concentration, mean hydrogen-ion concentration, and mean phenol level using the previously given equation. The logarithm of the adjusted values was used as the dependent variable (log Y) and the logarithm of the ash-free weight per individual as the independent variable (log X) in the calculation of a linear regression equation (Fig 3). The equation was

$$\log Y = 0.173 - 0.478 \log X.$$

Ash-free weight per individual was retained as an independent variable for further regressions.

Using the first equation calculated, values of oxygen uptake were adjusted for mean oxygen concentration, mean hydrogen-ion concentration, and mean ash-free weight per individual (Table XIII).

The exposure of the organisms to the various phenol levels at different times which was necessitated by the limited testing facilities did not seriously influence the uptake of oxygen by the larvae. A

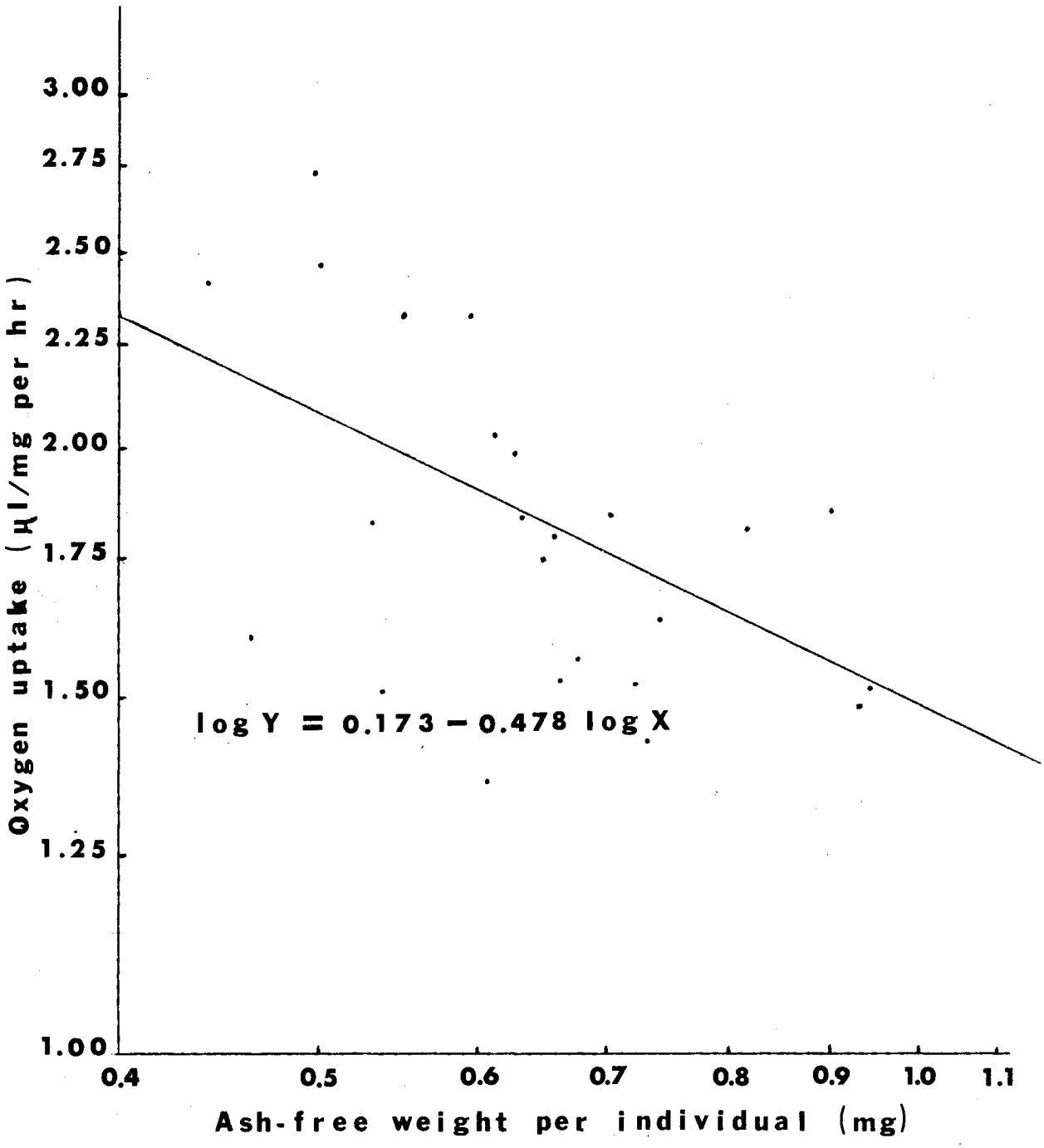

Figure 3. Regression of oxygen uptake (µl/mg per hr) on ash-free weight per individual (mg) in a laboratory population of Chironomus attenuatus fourth-instar larvae.

TABLE XIII

OXYGEN UPTAKE (μl/mg per hr) IN A LABORATORY POPULATION OF CHIRONOMUS ATTENUATUS ADJUSTED FOR MEAN OXYGEN CONCENTRATION, MEAN pH LEVEL, AND MEAN ASH-FREE WEIGHT/INDIVIDUAL

REPLICATE	SUBSAMPLE	Level of Phenol (ppm)					
		0	5	10	15	20	25
1	1	1.41	1.35	1.58	2.86	1.15	2.23
	2	1.35	2.71	1.36	2.23	2.18	2.31
	3	1.23	1.49	0.88	2.05	2.11	2.41
	4	1.30	1.75	1.39	2.11	1.63	2.67
	5	1.57	1.66	1.53	2.13	2.17	2.09
	$\bar{x}$	1.37	1.79	1.35	2.28	1.85	2.34
2	1	1.61	2.02	1.66	2.11	1.79	1.82
	2	1.47	1.88	1.10	2.75	1.70	2.25
	3	0.95	1.93	1.12	2.27	1.91	1.78
	4	1.68	1.36	1.08	1.90	1.72	1.80
	5	1.48	2.00	1.60	2.23	1.79	2.32
	$\bar{x}$	1.44	1.84	1.91	2.25	1.78	1.99

TABLE XIII (continued)

REPLICATE	SUBSAMPLE	Level of Phenol (ppm)					
		0	5	10	15	20	25
3	1	1.56	2.34	2.09	1.79	1.84	1.97
	2	1.91	1.84	2.03	2.61	1.87	1.94
	3	1.77	1.56	1.57	2.30	1.76	1.46
	4	1.86	1.81	1.83	2.39	2.39	2.06
	5	2.18	1.68	1.48	2.37	1.96	1.70
	$\bar{x}$	1.86	1.85	1.80	2.29	1.96	1.83
4	1	1.75	2.19	2.30	2.00	2.04	1.98
	2	1.76	1.90	2.09	2.22	1.75	1.77
	3	1.84	1.76	2.22	2.25	1.88	2.44
	4	1.72	1.98	1.82	2.25	1.95	2.08
	5	1.68	1.90	2.16	2.33	1.94	1.61
	$\bar{x}$	1.75	1.95	2.12	2.21	1.91	1.98

randomized block analysis of variance performed on the adjusted values revealed that the day of testing did not significantly affect the test results. However, the effect of mean phenol level was highly significant (Table XIV).

The adjusted values of oxygen uptake were used as the dependent variables (Y) and the logarithms of the mean phenol levels were used as the independent variables (X) in determining a regression line (Fig 4). The equation was

$$Y = 1.632 + 0.299 \log X.$$

The regression reveals that oxygen uptake by the larvae increased with the increase in phenol concentration. Whitely and Sikora (1970) found a similar logarithmic response in respiration of tubificid worms exposed to pentachloraphenol (PCP). They suggested that this was due to PCP's action as an uncoupler in oxidative phosphorylation as discussed in Weinback (1954). Other substituted phenol compounds are known to act as uncouplers in the cytochrome chain and produce similar increases in respiration (Rockstein, 1964). Phenol is less polar and therefore is less toxic than the substituted phenols (Brown, 1951). It is probable that the action of phenol is the same, an uncoupler of oxidative phosphorylation, but that it tends to increase respiration to a lesser degree than the substituted phenols.

An increase in oxygen uptake represents an increase in energy lost from the population through respiration. The average oxycalorific coefficient determined by Ivlev (1934) was used to calculate the number of calories released by an average larval individual in 1 hr (Table XV)

TABLE XIV

ANALYSIS OF VARIANCE FOR OXYGEN UPTAKE VALUES (µl/mg per hr) IN CHIRONOMUS ATTENUATUS ADJUSTED FOR MEAN OXYGEN CONCENTRATION, MEAN pH LEVEL, AND MEAN ASH-FREE WEIGHT/INDIVIDUAL

SOURCE	DF	SS	MS	F	SIGNIFICANCE LEVEL
Blocks	1	0.754	0.754	3.847	0.100
Replicates in Blocks	2	0.853			
Treatments	5	5.973	1.195	6.097	0.005
Experimental Error	12	2.349	0.196		
Sampling Error	96	7.109			
Total	116	17.038			

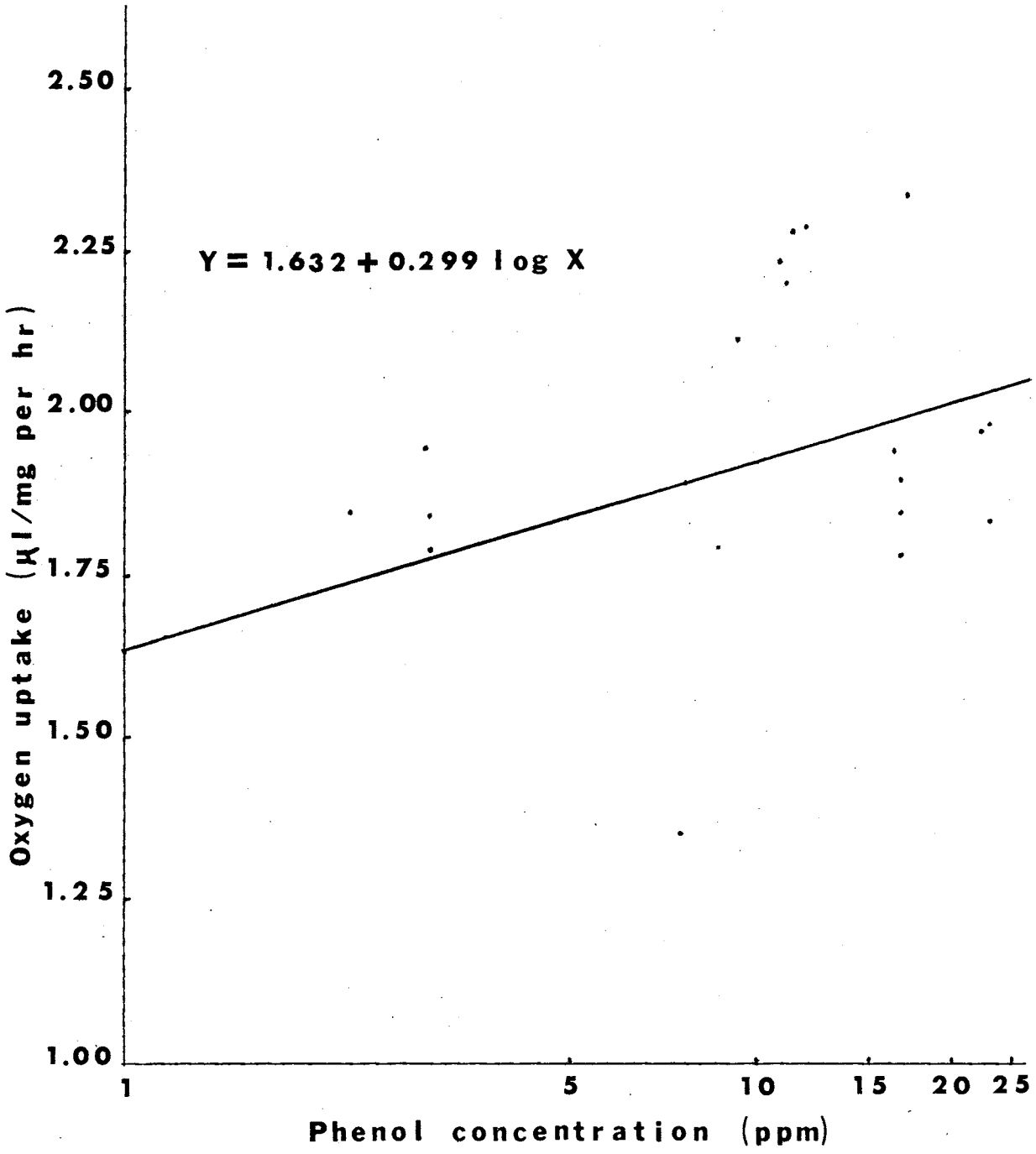

Figure 4. Regression of uptake of oxygen (µl/mg per hr) on the concentration of phenol (ppm) in a laboratory population of Chironomus attenuatus fourth-instar larvae.

TABLE XV

ENERGY (1×10^{-3} cal/hr) LOST TO ENTROPY THROUGH RESPIRATION BY INDIVIDUAL *CHIRONOMUS ATTENUATUS* IN ONE HOUR

REPLICATE	Level of Phenol (ppm)					
	0	5	10	15	20	25
1	1.78	2.31	1.74	2.94	2.39	3.02
2	1.86	2.37	1.70	2.90	2.30	2.57
3	2.40	2.39	2.32	2.95	2.53	2.36
4	2.26	2.52	2.73	2.85	2.46	2.55
$\bar{x}$	2.07	2.40	2.12	2.91	2.42	2.63

and in 24 hr (Table XVI). Ivlev's value of 3.38 cal/mg O_2 was used to compute a value of 1.29×10^{-3} cal/ul of O_2 consumed at the temperature and atmospheric pressure in the manometer flasks. The regression line in Fig 5 was determined using the calorific values calculated as the dependent variables (Y) and the logarithms of the phenol levels as the independent variables (log X). The regression equation was

$$Y = (2.10 \times 10^{-3}) + (3.86 \times 10^{-4}) \log X.$$

For a tenfold increase in phenol 3.86×10^{-4} cal are lost from the average individual per hr.

Effect of Phenol on Ash-free Weight

According to the energy flow model of Odum (1957), and increase in energy lost through respiration would be accompanied by an increase in energy assimilated, a decrease in energy in the standing crop, a decrease in energy passed on to the next trophic level, or a combination of these. Assuming that no change occured in the energy content per unit weight of the organisms, a change in the amount of energy in the standing crop would be reflected in a difference in the weight of the organisms. A linear regression analysis was performed with ash-free weight per individual as the dependent variable (Y) and the logarithm of the average phenol level as the independent variable (log X). The calculated regression equation was

$$Y = 0.753 - 0.114 \log X.$$

TABLE XVI

ENERGY (1×10^{-2} cal/day) LOST TO ENTROPY THROUGH RESPIRATION BY INDIVIDUAL *CHIRONOMUS ATTENUATUS* IN A 24-HOUR PERIOD

REPLICATE	Level of Phenol (ppm)					
	0	5	10	15	20	25
1	4.27	5.54	4.18	7.06	5.74	7.25
2	8.46	5.69	4.08	6.96	5.52	6.17
3	5.76	5.94	5.57	7.08	6.07	5.66
4	5.42	6.05	6.55	6.84	5.90	6.12
$\bar{x}$	4.94	5.76	5.09	7.01	5.83	6.29

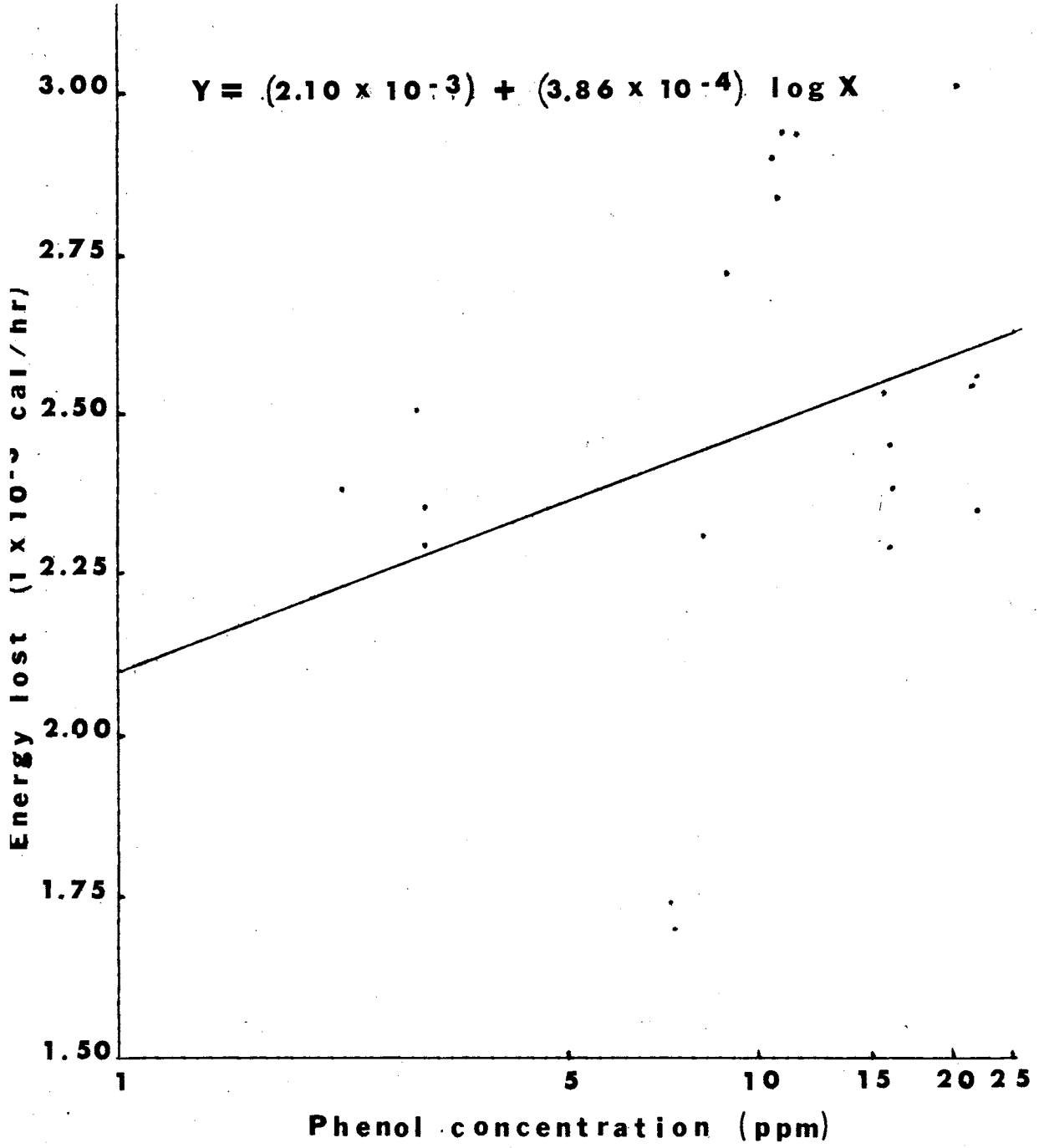

Figure 5. Regression of energy lost to entropy through respiration (cal/hr) on phenol concentration (ppm) in a laboratory population of Chironomus attenuatus fourth instar larvae.

The regression line (Fig 6) illustrates that an increase in phenol level causes a decrease in the ash-free weight of the larvae. Thus as phenol level rises, increasing amounts of energy are being lost in respiration, resulting in a reduced standing crop measured as a weight loss.

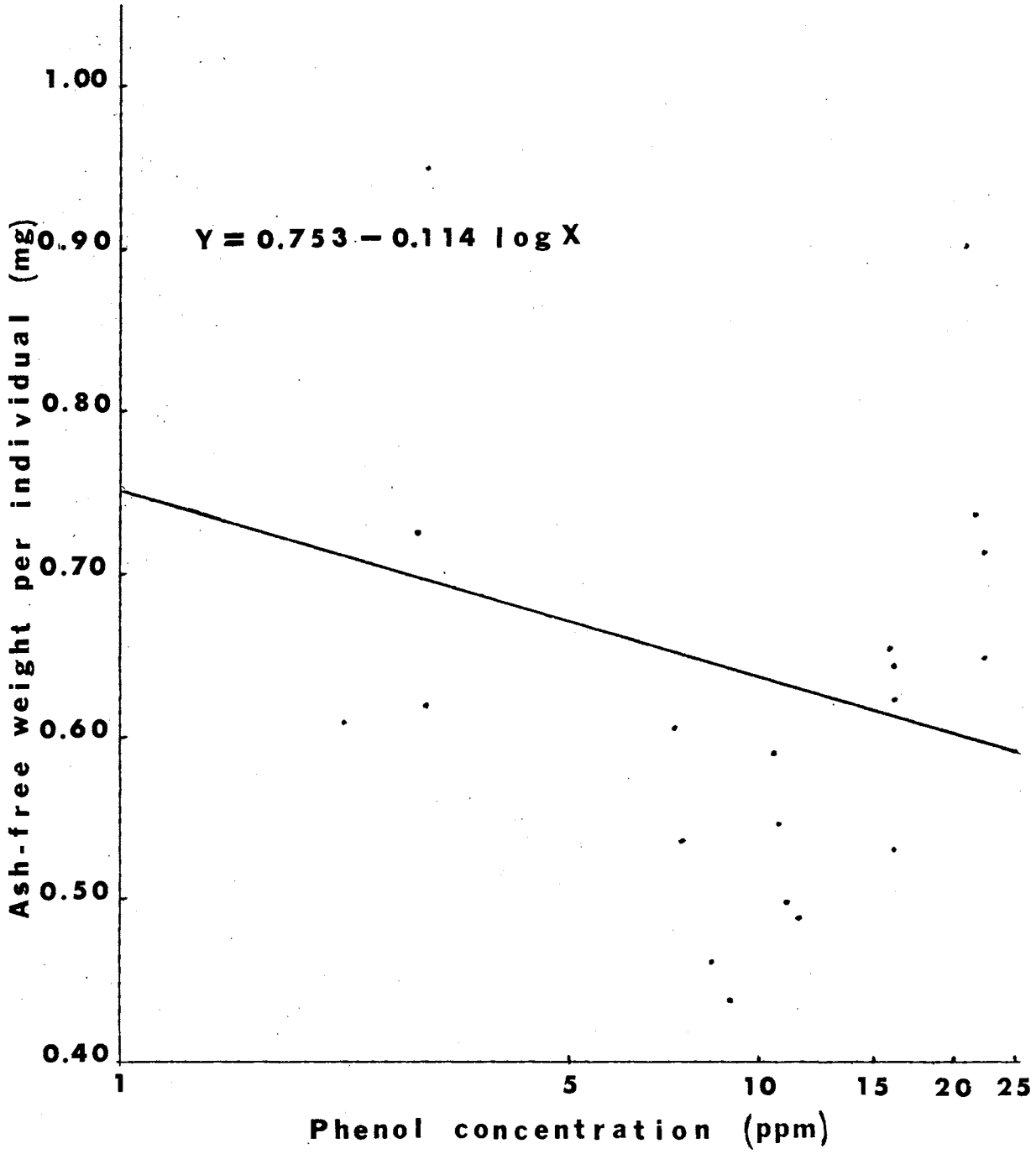

Figure 6. Regression of ash-free weight per individual (mg) on phenol concentration (ppm) in a laboratory population of Chironomus attenuatus fourth-instar larvae.

CHAPTER IV

SUMMARY

1. Oxygen uptake in a laboratory population of fourth-instar Chironomus attenuatus (Walk.) larvae exposed to six concentrations of phenol was found to be dependent on mean oxygen concentration, mean pH, mean ash-free weight per individual, and mean concentration of phenol.

2. The regression of log oxygen uptake adjusted for mean phenol level, mean pH, and mean oxygen concentration as dependent variable (log Y) and log mean ash-free weight as independent variable (log X) demonstrated an inverse relationship,

$$\log Y = 0.173 - 0.478 \log X.$$

3. The equation for the regression of oxygen uptake (Y) with the log of mean phenol concentration as the independent variable (log X) was

$$Y = 1.632 + 0.299 \log X,$$

when oxygen uptake values were adjusted for mean oxygen concentration, mean pH, and mean ash-free weight per individual.

4. Energy lost in respiration was calculated using the average oxycalorific coefficient determined by Ivlev (1934). It was found to be directly related to the logarithm of the mean phenol concentration,

cal lost (Y) increases with phenol concentration (log X):

$$Y = (2.10 \times 10^{-3}) + (3.86 \times 10^{-4}) \log X.$$

5. The ash-free weight per individual (Y) was found to decrease with an increase in the log of phenol concentration (log X),

$$Y = 0.753 - 0.114 \log X.$$

6. It was concluded that an increase in phenol concentration increases losses to entropy in Chironomus attenuatus fourth-instar larvae. This may be due to uncoupling of the cytochrome chain by phenol.

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VITA

2

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