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COMPARISON OF ENERGY FLOW PARAMETERS OF MIDGE POPULATIONS IN BIOLOGICAL OXIDATION PONDS (Effect of Phenol on Oxygen Uptake Rate of a Laboratory Population of Chironomus Attenuatus (Walk.) [1,2])

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by

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ABSTRACT

A laboratory population of fourth-instar larval forms of Chironomus attenuatus Wlaker received a continuous life-long exposure of 0, 3, 8, 11, 16, and 22 ppm phenol. Measurements were taken of water temperature, pH, and dissolved oxygen concentration. Larvae exposed to the different phenol concentrations were analyzed for oven-dry weight, ash-free weight, caloric content, and oxygen uptake. Caloric content per gram in larvae increased with phenol level. Maximum caloric content was 5869 cal/g in larvae exposed to 16 ppm phenol. The linear relationship of energy content (X) on phenol level (Y) was Y = 5751 + 12.9 + 3.1. A linear equation of energy content (X) on distance downstream below a sewage outfall (Y) was Y = 4832 + (-48.8)(X) + 3.76. Maximum oxygen uptake was 2.26 µl/mg per hr in larvae exposed to 11 ppm phenol. The regression of oxygen uptake (Y) adjusted for phenol level, pH, and oxygen concentration on ash-free weight (X) was log Y = 0.173 - 0.478 log X. The regression of adjusted oxygen uptake (Y) on phenol concentration (X) was Y = 1.632 + 0.299 log X. Calories lost through respiration (X) and phenol level (Y) were related by the equation, $Y = (2.10 \times 10^{-3}) + (3.86 \times 10^{-4}) \log X$.

The effect of salt on survival of fourth instar larvae was determined by exposing larvae to six NaCl concentrations between 0 and 342 mM/l for four different time periods. The TL_m for larvae exposed for 12 hr was 171 mM/l. Mortality larvae exposed for 12, 24, and 36 hr was low at the three lower salt concentrations and equaled or

approached 100% at the three higher levels. Sodium and chlorine concentrations in the haemolymph and total body water and caloric content were studied by exposing larvae to NaCl concentrations from 0 to 171 mM/l. The concentration of sodium in the haemolymph was similar in larvae exposed to the three lower salt concentrations and increased progressively at the three higher concentrations. Chloride concentrations in the haemolymph increased with the level of NaCl in the medium. Total body water was similar in larvae at all salt concentrations; whereas, caloric content varied with salt concentrations. Maximum caloric content was 7295 cal/ash free g in larvae exposed to a NaCl concentration of 68.4 mM/l.

INTRODUCTION

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 effluents. 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.

Phenol

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 (McKinney, Tomlinson, and Wilcox, 1956). Phenol has strong bactericidal action (Klein, 1957)

for example, carries approximately 15,000 tons of sodium chloride per day (Hynes, 1966). In Oklahoma, salt pollution arises from natural erosion of salt beds, oil field production, domestic, municipal and industrial effluents. The Cimarron River in northern Oklahoma contains approximately 0.47% NaCl (Eley, 1970).

Laboratory Approach

The many biotic and abiotic variables may lead to problems in experimental design and interpretation of results. We used the laboratory approach to evaluate the effects of phenol and salts. The use of a laboratory study permits (1) rigid control of boundary conditions so any variation above error can be attributed to the response under investigation; (2) study of regulatory factors independently or in a factorial arrangement; (3) replication of experimental units and measurement of precision; and (4) sacrifice of units at each observation, so random error will be independent.

Chironomus attenuatus Walker

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 past history as well as present conditions of the environment (Paine and Gaufin, 1956). Although microinvertebrates share some of these advantages, macroinvertebrates are often preferred for study because of their larger size and longer life histories (Gaufin, 1958).

Species of Chironomus are often the most abundant benthic

macroinvertebrate in receiving streams below industrial and domestic sewage outfalls. Over 20,000 individuals/m^2 of Chironomus attenuatus Walker were collected from an enriched area of Skeleton Creek, Oklahoma (Wilhm and Dorris, 1966).

The life cycle of C. attenuatus consists of the egg, four larval instars, pupa, and adult. Eggs are enclosed in gelatinous masses containing about 1,000 eggs each. After oviposition in the water by the female, the egg mass swells and sinks to the bottom. Eggs hatch in a few days into first larval instars.

C. attenuatus larvae haemolymph contains the pigment hemoglobin which is responsible for the common name, bloodworm. The first instars are colorless and a red color increases in intensity throughout the larval stages. Fourth-instar larvae are a deep red. The concentration of hemoglobin changes in response to low oxygen conditions (Fox and Taylor, 1954) and the pigment probably serves either as a transport or a storage pigment (Walsche, 1947c). The tube is open at both ends with a net spun across one opening. Undulations of the body cause a current to flow through the tube and particles are trapped in the net. The larva eats the food net and its contents and then spins a new net. The larva also leaves the tube and forages along the bottom.

At the end of the fourth instar the thorax becomes enlarged and the larva develop into a pupa. In our laboratory populations, this occurs about 21 days after the egg hatches at a mean temperature of 22.5 C. Pupae emerge as adults 2 to 5 days later. Adults usually live from 3 to 5 days during which time mating and oviposition occurs. Although mating generally occurs in flight, adults will mate in confined spaces.

Chironomus as an Experimental Animal

Populations of chironomids undoubtedly have a pronounced effect on many aquatic ecosystems because of their extreme abundance and filter feeding. The larvae serve as a source of food for many species of invertebrate predators and fish. C. attenuatus not only filters particulate matter from the water but also forages on detrital material on the bottom. Chironomids have been shown to be important in reducing the turbidity of refinery effluents (Tubb and Dorris, 1965).

C. attenuatus is a valuable test species for the following reasons: (1) ease of laboratory culture, (2) relatively short life cycle, (3) ready mating in confinement, (4) relatively large size of the individuals, (5) wide distribution, and (6) importance in the aquatic ecosystem. These characteristics permit study of the long term effects of environmental contaminants in addition to short term studies.

Energy Content

The caloric content of animals in many different taxonomic groups has been calculated (Comita and Schindler, 1963; Golley, 1961; Slobodkin and Richman, 1961) to provide data for energy flow studies. Environmental stress results in a change in the caloric content of an organism. In Chlorella pyrenoidosa R-valves, which are proportional to the heat of combustion of organic matter and therefore to energy content, were correlated with changes in carbon dioxide and oxygen content of the atmosphere, nutrient level of the media, light intensity, and temperature (Spoehr and Milner, 1949). The effects of phenol on energy content has received little attention.

Oxygen Uptake by Chironomus

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 lotic habitats consume more oxygen than do those taken from lentic habitats. 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 (Ewer, 1942; Walshe, 1947a and 1947b; Platzer-Schultz, 1968). The effects of phenol on oxygen uptake has not been reported.

Salt Regulation

Salt and water regulation in Aedes aegypti has been studied by Wigglesworth (1933, 1938), Ramsey (1950, 1951, 1953) and Stobbart (1959, 1960, 1965). From these studies on Aedes aegypti, it was evident the active uptake of sodium and chloride ions occurred through the anal papillae. The Malpighian tubules contributed to sodium retention when environmental concentrations were less than haemolymph concentrations. The Malpighian tubules were, however, incapable of secreting more sodium in excess of environmental concentrations. The larvae were capable of sodium and chloride regulation until the external concentration of sodium chloride reached 0.75 - 1.0%. At these external concentrations, regulation broke down. The net transport and exchange of sodium were probably brought about by a carrier mechanism in the anal papillae related to the metabolism of the larva. Koch (1938) demonstrated active uptake of chloride by the anal papillae in Culex sp. and Chironomus sp. Lauer (1969) found larvae of Chironomus plumosus were

able to tolerate salt concentrations hypertonic to their haemolymph for only a few days and postulated this tolerance was due to the impermeability of the cuticle rather than any ability to osmoregulate in hypertonic medium.

Objective

The objective of this study is to study (1) the effects of phenol on energy content and oxygen uptake and (2) salt regulation in laboratory populations of Chironomus attenuatus Walker.

METHODS AND MATERIALS

Laboratory Population

The C. attenuatus larvae used in this study were taken from a laboratory population originally collected from an enriched area of Skeleton Creek, Oklahoma, by Wilhm and Dorris (1966). The organisms are reared in six metal trays 88 x 56 x 18 cm painted with a nontoxic epoxy paint. Each tray is enclosed in a screened cage, 90 x 57 x 42 cm, to provide space for emergence and mating of adults. A 40-watt light source in each cage is controlled by a timer on a 12-hr photoperiod. Forced air is continuously bubbled into the trays. Sand is placed on the bottom of each tray. Two g of dog kisses (Hartz Mountain Products Corp.) are ground into paste with water and fed in each tray every 3 days. Trays are drained and cleaned every 6 months. Adults and larvae from each tray are mixed with those of other trays to prevent inbreeding.

Continuous-flow System for Phenol Experiments

Two continuous-flow systems were constructed to provide a

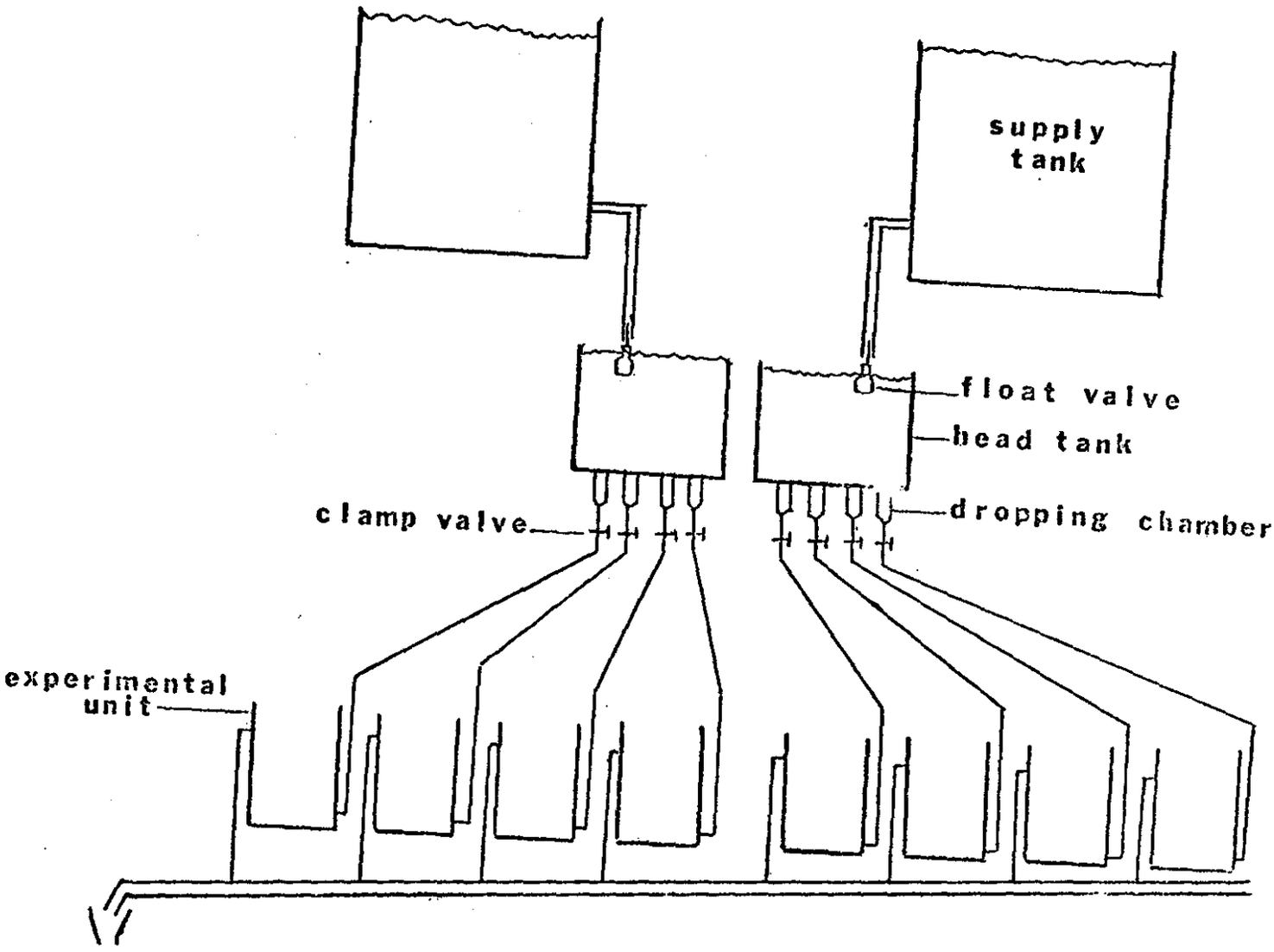

Figure 1. Continuous-flow systems

Table 1

Mean temporal decrease of phenol (ppm) of three replicate samples each of 0, 40 dead, and 40 living *Chironomus attenuatus* fourth-instar larvae

Number of Organisms	Time (hr) 0	18.0	41.5	65.5	89.5
None	40.0	38.1	37.5	33.6	24.9
40 Dead	40.0	36.6	29.4	8.8	0.2
40 Living	40.0	38.4	28.8	13.3	2.5

to the fourth-instar if raised in a phenol solution exceeding 25 ppm. Therefore, the levels of phenol in the treatments selected for study were 0, 5, 10, 15, 20, and 25 ppm. Although these levels were produced in the head tank, decomposition resulted in lower mean values of phenol in the experimental units (Table 2). Responses reported in this study are given according to the mean level of phenol in experimental units. All phenol solutions were made using reagent grade, crystalline phenol and deionized water.

Operation of System

Since only two treatments could be run simultaneously, two experimental units were started for each treatment with two more being started 2 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.

Table 2

Phenol concentration produced in the head tanks and means of four replicate experimental units

Site	Phenol Concentration (ppm)					
Head Tank	0	5.00	10.00	15.00	20.00	25.00
Experimental Units	0	2.82	8.18	11.20	16.32	22.36

Fourth instar larvae were removed for caloric content determinations and oxygen uptake measurements 21 days after the egg masses were added to the experimental units. Randomization procedures were used to specify organisms in an experimental unit for analysis. Larvae of C. attenuatus from an experimental unit were placed in an enamel pan containing 100 numbered grid squares on the bottom. A table of random numbers was used to specify grid squares.

Energy Content

Four samples of 10 organisms each were taken from each experimental unit for determination of energy content. Organisms were placed in a tared crucible and dried in an oven at 100 C. The crucible was allowed to equilibrate in a dessicator at room temperature for 24 hr. The sample was weighed to obtain the dry weight per individual and then pelleted. The pellet was weighed on a tared platinum foil disk and fired in a Phillipson Oxygen Microbomb Calorimeter (Gentry and Wiegert Instruments, Inc.) connected to a Honeywell 194 multi-range potentiometer with 0.5 mV span. The graph generated by the recorder was used to calculate the caloric content as calories/gram and calories/gram ash free weight. The apparatus was calibrated using benzoic acid.

Gravimetric Analyses

Samples were analyzed for oven dry weight by drying at 103 C for 2 hr and for ash free weight by muffling at 500 C for 3 hr. The numbers and samples analyzed were the same as those described for energy content.

Oxygen Uptake Tests

From each experimental unit, 15 fourth-instar larvae from the designated grid square were added to a manometer flask of 15 ml volume. A 14-station Warburg manometer apparatus with controlled temperature water bath was used throughout the study. 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^{o} C. A shaking rate of 106/min with flasks describing arcs of 4.8 cm was used. 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). After oxygen uptake measurements were made, larvae from each flask were analyzed gravimetrically

Corrections for extrinsic oxygen uptake were determined by placing 5 ml of solution from the unit being tested in each of two flasks. Average oxygen uptake values obtained were subtracted from those of the larvae in solution to correct for the non-larval oxygen uptake in the flasks that contained larvae.

Effects of Sodium Chloride on Mortality

Watch glasses were used as experimental units for studying the effects of sodium chloride on survival of C. attenuatus. The bottom of each unit was covered with washed, ashed sand. Twenty-five ml of the appropriate treatment solution was added to each watch glass. Five

fourth-instar larvae were placed in each of six concentrations of NaCl: 0.0, 68.4 (0.4%), 136.8 (0.8%), 205.2 (1.2%), 273.6 (1.6%), and 342 (2.0%) mM/l. Mortality was observed after 12, 24, 36,and 48 hr. Six replications of each treatment were performed. The experiments were conducted in a constant temperature chamber at 25 C. In each experiment, the units were monitored over the 48 hr period and any loss of water from the units due to evaporation was replaced. The experiments were of a completely randomized design, one-way classification.

Sodium and Chloride Concentrations in the Haemolymph

Five larvae were placed in each of six concentrations of NaCl in watch glasses: 0.0, 34.2 (0.2%), 68.4 (0.4%), 102.6 (0.6%), 136.8 (0.8%), and 171 (1.0%) mM/l with six replications of each treatment. The larvae were removed from the units after 48 hr and adsorbed water was removed with absorbant paper. A minute puncture was made in the posterior area of the larva near the anal papillae and 0.2 µl of haemolymph was drawn into a calibrated capillary tube. For sodium analysis, the sample was diluted in 700 µl of deionized water. The sodium concentration of the sample was determined using a Beckman 440 Atomic Absorption Flame Spectrophotometer. Ten samples were pooled to yield a 2.0 µl sample for chloride analysis. The samples were pooled to obtain a sample large enough to be titrated by a Fiske/Marius Micro Chloro-counter.

Total Body Water and Caloric Content

The experimental units for determining total body water were finger bowls containing 100 ml of the specified treatment solution and a layer of washed, ashed sand. Fifty larvae were placed in the same concentrations of NaCl as were used in the haemolymph determinations

with three replications of each treatment. The larvae were removed from the units after 48 hr and adsorbed water removed. Wet weights were determined by placing the larvae in a tared sealed vial containing 4 ml of the appropriate test solution. The test solution prevented desiccation of the larvae during weighing. The larvae were transferred to tared crucibles and oven dry weight determined. Dry weights were corrected for water loss from the crucibles before the total body water was computed. The dried larvae were formed into pellets and caloric content determined. Ash weight was determined by combusting half of each sample.

Laboratory Populations

The length of the C. attenuatus larvae increases from 1 mm in the first instar to 12 mm in the fourth instar, while oven dry weight increases from 0.004 to 0.724 mg. The length and biomass of the different instars overlap and cannot be used to distinguish the instars. However, head capsule widths increase uniformly with no overlaps as follows:

1st instar = 0.08 to 0.10 mm

2nd instar - 0.12 to 0.15 mm

3rd instar - 0.25 to 0.30 mm

4th instar - Over 0.40 mm

Mean caloric content of the stock populations of C. attenuatus ranged from 5473 cal/ash free g in the pupae to 6028 cal/ash free g in third instar (Table 3). Although an analysis of variance on the four larval instar stages showed a significance level of only $p = 0.13$, no overlap among these values existed. A student's t test between the third and fourth instars showed a significance level of $p = 0.075$. Energy

Table 3

Caloric values of the life stages of *Chironomus attenuatus*

Life State	cal/g	cal/ash-free g
Egg Mass	4914	5518
First Instar	4683	5871
Second Instar	5056	5976
Third Instar	5276	6028
Fourth Instar	5458	5890
Pupa	5172	5473
Adult	5354	5769

content decreased from the fourth instar to the pupal stage. Pupae do not feed and the pupal exoskeleton is composed of material low in caloric content. Adults had a greater energy content per gram than pupae even though adults do not feed. The weight of adults is 21% less than that of the pupae and only 6% of this loss is attributed to loss of pupal exuviae. Apparently, the other 15% of the weight lost during the transformation was composed of material of low energy content.

The oven-dry weight of the fourth instar larvae in the laboratory populations used in this experiment was 0.880 mg per individual, while ash-free weight per individual averaged 0.801 mg. Fourth instar larvae of the stock populations averaged 5458 cal/g and 5890 cal/ash-free g.

Average value of oxygen uptake for fourth-instar larvae in the laboratory populations was 1.65 µl/hr per mg ash free weight (Table 4). This falls within the range of values measured for chironomid larvae by other investigators (Table 5). 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. Ash-free weight and oven-dry weight were determined in this study.

EFFECTS OF PHENOL

Physicochemical Conditions in Experimental Units

Mean values of oxygen concentration in replicate units used in the phenol studies by level of phenol ranged from 6.22 to 6.98 ppm with an overall mean of 6.58 and a standard deviation of 0.44 for all units (Table 6). Variation among units was due to differences in air line

pressure in the aeration system, in room temperature, and atmospheric pressure. Average hydrogen-ion concentration of replicate units varied from a pH of 6.00 to 7.88 (Table 6). 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 were below 7.0.

Ta

Uptake of oxygen (µl/hr) per mg ash-free weight in fourth instar larvae of Chironomus attenuatus

	Sample			
Subsample	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
$\bar{x}$	1.47	1.70	1.90	1.51

Average water temperature of replicate units varied from 20.8 to 23.9° C (Table 6). Mean temperature for all units was 22.5° C with a standard deviation of 0.98. Higher temperatures reduce the maturation time of midge larvae (Hilsenhoff, 1966). No emergences occurred in water with an average temperature of less than 22.3° C, whereas emergences occurred in all units in which mean temperature exceeded 22.7° C. Variation in maturity of the populations caused by temperature difference did not affect the results of the tests since measurements were made only on fourth-instar larvae.

Table 5

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, 1958	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	C. plumosus	*	Micro-Winkler	17° C	*	3.19

* Not given

Table 6

Mean responses of samples in four replicate microcosms by level of phenol

Response	Units	Samples per Replicate	Level of Phenol (ppm) 0	3	8	11	16	22
Dissolved Oxygen	ppm	6	6.35	6.98	6.64	6.87	6.43	6.22
Hydrogen-ion Concentration		6	6.00	7.81	7.55	7.02	7.88	7.66
Temperature	^{o}C	6	20.8	22.8	22.4	22.0	22.8	23.9
Oven-dry weight per individual	mg	5	.880	.812	.559	.580	.694	.817
Ash-free weight per individual	mg	5	.801	.729	.513	.536	.618	.755
Calorie Content	cal/g	4	5614	5687	5753	5812	5869	5479
Oxygen Uptake*	ul/mg per hr	5	1.60	1.86	1.80	2.26	1.87	2.03
Energy lost by Respiration	1×10^{-3} cal/hr	5	2.07	2.40	2.12	2.91	2.42	2.63

*Adjusted for mean oxygen concentration, pH level, and ash free weight per individual.

Biomass

Oven-dry weight per individual over the phenol levels used in this study was directly related to ash-free weight per individual. Ash-free weight (Y) was 86% of oven-dry weight (X) and the regression equation was (Fig. 2): $Y = 0.033 + 0.865\ X$

Both oven-dry weight and ash-free weight per individual decreased through 8 ppm phenol and then increased progressively as phenol level increased (Table 6). The reason for this pattern was not determined.

Energy Content

A high level of significance exists between energy content and phenol level. The energy content of fourth instar larvae of C. attenuatus increased from 5614 cal/g dry weight at a concentration of 0 ppm phenol to 5879 cal/g at 16 ppm phenol. The linear relationship of energy content in cal/g (Y) on phenol level (X) between 0 and 16 ppm phenol can be expressed by the following regression equation (Fig. 3):

$$Y = 5751 + 12.9X + 3.1$$

The value of 5751 is the mean energy content and 3.1 is the estimate of experimental error. The standard deviation of Y for fixed phenol level is 56 cal/g. At 22 ppm phenol, a decrease in energy content occurred. This level is approaching the limits of tolerance and population numbers were much lower than at smaller concentrations. At 30 ppm phenol, mortality was almost 100%.

Variation in energy content was also measured in field populations of C. attenuatus in Skeleton Creek, Oklahoma. Energy contents of fourth instar larvae collected from five stations below a domestic sewage and oil refinery outfall decreased progressively downstream with

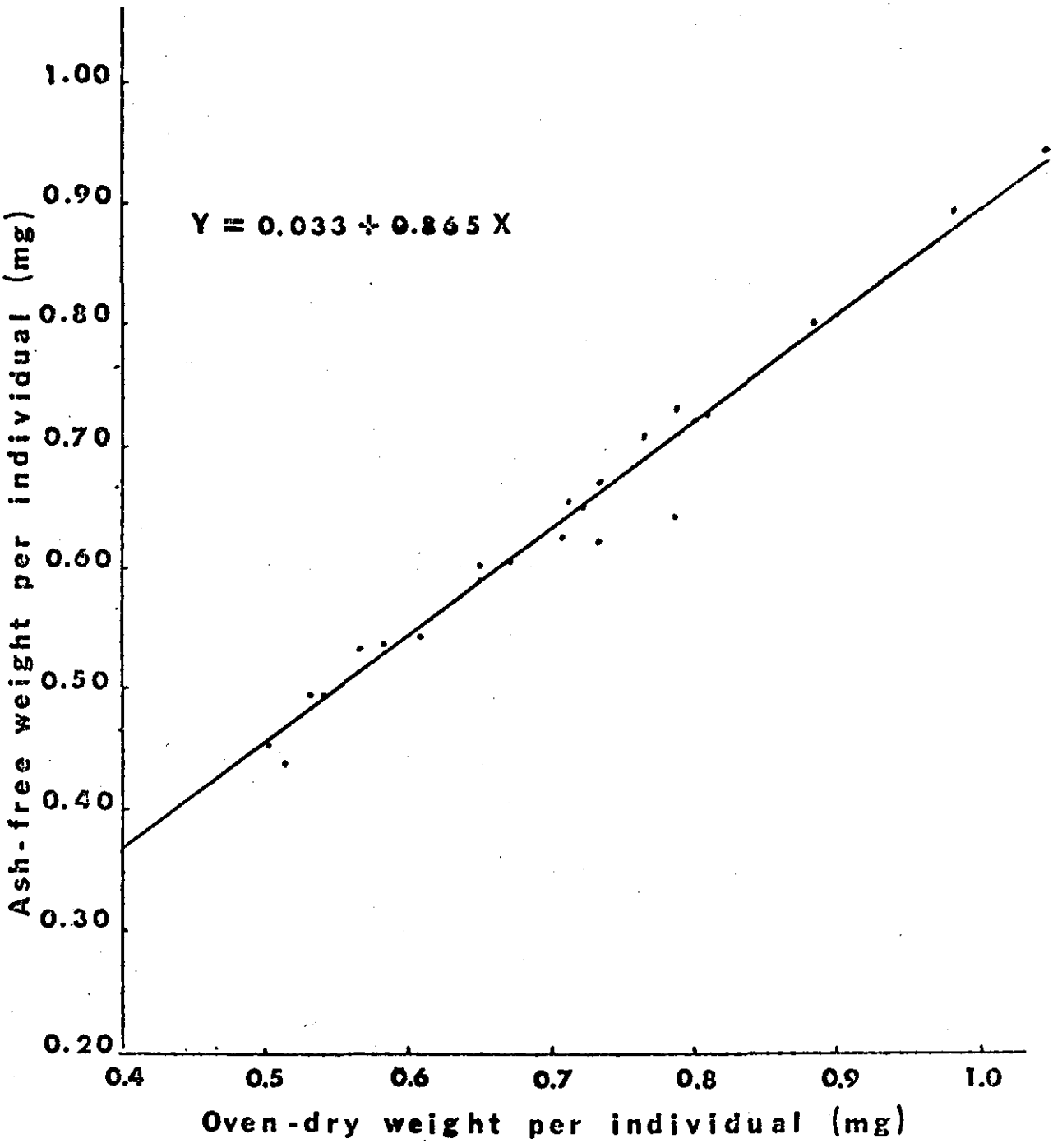

Figure 2. Regression of ash-free weight per individual on oven-dry weight per individual of Chironomus attenuatus fourth-instar larvae

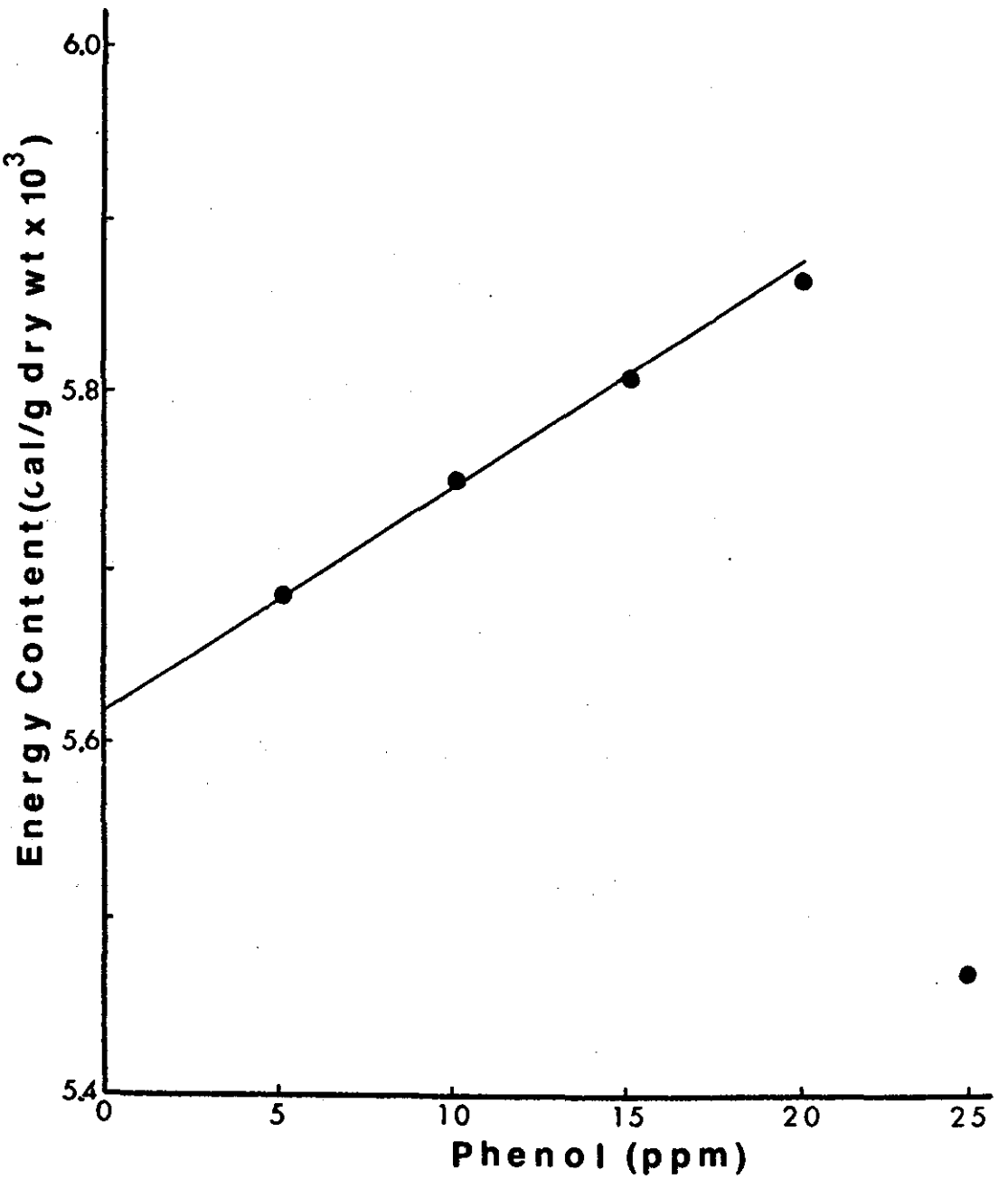

Figure 3. Effect of phenol on the energy content of fourth instar larvae of Chironomus attenuatus.

a highly significant effect of distance downstream. A linear regression of energy content in cal/g (Y) on distance below the sewage outfall (X) can be expressed by the equation (Fig. 4):

$$Y = 4832 + (-48.8)(X) + 3.76$$

The standard deviation of Y for a fixed distance downstream is 61.3 cal/g. Although the slopes of the regression lines for larvae raised in the laboratories and those collected in the stream are not directly comparable, they both indicate an increase in calorie content with pollution.

Oxygen Uptake

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 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 at the .005 level.

Multiple regression analysis was used to determine which variables were affecting the regression of oxygen uptake by the larvae. The first regression included mean water temperature, oxygen concentration, hydrogen-ion concentration, ash-free weight, individual and phenol concentration in the experimental units as independent variables. Mean water temperature (^{o}C) was found to be insignificant in the regression. 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. Thus, temperature was not

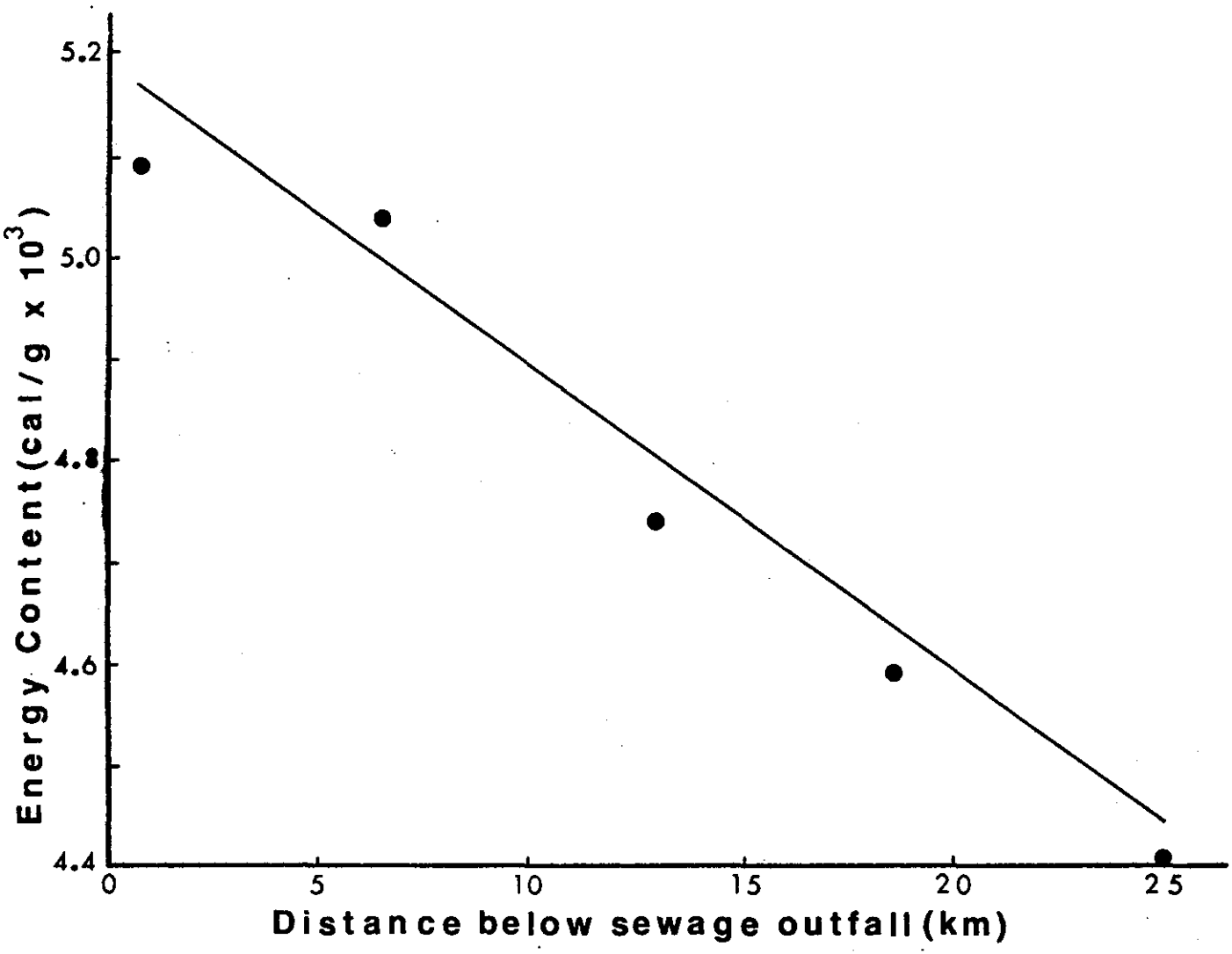

Figure 4. Energy content of fourth instar larvae of Chironomus attenuatus collected from different stations on Skeleton Creek.

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 7). 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 since it was significant at the 0.005 level (Table 7). 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 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 and was included as an independent variable (Table 7). Using mean oxygen concentration, mean hydrogen-ion concentration, mean ash-free weight per individual, and mean phenol level as the independent variables, 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 pH

Table 7

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

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

X_4 = mean level of phenol (ppm)

The rate of oxygen uptake per unit weight by aquatic insects 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, hydrogen-ion concentration, and phenol level using the previously given equation. The regression of adjusted values of oxygen uptake as the dependent variable (Y) on the ash-free weight per individual as the independent variable (X) was (Fig. 5), log Y - 0.173 - 0.478 log X.

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. 6). 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.

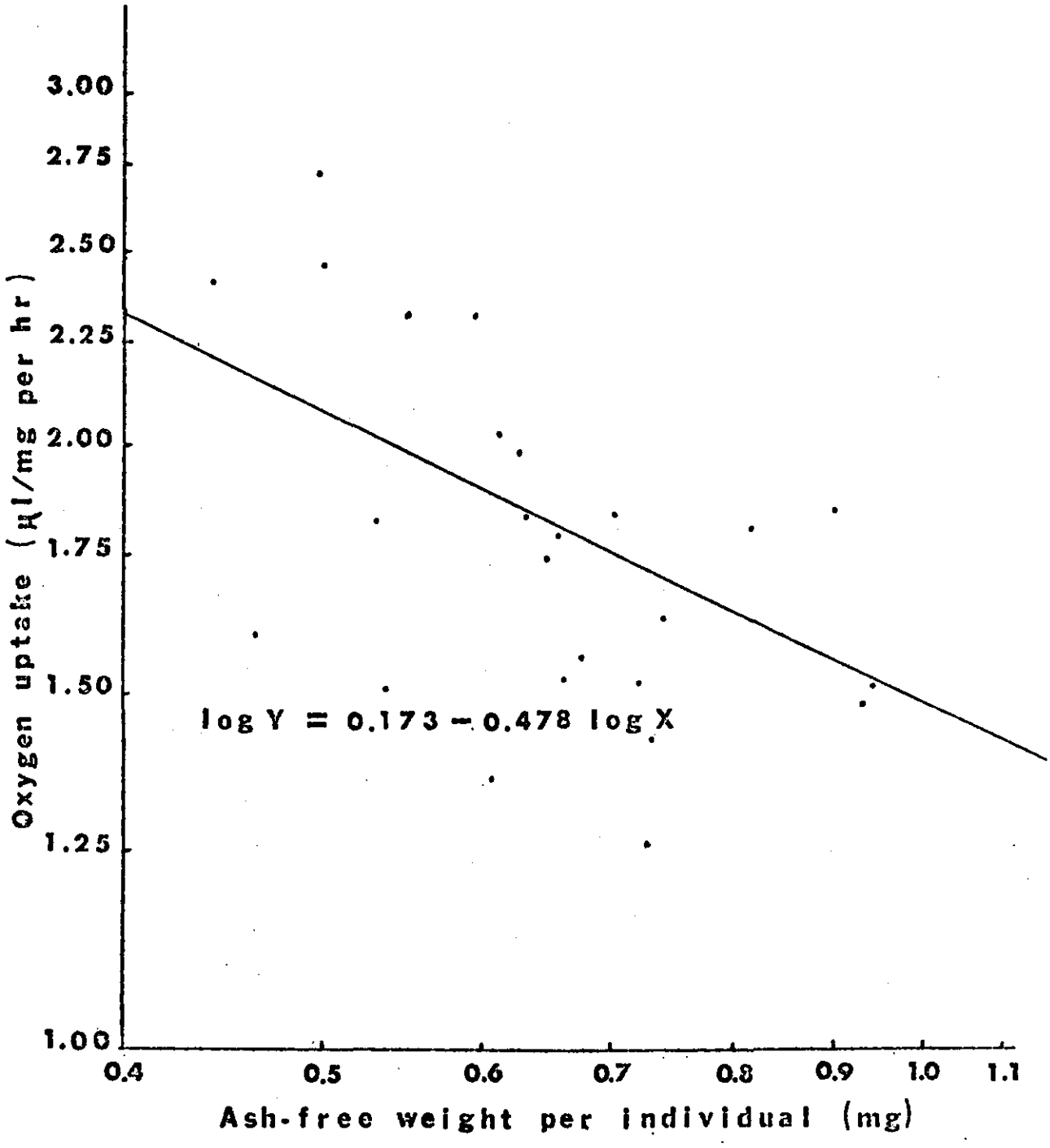

Figure 5. Regression of oxygen uptake on ash-free weight per individual in a laboratory population of Chironomous attenuatus fourth-instar larvae

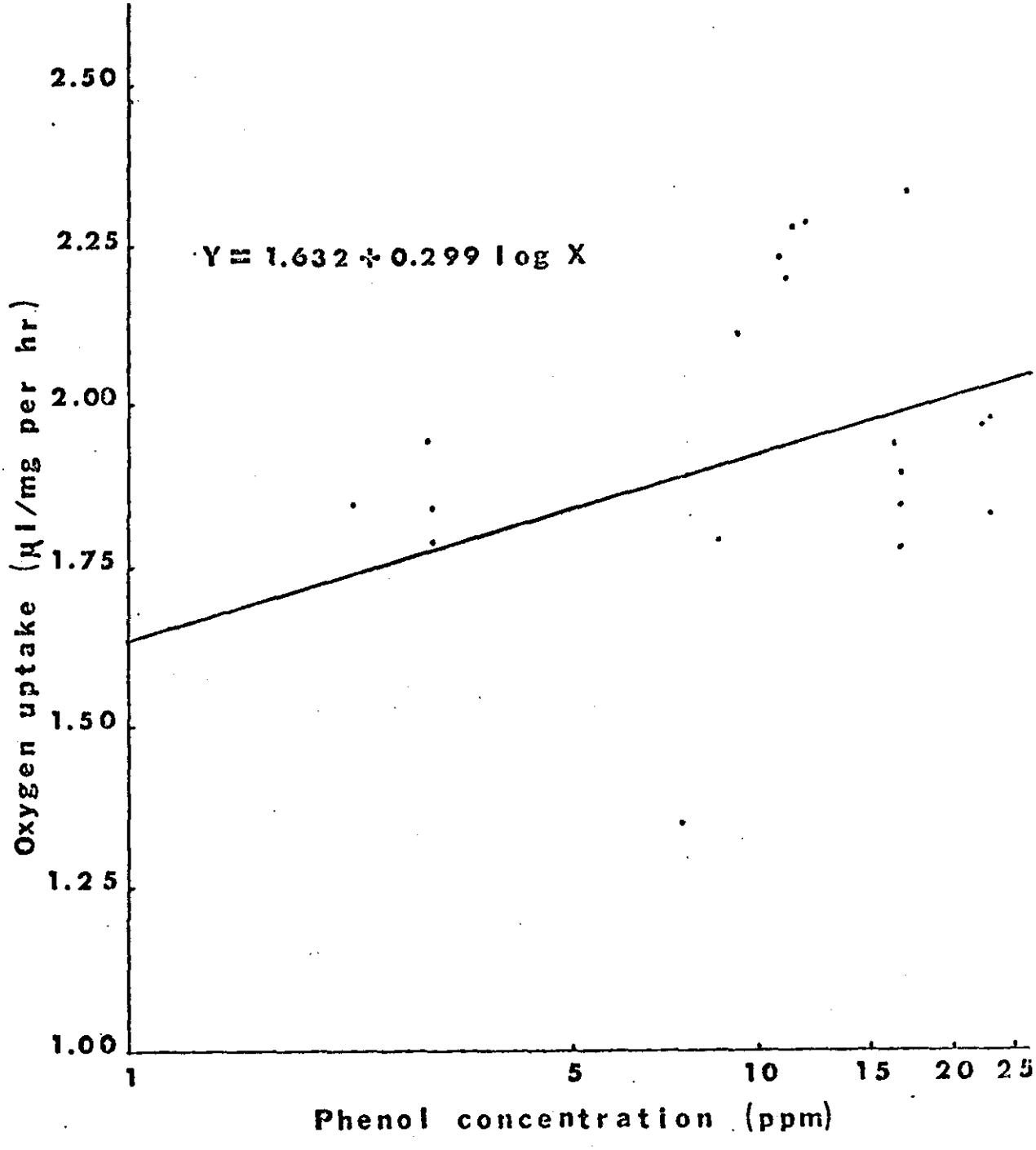

Figure 6. Regression of oxygen uptake on the concentration of phenol in a laboratory population of Chironomus attenuatus fourth-instar larvae

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 3). Ivlev's value of 3.38 cal/mg O_2 was used to compute a value of 1.29×10^{-3} cal/µl of O_2 consumed at the temperature and atmospheric pressure in the manometer flasks. The regression line in Fig. 7 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.

SALT REGULATION

Mortality

Mortality at any NaCl concentration was similar for the 12, 24, and 36 hr periods (Fig. 8). The 48 hr period resulted in greatly increased mortaility at 136.8 (0.8%) mM/1 NaCl. Mortality was 100% at all time periods at or above 205.2 (1.2%, 12 g/1) mM/1 NaCl. The median tolerance limits (TL_m) are expressed in Table 8.

Table 8

Median Tolerance limits of Chironomus attenuatus exposed for different time periods to concentrations of NaCl

	Time (hr)			
	12	24	36	48
TL_m (mM/1)	171	168	164	136.8

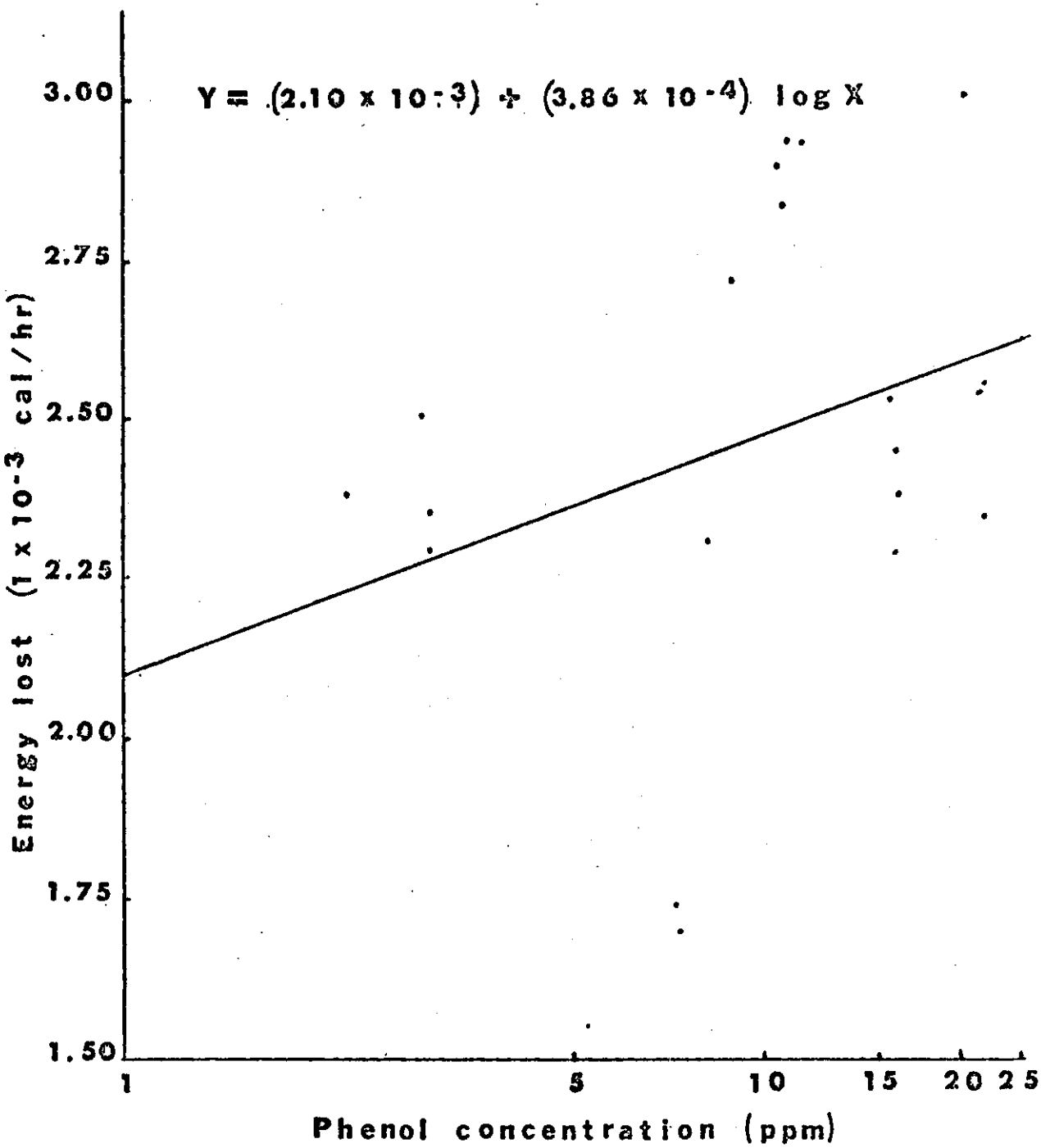

Figure 7. Regression of energy lost to entropy through respiration on phenol concentration in a laboratory population of Chironomus attenuatus fourth-instar larvae

This implies Chironomus attenuatus is incapable of surviving extended periods in saline waters. Chironomus plumosus, however, was able to tolerate 35 g/l of salts for 5 days with 90% mortality (Lauer, 1969). NaCl constituted 16 g/l of the 35 g/l total. The importance of using known species in toxicity studies is evident since tolerance may differ widely among species within the same genus.

Sodium and Chlorine in the Haemolymph

The concentration of sodium in the haemolymph at various NaCl concentrations in the medium is shown depicted in Figure 9. Although an analysis of variance (AOV) showed no significant difference among treatments, the concentration of sodium in the haemolymph was similar at the three lower salt concentrations and then tended to increase progressively. The straight line in Figure 9 indicates where the means would lie if the haemolymph and medium had the same composition. The trends in Figures 8 and 9 suggest that C. attenuatus is able to regulate sodium up to a medium concentration of 102.6 (0.6%) mM/l. Harnisch (1934) reported Chironomus thummi actively secreted sodium into the haemolymph through the anal papillae. Assuming this mechanism is active in C. attenuatus, it is probable the Malpighian tubules are capable of maintaining the haemolymph sodium level by secreting superfluous sodium into the intestinal lumen. Above medium salinities of 102.6 mM/l, the uptake of sodium through the anal papillae is greater than its excretion and sodium levels increase in the haemolymph. The larvae apparently have no mechanism for preventing the uptake of sodium by the anal papillae.

Haemolymph concentrations of chloride are shown in Figure 10. An AOV (Table III) showed a significant difference between treatments

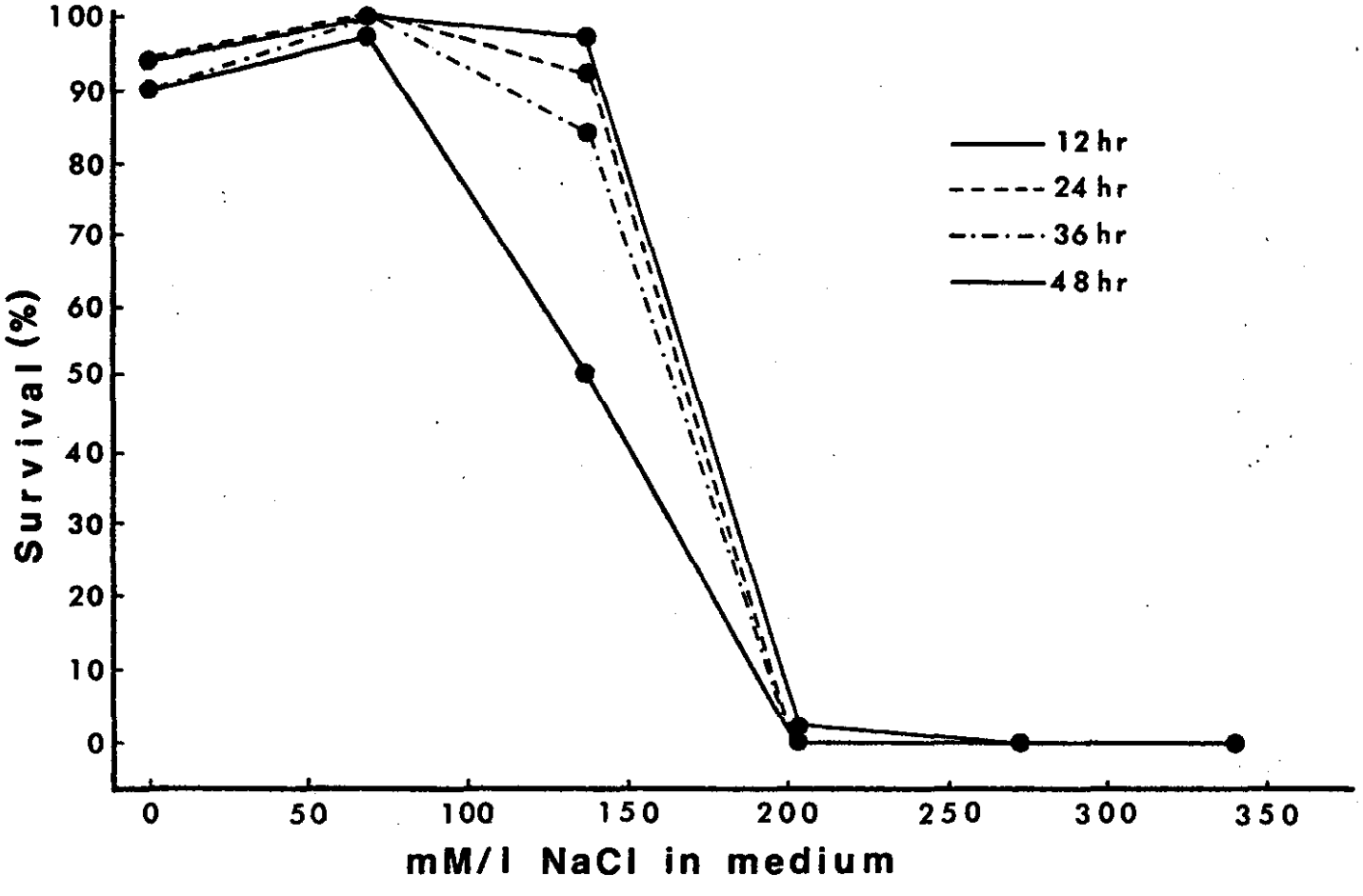

Figure 8. Survival (%) after 12, 24, 36 and 48 hrs in different concentrations of NaCl in the medium.

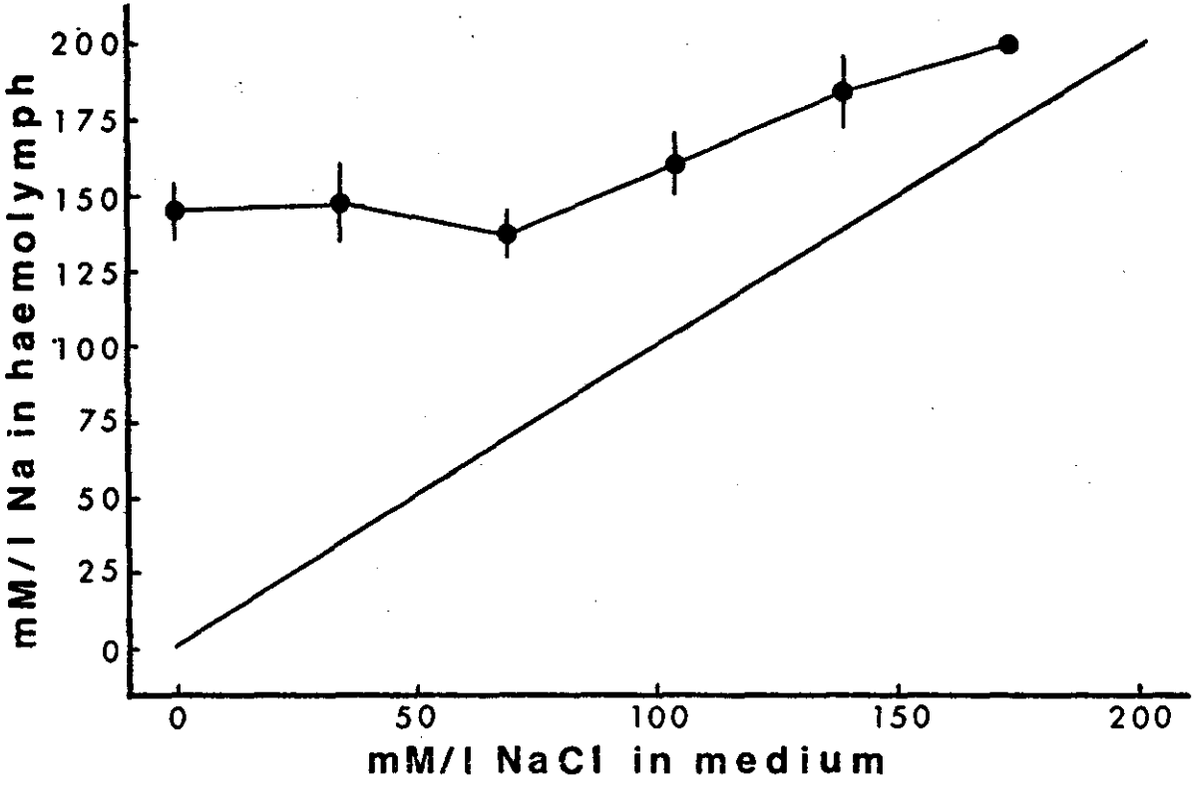

Figure 9. Sodium in the haemolymph at different concentrations of NaCl in the medium. Vertical lines denote standard errors.

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