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Daily Patterns of Oxygen Consumption in *Limnodrilus Hoffmeisteri*

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DAILY PATTERNS OF OXYGEN CONSUMPTION
IN LIMNODRILUS HOFFMEISTERI

by

Thomas Charles Beck

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

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TABLE OF CONTENTS

CHAPTER		PAGE
I	INTRODUCTION	1
II	LITERATURE REVIEW	2
	Tubificid Respiratory Physiology	2
	Rhythms of Respiration in Invertebrates	3
	The Effect of Continuous Light on Invertebrate Rhythms	5
	Seasonal Variations in Oxygen Consumption and Respiration Rhythms in Invertebrates	6
III	MATERIALS AND METHODS	9
	Experimental Procedures	9
	Statistical Procedures	12
IV	RESULTS	17
	Results of 24 Hour Respiration in a Photoperiod of L:D (14:10)	18
	Results of 24 Hour Respiration in Constant Light L:L	32
V	DISCUSSION	53
VI	SUMMARY	62
VII	APPENDIX	63
VIII	LITERATURE CITED	68

LIST OF TABLES

TABLE		PAGE
1	Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), A	20
2	One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), A	21
3	Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), A	22
4	Cosine Model of Oxygen Consumption vs Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), A	23
5	Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), B	26
6	One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), B	27
7	Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), B	28
8	Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:D (14:10), B	29
9	Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, C	34
10	One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, C	35
11	Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, C	36

TABLE	PAGE
12	Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, C 37
13	Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, D 40
14	One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, D 41
15	Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, D 42
16	Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, D 43
17	Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, E 46
18	One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, E 47
19	Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, E 48
20	Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration of <u>L. hoffmeisteri</u> , in L:L, E 49

LIST OF FIGURES

FIGURE		PAGE
1	Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of <u>L. hoffmeisteri</u> , in a Photoperiod of L:D (14:10), Showing best-fit Quartic and Cosine Curves, A	25
2	Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of <u>L. hoffmeisteri</u> , in a Photoperiod of L:D (14:10), Showing best-fit Quartic and Cosine Curves, B	31
3	Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of <u>L. hoffmeisteri</u> , in Constant Light, L:L, Showing best-fit Quartic and Cosine Curves, C	39
4	Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of <u>L. hoffmeisteri</u> , in Constant Light, L:L, Showing best-fit Quartic and Cosine Curves, D	45
5	Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of <u>L. hoffmeisteri</u> , in Constant Light, L:L, Showing best-fit Quartic and Cosine Curves, E	51
6	Graph Showing Comparison of Oxygen Consumption vs. Time for Respiration Data of <u>L. hoffmeisteri</u> , in L:D (14:10) and L:L	52

INTRODUCTION

Tubificid worms have become increasingly important in environmental physiology because of their ability to adapt to stressful environments. They have been the subject of many physiological studies, which include investigations of their respiratory physiology, tolerance to common pollutants and pesticides, reaction to high oxygen stress, and their ability to accumulate and retain radionuclides. The daily pattern of respiration of these organisms has not been studied. Regular daily changes in metabolism could be an important aspect of their physiology. These fluctuations would affect their responses to various conditions imposed on them in laboratory experiments, for example, the metabolically related uptake and retention of certain radionuclides. More importantly, knowledge of these metabolic adjustments might help in understanding the adaptations so successfully utilized by these organisms.

This study was undertaken to determine the daily metabolic pattern, expressed as oxygen consumption, of the tubificid, Limnodrilus hoffmeisteri, under a normal photoperiod, and under conditions of constant light.

LITERATURE REVIEW

Tubificid Respiratory Physiology

Limnodrilus hoffmeisteri is a species of small aquatic oligochaete in the Tubificidae. These worms are often found in great numbers in waters of low oxygen content. They have several adaptations which enable them to survive in such habitats. They require a low minimum of oxygen, (1.1-1.5% O₂), and they show a wide range of oxygen independence. In reduced environmental O₂ they regulate their oxygen consumption down to the critical pressure, (1.1-1.5%), below which their oxygen consumption declines rapidly (Palmer, 1968). Hemoglobin transports oxygen particularly at high partial pressures in the normal range of oxygen, and at steep gradients. As oxygen pressure decreases, a behavioral modification is displayed. With the anterior end buried in the substrate, they elongate and wave the posterior end. Under very low oxygen tensions, corkscrew motions tend to pull upper layers of water down to the worm (Prosser and Brown, 1961). The principle route of oxygen exchange is reported to be through the highly vascular integument.

Members of the family Tubificidae are of special interest as pollution indicators. In the past few years, many physiological studies have been done with this group

of worms in order to understand the adaptations needed by organisms to withstand stressful environments. Fowler and Goodnight (1965) studied the effects of environmental factors on the respiration of Tubifex tubifex, a tubificid which occupies the same ecological niche as does L. hoffmeisteri. They found a variety of respiratory responses to environmental situations. Population size had an effect on respiration; oxygen consumption per individual decreased as population size increased up to 50 worms. However, a group of 100 worms consumed more oxygen per individual than any of the smaller groups. A pH shift from 7.5 to 6.1 did not significantly change respiration. Oxygen consumption occurred at a very low level at 0°C.

Osmotic concentration of the environmental medium seems not to be an important factor in the response of T. tubifex to oxygen. Palmer (1968) found no change in the oxygen consumption of T. tubifex to variation in salinity.

Rhythms of Respiration in Invertebrates

The circadian nature of respiration has been studied in many organisms, including invertebrates. Fingermann and Lago (1957) found higher rates of oxygen consumption between midnight and noon than between noon and midnight in the crawfish, Orconectes clypeatus. Persistent daily rhythms of oxygen consumption were found in fiddler crabs by Brown et. al. (1954). They showed a diurnal cycle of

oxygen consumption with maximum rates from 6:00 A.M. to 8:00 A.M. and 11:00 A.M. to 12:00 P.M.; minimum rates occurred at noon and midnight. Sandeen et. al. (1954) investigated rhythms of oxygen consumption in two species of marine snails. Peaks in respiration occurred from 8:30 to 9:30 A.M. and 8:30 to 10:30 P.M., and lows occurred at 2:30 A.M. and 1:30 P.M. in Littorina littorea. Urosalpinx cinereus consumed oxygen the greatest from 4:30 to 6:30 A.M. and 7:30 to 9:30 P.M., with a lesser maximum at 2:30 P.M.; minima occurred from 12:30 to 1:30 A.M. and from 11:30 A.M. to 1:30 P.M.

Rhythms of oxygen consumption have been reported from the Annelida. Magnum and Miyamoto (1970) measured the oxygen consumption of the polychaete bloodworm Glycera di-branchiata under constant dim light. Aerobic metabolism was significantly greater during daylight hours than at night, regardless of the spontaneous activity cycles. The polychaete Arenicola displays a tidal rhythm of oxygen consumption, with maximum at high tide, and minimum at low tide (Gompel, 1937).

Ralph (1957) has described rhythms of oxygen consumption in the oligochaete Lumbricus terrestris; he showed cycles which were diurnal (24 hour), lunar day (24.8 hr.), and lunar (29.5 day) in duration. Peaks in respiration occurred from 6:00 to 8:00 A.M., and about 7:00 P.M.; minima occurred at 4:00 P.M. and 10:00 P.M. These measure-

ments were made in continuous dim light, and did not merely reflect peaks in the locomotor activity of the worms.

The Effect of Continuous Light on Invertebrate Rhythms

Investigations of various biological rhythms usually include experiments in which obvious and controllable environmental conditions can be made constant. This procedure eliminates such significant environmental clues as temperature, pressure, and photoperiod from being used as rhythm setters by the organism under study. The effect of continuous light has been studied in several invertebrate rhythms, with very different responses from different organisms.

In her review article, Harker (1958) showed that one effect of constant light was the loss of various behavioral rhythms that existed under normal conditions of light and dark. *Euglena* ceased to show a rhythm of phototactic sensitivity in constant light. The cockroach, *Periplaneta*, lost a 24 hour activity rhythm after a few days in continuous light, and the emergence rhythm of *Psuedosmitta arem-aria* persisted for only one cycle under constant light conditions. Bruce and Pittendrigh (1957) reported the loss of activity rhythms of the stick insect, *Dixippus morosus*, and the spider beetle, *Ptinus tectus*, under conditions of continuous light.

The persistence of respiration rhythms in constant

light has been shown. Ulanoski and McDiffett (1972) studied the respiration of the mayfly nymph, Isonychia sp. They found higher respiration during darkness of the normal photoperiod, with peaks at 10:00 P.M. and 2:00 A.M. Under constant light, the oxygen consumption of these aquatic insects was still higher during what would be the dark period under normal conditions, but the peaks occurred at 6:00 P.M. and 10:00 P.M. No differences were found in the oxygen consumption period length of the leaf-cutter bee held in constant light and under normal conditions (Tweedy and Stephen, 1971).

Seasonal Variations in Oxygen Consumption and Respiration Rhythms in Invertebrates

The effect of changing seasons on oxygen consumption in invertebrates has been demonstrated by several investigators. Edwards and Irving (1943) found that the respiration of the sand crab, Emerita talpoida, was four times as great in winter than in summer at 3°C. Oxygen consumption was greater in winter at all temperatures less than 20°C., and these higher rates corresponded to a continuation of growth and activity.

Seasonal changes in oxygen consumption were shown to be different for the same species of copepod collected from different ponds by Siefken and Armitage (1967). A population of Diaptomus clavipes taken from a fish labor-

atory pond had a low rate of respiration in early summer, followed by a late summer peak, and a steady decline into winter. A population of the same species taken from a farm pond showed a late spring peak in oxygen consumption, followed by a summer decrease; another peak occurred in late autumn, followed by a steady decline into winter.

Berg, Lumbye, and Ockelmann (1957) found an annual cycle of respiration in the limpet, Ancylus fluviatilis, that closely followed seasonal changes in temperature. Oxygen consumption in this invertebrate gradually increased from a winter low to a mid-summer peak, followed by a steady decline in respiration to another winter low.

Seasonal variations in the daily respiration rhythms of invertebrates have also been demonstrated. In summer, the beetle, Dytiscus marginalis, had a rhythm of oxygen consumption with the maximum at 9:00 P.M., and a secondary maximum at 5:00 A.M. In winter, the hourly changes in respiration became less marked, and a single maximum occurred from 11:00 to 12:00 P.M. The summer peaks in respiration were more than three times greater than the winter peaks (Poljakov, 1938).

Ralph (1957) found that maxima and minima in the oxygen consumption rhythm of the oligochaete Lumbricus terrestris, shifted between late spring and late autumn. In May, maximum rates of respiration occurred at 6:00 A.M.

and 7:00 A.M., and minimum rates occurred at 4:00 P.M.
and 10:00 P.M. In late November, maximum rates occurred
at 10:00 A.M. and 6:00 P.M., and minimum rates occurred at
about noon and 9:00 P.M.

MATERIALS AND METHODS

Experimental Procedures

Tubificid worms of the species Limnodrilus hoffmeisteri and Tubifex tubifex were collected from a section of the Kalamazoo River, in the City of Kalamazoo, downstream from a sewage treatment plant, and from one of its tributaries, Portage Creek, near a paper mill. The worms were taken from the mud in a bottom net, and the fine silt was washed out in the water. The worms and other benthos were brought back to the lab with the larger debris, mostly cellulose fibers, and placed in small aquaria. As the medium putrified, the worms moved to the surface in large balls and were placed in finger bowls containing .01% Knops solution (a .01% Knops solution contains 57.1 mg/l. $\text{Ca}(\text{NO}_3)_2$, 21.4 mg/l. KNO_3 , and 21.4 mg/l. MgSO_4). Worms collected from the Kalamazoo River were used in the L:D trials, and worms obtained from Portage Creek were used in the L:L trials.

Morphological features listed by Brinkhurst and Johnson (1971) were used to separate Limnodrilus hoffmeisteri from the Tubifex obtained in field collections. Initially, L. hoffmeisteri was separated from the Tubifex by the absence of hair setae that are present on the T. tubifex.

To confirm the identification, worms from this initial population of L. hoffmeisteri were fixed on slides, and the shape of the penis sheath was noted. The penis sheath of Limnodrilus hoffmeisteri is much longer than it is broad and has a hood at right angles to the shaft. In Tubifex tubifex, the penis sheath is short, thin, and tub-shaped. During the time of separation and identification, the worms were kept in Knops solution in a dark refrigerator, at a temperature of 55^oF.

For each experiment, groups of 50 L. hoffmeisteri were selected of uniform size and placed in finger bowls, with enough Knops solution to completely cover the ball of worms. They were kept in an environmental chamber at 60⁺⁵^oF., either under continuous fluorescent lighting, or a photoperiod of 14 hours of light, beginning at 6:00 A.M. This adaptation period lasted for one week prior to respiration measurements.

The Gilson Differential Respirometer was used to measure oxygen consumption in each experiment. This apparatus consists of a reaction vessel, which contains the experimental organism, connected to a closed manometer. Gas exchange in the system is reflected in a change in the height of the manometer fluid. Gas evolution or consumption is measured directly in microliters by adjusting the height of the manometer fluid to an index line. A refer-

ence flask is connected to the opposite side of the manometer, so that changes in the vapor pressure of water are applied equally on both sides of the fluid. The reaction vessels and reference flask are submerged in a temperature controlled water bath; a stirring motor mixes the water constantly, to maintain an equal temperature throughout the bath. A variable-speed shaking motor allows for the shaking of the reaction vessels and reference flask; this facilitates maximum gas exchange at the air-water interface in the vessels and flask.

Each 15 milliliter, single sidearm reaction vessel contained 3 ml. of Knops solution, and 0.5 ml. of 6N KOH in the center well, with pleated filter paper to absorb CO₂. The worms were transferred from the finger bowls to filter paper. The entire ball of 50 worms was lifted with a teasing needle and placed in the reaction vessel. Five vessels of fifty worms were used in each run, so that each experiment consisted of a sample population size of 250 worms.

The sidearms of the vessels were stopcocked, and the vessels were greased and joined to the manometers. The main heater and the refrigerator maintained 15°C. with the aid of the stirring motor. The vessels were not shaken at any time during the experimental period. Following a 30 minute equilibration, the system was closed and the index

lines adjusted to zero. Readings of oxygen consumption were taken every two hours for twenty-four hours. The barometric pressure was recorded every twelve hours.

Two-hour oxygen consumption values were converted to standard temperature and pressure by the correction factor determined in the following equation:

$$\text{Correction factor} = \frac{(273) (P_b - 3 - P_w)}{(t + 273) (760)}$$

where, P_b = barometric pressure in mm Hg

P_w = vapor pressure of water at temperature t

t = temperature of the experimental medium in °C.

Each set of worms was used for only one twenty-four hour respiration experiment. At the end of each trial, the worms were examined to determine their general condition. No deaths were recorded, but the worms were not as active as they had been at the beginning of the run.

For the trials in which respiration was measured in a photoperiod of L:D (14:10), the top of the respirometer was covered with aluminum foil, the room lights were kept off, and the micrometers were read with a flashlight during the dark period. For the constant light, L:L, trials, the room lights were kept on for the entire run.

Statistical Procedures

Several different statistical analyses were used on the data from the twenty-four hour respiration studies on

L. hoffmeisteri. The data from the various trials were treated separately, and no attempt was made to pool data from the different days.

The mean oxygen consumption was computed for the flask readings at 2 hour intervals. These means were used to compare oxygen consumption at individual times within the 24 hour period. Standard deviations and coefficients of variation, the ratio between the standard deviation and the mean, were calculated to give a measure of the variability that existed between the flasks in an individual run.

A one-way repeated measure analysis of variance was used to measure significant variation between the mean oxygen consumption of the worms at the various times of the day. In each 24 hour respiration study, measurements of oxygen consumption were made on the same individuals for the entire 24 hour period. Hence, these observations were not independent, and the repeated measurement design was appropriate. In this analysis, the total variation is divided into variation between flasks and within flasks; the within flask variation is further divided into variation due to time and residual variation, or error. Since variation due to time was critical, the null hypothesis tested was:

$$H_0; u_1 = u_2 = \dots u_{12}$$

where, u_1 = mean O_2 -consumption at time 1

The alternative hypothesis was that at least one pair of oxygen consumption means was not equal (Winer, 1962, sect. 4.1-4.4).

Inspection of the patterns of the oxygen consumption data indicated that a fourth degree, (quartic), equation might be a good approximation of the relationship between oxygen consumption and time for L. hoffmeisteri. A repeated measurement test for trend was conducted to determine the significance of various polynomial components in explaining this relationship. In this analysis, the within flask variation due to time was divided into five components. Linear, quadratic, cubic, quartic, and greater than quartic components were tested for significance. An F statistic was computed for each of the five trends, to test the null hypothesis:

H_0 ; x-degree component not significant

where, x = linear, quadratic, cubic, etc.

This analysis was taken after Winer (1962, sect. 4.6).

A transformation feature was used in a stepwise regression analysis to generate the quartic model. The important features of this analysis were the coefficient of determination, a measure of the variation in oxygen consumption due to time explained by the quartic model, and the best-fit quartic curve (Anema, 1971).

Since it was the purpose of this study to consider the existence of a rhythmic nature of respiration of L. hoffmeisteri, and to be able to predict the daily pattern of oxygen consumption of this organism, a non-linear least squares regression analysis was used to attempt to fit a cosine curve to the data. The cosine model was chosen because of its use in describing rhythmic biological functions by Halberg et. al. (1965).

In the first step, a cosine curve of the equation,

$$F = \alpha + \beta \cos(\omega t + \phi)$$

where, F = predicted mean O_2 -consumption at time t

α = 0-line

β = amplitude

ω = angular frequency

ϕ = phase (location of the first peak in time units)

was fitted to data within each 24 hour period. Coefficients of determination were computed to measure the variation accounted for by the cosine model. Estimates of the components of the cosine equation were calculated (Dixon, 1970). From the estimate of the angular frequency, ω , the length of the cosine cycle (c) in hours, was calculated according to the relationship,

$$c = \frac{2\pi}{\omega}$$

A second step was conducted to detect a cosine rhythmic function in the data. Estimates of the amplitude and phase were made for the oxygen consumption data from each flask in a trial. These estimates were then used to compute the transformed values x' and y' , where,

$$x' = \beta \cos \phi$$

$$y' = \beta \sin \phi$$

The several x' and y' were averaged to obtain \bar{x} and \bar{y} , the center of an error ellipse in rectangular coordinates. The major and minor axes of the ellipse, and the angle that the major axis made with the x-axis were calculated. The error ellipse was then drawn. This procedure is the imputation procedure described by Halberg et. al. (1965).

The cosine model tested the null hypothesis that x' and y' were randomly distributed. If the error ellipse included the origin, (0,0), the null hypothesis was accepted, and implied that a cosine rhythm with angular frequency ω was not detected in the sample analyzed. Rejection of the null hypothesis implied that a cosine rhythm with angular frequency ω was detected in the sample analyzed.

RESULTS

The following data tables and graphs show the results of the 24 hour respiration studies on Limnodrilus hoffmeisteri. The results are given in two sections. The first reports the data of 24 hour respiration trials conducted in a photoperiod of L:D (14:10), and the second section shows the data of 24 hour respiration trials conducted in constant light, L:L.

In all tables and graphs where oxygen consumption is given, it is reported in mean microliters of oxygen for fifty worms, for two hours, and is corrected to standard temperature and pressure.

Each set of 24 hour respiration data is treated separately; no attempt was made to combine data from different days, because of possible shifts in the pattern of oxygen consumption.

The tables and graphs are accompanied by an identifying letter, which corresponds to the date of the respiration study:

L:D (14:10)

A. July 26-27, 1972 (Tables 1-4 and Figure 1)

B. July 27-28, 1972 (Tables 5-8 and Figure 2)

L:L

C. September 1-2, 1971 (Tables 9-12 and Figure 3)

D. October 21-22, 1971 (Tables 13-16 and Figure 4)

E. November 18-19, 1971 (Tables 17-20 and Figure 5)

Means, standard deviations, and coefficients of variation are found in Tables 1, 5, 9, 13, and 17.

The one-way repeated measure analyses of variance are given in Tables 2, 6, 10, 14, and 18.

The results of the quartic model analyses are reported in Tables 3, 7, 11, 15, and 19.

The results of the cosine model analyses are found in Tables 4, 8, 12, 16, and 20.

A graph comparing the 24 hour oxygen consumption of L. hoffmeisteri in L:D (14:10) and in L:L is shown in Figure 6.

Results of 24 Hour Respiration in a Photoperiod of L:D (14:10)

Two determinations of the daily pattern of oxygen consumption of Limnodrilus hoffmeisteri, in a photoperiod of L:D (14:10), were made in July, 1972.

The mean oxygen consumption was highest between 10:00 A.M. and 12:00 P.M. It decreased steadily to a low around midnight, and reached a secondary maximum between 6:00 A.M. and 8:00 A.M. (Tables 1 and 5, Figures 1 and 2).

The variation in oxygen consumption between individual flasks was greater in B, as measured by the coefficients of variation (Tables 1 and 5).

There was significant variation in oxygen consumption due to time in both studies, with 99% confidence. Linear and quadratic trends were significant in explaining the relationship between oxygen consumption and time in both (Tables 2 and 6), and a greater than quartic trend was significant in B (Table 6).

Eighty percent of the variation in oxygen consumption due to time was accounted for by the quartic model in A (Table 3). The quartic model accounted for 55% of this variation in B (Table 7).

The results of the cosine analysis were very different between the two trials. The amplitude and phase in A were $5.3 \mu\text{LO}_2$ and 4.4 hours; in B, the amplitude was $17.0 \mu\text{LO}_2$, and the phase was 14.4 hours. The length of the cosine cycle was 11.1 hours in A, and 42 hours in B. The cosine model accounted for 58% of the variation in oxygen consumption due to time in A, and for 15% in B (Tables 4 and 8). The best-fit cosine curves were very dissimilar (Figures 1 and 2).

The cosine fitness test resulted in acceptance of the null hypothesis in both studies (Tables 4 and 8), hence, a cosine rhythm of oxygen consumption was not detected in either trial.

Table 1. Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of Limnodrilus hoffmeisteri, in a Photoperiod of L:D (14:10)

Hour	Mean μO_2 / 2hr./ 50worms	Std. Dev.	Coeff. Var.
8-10am	51.2	5.9	.11
10-12pm	56.1	3.7	.07
12-2	48.4	4.4	.09
2-4	40.5	3.4	.08
4-6	35.6	2.1	.06
6-8	34.7	1.9	.06
8-10	33.8	4.6	.13
10-12am	30.1	2.7	.09
12-2	30.5	2.1	.07
2-4	29.3	3.5	.12
4-6	34.0	4.0	.12
6-8am	36.3	7.1	.19

Table 2. One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in a Photoperiod of L:D (14:10)

Source	SS	DF	MS	F
Between Flasks	54.33	4		
Within Flasks	5041.44	55		
Time	4305.03	11	391.37	23.38*
Linear	2781.15	1	2781.15	106.17*
Quadratic	995.55	1	995.55	59.48*
Cubic	108.26	1	108.26	6.47
Quartic	65.48	1	65.48	3.91
> Quartic	354.42	7	50.66	3.03
Residual	736.42	44	16.74	
Total	5095.77	59		

$$F_{11,44}(\text{Time}) = \underline{2.72} \text{ at } .01 \text{ prob level}$$

$$F_{1,44}(\text{linear-quartic trend}) = \underline{7.30} \text{ at } .01 \text{ prob level}$$

$$F_{7,44}(> \text{quartic trend}) = \underline{3.10} \text{ at } .01 \text{ prob level}$$

* F statistic significant

Table 3. Quartic Model of Oxygen Consumption vs. Time for
24 Hour Respiration of Limnodrilus hoffmeisteri,
A in a Photoperiod of L:D (14:10)

$$\text{Quartic model: } \bar{Y}_p = \alpha + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \beta_4 t^4 + \epsilon$$

whose
estimates are: $\bar{Y}_p = 50.3 + 5.7t + 3.1t^2 + 0.4t^3 + 0.0t^4$

where \bar{Y}_p = predicted mean O₂-consumption at time t

β_1 = partial regression coefficient*

% Variability accounted for by the quartic model = 80%

Predicted vs. Actual Results

Hour	Actual (\bar{Y}_a)	Predicted (\bar{Y}_p)	Deviation ($\bar{Y}_p - \bar{Y}_a$)
8-10am	51.2	53.2	2.0
10-12pm	56.1	51.9	- 4.1
12-2	48.4	48.1	- 0.3
2-4	40.5	43.1	2.8
4-6	35.6	38.1	2.5
6-8	34.7	33.9	- 0.8
8-10	33.8	31.0	- 2.8
10-12am	30.1	29.8	- 0.3
12-2	30.5	30.0	- 0.5
2-4	29.3	31.6	2.3
4-6	34.0	33.8	- 0.2
6-8am	36.3	35.8	- 0.5

The differences between the actual \bar{Y}_a and the
predicted \bar{Y}_p are shown graphically in Fig. 1.

* Significance of coefficients shown in Table 2

Table 4. Cosine Model of Oxygen Consumption vs. Time for
24 Hour Respiration Data of Limnodrilus hoff-
meisteri, in a Photoperiod of L:D (14:10)

A

$$\text{Cosine Model: } F = \alpha + \beta \cos(\omega t + \phi)$$

$$\text{whose estimates are: } F = 38.2 + 5.3\cos(1.1t + 2.2)$$

where, F = predicted mean O_2 -consumption at time t

α = 0-line

β = amplitude

ω = angular frequency

ϕ = phase (location of first peak in time units)

$$\text{cycle in hours (c)} = \frac{2\pi}{\omega} = 11.1 \text{ hr.}$$

% Variability accounted for by the cosine model = 38%

Predicted vs. Actual Results

Hour	Actual \bar{X}	Predicted \bar{F}	Deviation ($\bar{F} - \bar{X}$)
8-10am	51.2	40.8	- 10.4
10-12pm	56.1	43.5	- 12.6
12-2	48.4	40.1	- 8.3
2-4	40.5	34.5	- 6.0
4-6	35.6	33.2	- 2.4
6-8	34.7	37.6	2.9
8-10	33.8	42.7	8.9
10-12am	30.1	42.6	12.5
12-2	30.5	37.5	7.0
2-4	29.3	33.1	3.8
4-6	34.0	34.6	0.6
6-8am	36.3	40.2	3.9

The differences between the actual \bar{X} and the predicted \bar{F} are shown graphically in Fig. 1.

Table 4 (cont.). Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in a Photo-period of L:D (14:10)

A

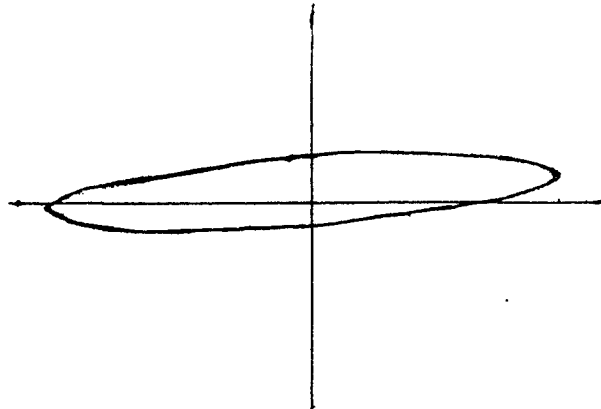
Cosine Fitness Test		
Flask*	β (radians)	ϕ (radians)
1	4.0	3.8
2	5.6	- 2.3
3	12.8	- 0.1
4	-6.4	0.9

center of error ellipse (\bar{x} , \bar{y}) = (0.3, -3.4)

angle of major axis from x-axis = 8°

length of major axis = 126.3, of minor axis = 13.7

Graphic Display of 95% Error Ellipse



The origin, (0, 0) is contained in the 95% error ellipse; the null hypothesis is accepted.

* The data from one flask was eliminated in this analysis because of exceptional values of β and ϕ .

Figure 1. Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in a Photoperiod of L:L (14:10), Showing best-fit Quartic and Cosine Curves

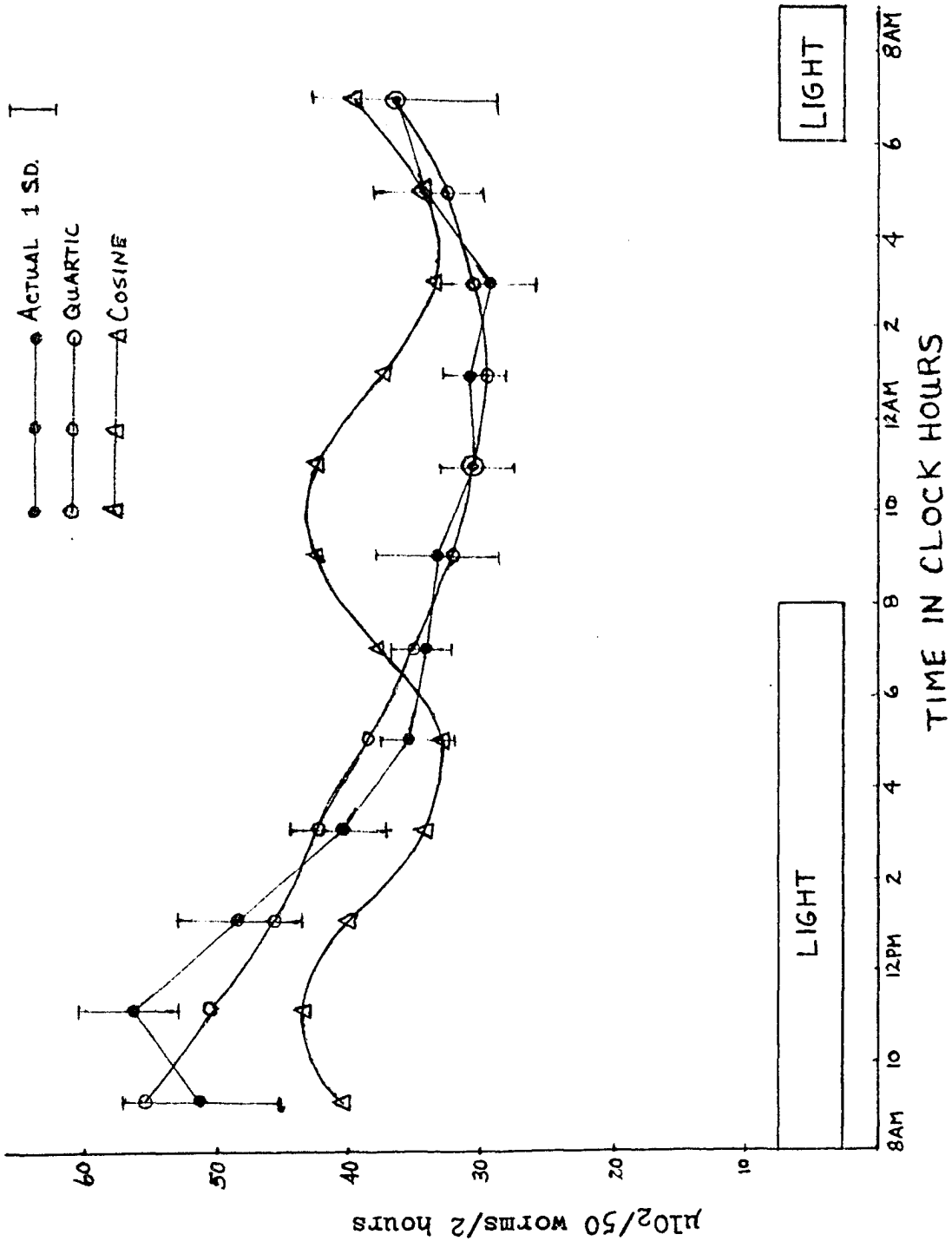


Table 5. Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of Limnodrilus hoffmeisteri, in a Photoperiod of L:D (14:10)

Hour	Mean μO_2 / 2hr./2/ 50worms	Std. Dev.	Coeff. Var.
10-12pm	52.4	6.8	.13
12-2	49.1	5.7	.12
2-4	42.3	4.8	.11
4-6	38.9	7.1	.18
6-8	37.6	3.6	.10
8-10	34.4	11.8	.34
10-12am	30.9	3.5	.11
12-2	23.9	4.7	.20
2-4	24.6	7.3	.30
4-6	37.7	4.7	.13
6-8	32.1	7.3	.23
8-10am	31.5	11.3	.36

Table 6. One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in a Photoperiod of L:D (14:10)

Source	SS	DF	MS	F
Between Flasks	1670.00	4		
Within Flasks	4873.85	55		
Time	4160.81	11	378.26	23.34*
Linear	2466.37	1	2466.37	152.19*
Quadratic	923.72	1	923.72	57.00*
Cubic	9.45	1	9.45	0.58
Quartic	64.33	1	64.33	3.97
> Quartic	696.94	7	99.56	6.14*
Residual	713.04	44	16.21	
Total	6543.85	59		

$$F_{11,44}(\text{Time}) = \underline{2.72} \text{ at } .01 \text{ prob. level}$$

$$F_{1,44}(\text{linear-quartic trend}) = \underline{7.30} \text{ at } .01 \text{ prob. level}$$

$$F_{7,44}(> \text{quartic trend}) = \underline{3.10} \text{ at } .01 \text{ prob. level}$$

* P statistic significant

Table 7. Quartic Model of Oxygen Consumption vs. Time for
 24 Hour Respiration of Limnodrilus hoffmeisteri,
 B in a Photoperiod of L:D (14:10)

$$\text{Quartic Model: } \bar{Y}_p = \alpha + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \beta_4 t^4 + \epsilon$$

whose
 estimates are: $\bar{Y}_p = 50.5 + 3.7t + 2.8t^2 + 0.4t^3 + 0.0t^4$

where \bar{Y}_p = predicted mean O_2 -consumption at time t

β_1 = partial regression coefficient*

% Variability accounted for by the quartic model = 55%

Predicted vs. Actual Results

Hour	Actual (\bar{Y}_a)	Predicted (\bar{Y}_p)	Deviation ($\bar{Y}_p - \bar{Y}_a$)
10-12pm	52.4	51.7	- 0.7
12-2	49.1	49.2	0.1
2-4	42.3	44.7	2.4
4-6	38.9	39.6	0.7
6-8	37.6	34.9	- 2.7
8-10	34.4	31.3	- 3.1
10-12am	30.9	29.3	- 1.6
12-2	23.9	28.8	4.9
2-4	24.6	29.6	5.0
4-6	37.7	31.1	- 6.6
6-8	32.1	32.5	0.4
8-10am	31.5	32.4	0.9

The differences between the actual \bar{Y}_a and the
 predicted \bar{Y}_p are shown graphically in Fig. 2

* Significance of coefficients shown in Table 6

Table 8. Cosine Model of Oxygen Consumption vs. Time for
24 Hour Respiration Data of Limnodrilus hoff-
B meisteri, in a Photoperiod of L:D (14:10)

$$\text{Cosine Model: } F = \alpha + \beta \cos(\omega t + \phi)$$

$$\text{whose estimates are: } F = 45.6 + 17.0\cos(0.3t + 7.2)$$

where, F = predicted mean O₂-consumption at time t

α = 0-line

β = amplitude

ω = angular frequency

ϕ = phase (location of first peak in time units)

$$\text{cycle in hours (c)} = \frac{2\pi}{\omega} = 42 \text{ hr.}$$

% Variability accounted for by the cosine model = 15%

Predicted vs. Actual Results

Hour	Actual \bar{X}	Predicted \bar{F}	Deviation $(\bar{F} - \bar{X})$
10-12pm	52.4	52.3	- 0.1
12-2	49.1	48.2	- 0.9
2-4	42.3	43.8	1.5
4-6	38.9	39.6	0.7
6-8	37.6	35.8	- 1.6
8-10	34.4	32.6	- 1.8
10-12am	30.9	30.3	- 0.6
12-2	23.9	28.9	5.0
2-4	24.6	28.6	4.0
4-6	37.7	29.5	- 8.2
6-8	32.1	31.4	- 0.7
8-10am	31.5	34.2	2.7

The differences between the actual \bar{X} and the predicted \bar{F} are shown graphically in Fig. 2.

Table 8 (cont.). Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in a Photo-period of L:D (14:10)

B

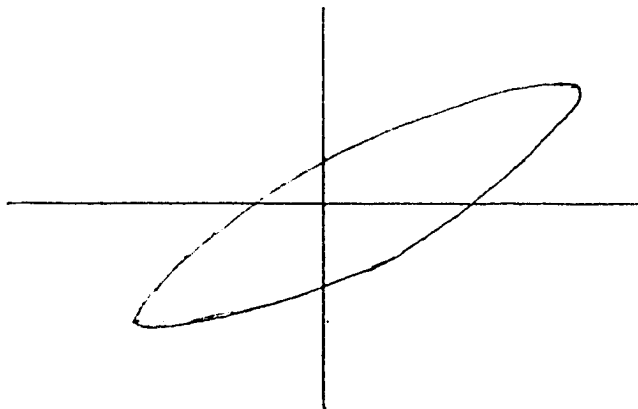
Cosine Fitness Test		
Flask*	β (radians)	ϕ (radians)
1	10.4	6.3
2	10.9	6.4
3	7.2	- 2.1
4	13.1	6.0

center of error ellipse (\bar{x} , \bar{y}) = (7.6 , -2.1)

angle of major axis from x-axis = 20°

length of major axis = 119.1, of minor axis = 29.8

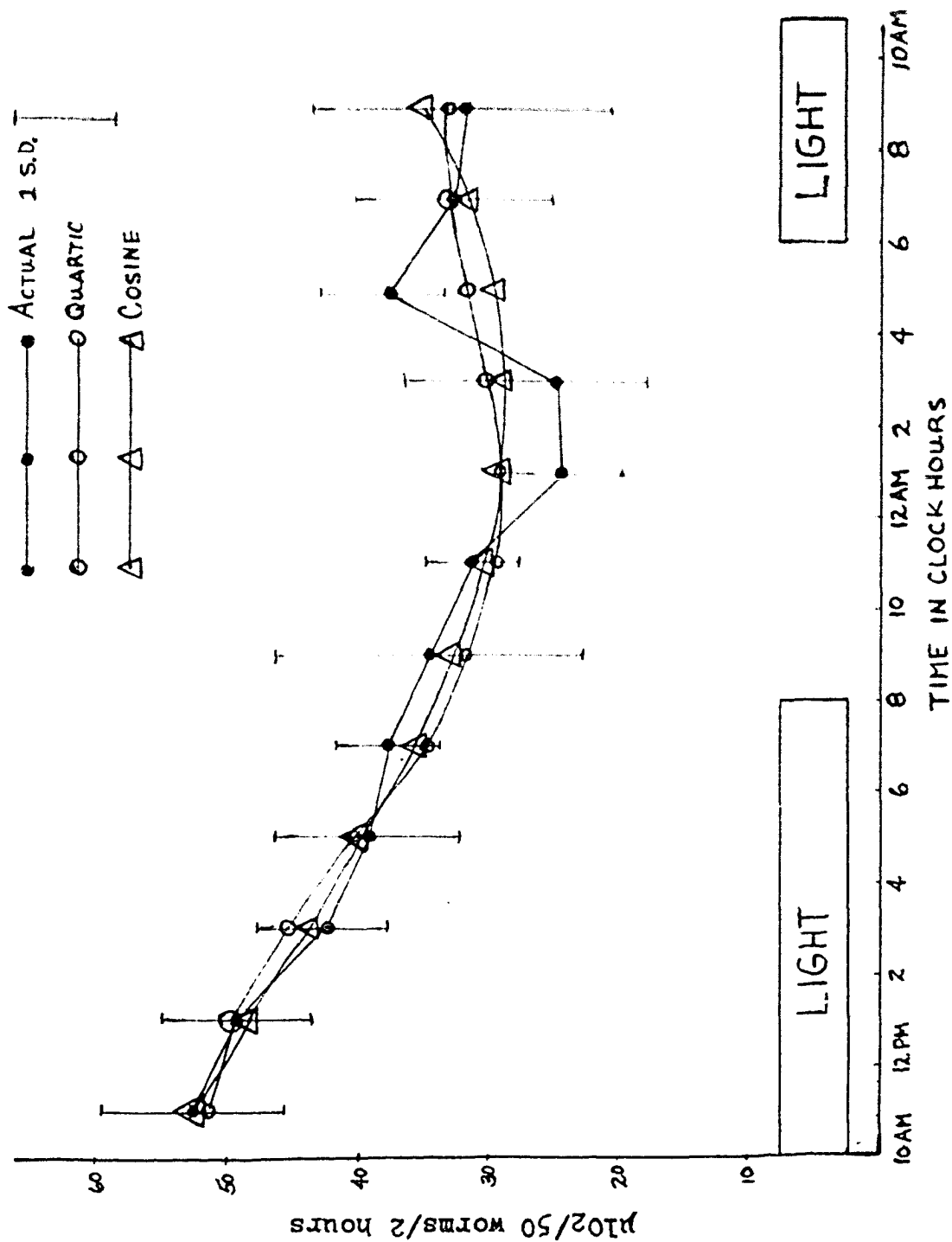
Graphic Display of 95% Error Ellipse



The origin, (0 , 0) is contained in the 95% error ellipse; the null hypothesis is accepted

* The data from one flask was eliminated in this analysis because of exceptional values of β and ϕ .

Figure 2. Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in a Photoperiod of L:D (14:10), Showing best-fit quartic and Cosine Curves



Results of 24 Hour Respiration
in Constant Light L:L

The oxygen consumption of Limnodrilus hoffmeisteri was measured over twenty-four hours, three times in constant light, L:L, from September to November, 1971.

Maxima in mean oxygen consumption occurred at 5:00 P.M. and 7:00 A.M., with a lesser maximum at 11:00 P.M. Minima occurred at 9:00 P.M. and 1:00 A.M. in C (Table 9 and Figure 3). In D, maxima occurred at 3:00 P.M. and 1:00 A.M., with minima at 11:00 P.M. and 5:00 A.M. (Table 13 and Figure 4). Maxima in mean oxygen consumption occurred at 3:00 P.M. and 3:00 A.M. in E, and minima occurred at 11:00 A.M. and 7:00 P.M. (Table 17 and Figure 5).

The variation in oxygen consumption between flasks was higher in C than in D, and higher in D than in E (Tables 9, 13, and 17).

In all trials, there was significant variation in oxygen consumption due to time (Tables 10, 14, and 18). The linear, quadratic, quartic, and greater than quartic trends were significant in C (Table 10). The linear, quadratic, cubic, and greater than quartic trends were significant in D (Table 14). The quadratic, cubic, quartic, and greater than quartic trends were significant in E (Table 18).

The quartic model accounted for 56% of the variation in oxygen consumption due to time in C (Table 11), for 44% of the variation in D (Table 15), and it accounted for 47% of the variation in E (Table 19).

The cosine model accounted for 19% of the variation in oxygen consumption due to time in C. The amplitude was $2.4 \mu\text{O}_2$, the phase was 3.5 hours, and the length of the cosine cycle was 12.3 hours. A cosine rhythm of oxygen consumption was not detected in this set of data (Table 12).

Ten percent of the variation in oxygen consumption due to time was accounted for by the cosine model in D. The amplitude was $4.6 \mu\text{O}_2$, the phase was 3.8 hours, and the length of the cosine cycle was 12.6 hours. A cosine rhythm of oxygen consumption was not detected in this set of data (Table 16).

The cosine model accounted for 58% of the variation in oxygen consumption due to time in E. The amplitude was $5.9 \mu\text{O}_2$, the phase was 7.2 hours, and the length of the cosine cycle was 13.8 hours. A cosine rhythm of oxygen consumption was detected in this set of data (Table 20).

Table 9. Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of Limnodrilus hoffmeisteri, in Constant Light, LL

Hour	Mean μO_2 / 2hr./2/ 50worms	Std. Dev.	Coeff. Var.
10-12pm	6.5	4.3	.65
12-2	7.2	2.8	.39
2-4	10.8	2.4	.23
4-6	12.4	2.8	.23
6-8	11.1	1.3	.12
8-10	10.4	1.8	.17
10-12am	12.6	1.2	.09
12-2	12.6	1.1	.08
2-4	13.0	1.7	.13
4-6	14.3	1.8	.13
6-8	18.0	1.8	.10
8-10am	12.7	1.8	.14

Table 10. One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, LL

Source	SS	DF	MS	F
Between Flasks	91.58	4		
Within Flasks	701.23	55		
Time	541.20	11	49.20	13.53*
Linear	367.78	1	367.78	101.12*
Quadratic	38.33	1	38.33	10.54*
Cubic	0.75	1	0.75	0.00
Quartic	40.68	1	40.68	11.19*
> Quartic	94.40	7	13.49	3.71*
Residual	160.03	44	3.64	
Total	792.80	59		

$$F_{11,44}(\text{Time}) = \underline{2.72} \text{ at } .01 \text{ prob. level}$$

$$F_{1,44}(\text{linear-quartic trend}) = \underline{7.30} \text{ at } .01 \text{ prob. level}$$

$$F_{7,44}(> \text{quartic trend}) = \underline{3.10} \text{ at } .01 \text{ prob. level}$$

* F statistic significant

Table 11. Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L

$$\text{Quartic Model: } \bar{Y}_p = \alpha + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \beta_4 t^4 + \epsilon$$

whose estimates are: $\bar{Y}_p = 1.4 + 9.1t + 2.4t^2 + 0.3t^3 + 0.0t^4$

where \bar{Y}_p = predicted mean O₂-consumption at time t

β_1 = partial regression coefficient*

% Variability accounted for by the quartic model = 56%

Predicted vs. Actual Results

Hour	Actual (Y _a)	Predicted (Y _p)	Deviation (Y _p - Y _a)
10-12pm	6.5	5.6	- 0.9
12-2	7.2	9.2	2.0
2-4	10.8	10.6	- 0.2
4-6	12.4	11.0	- 1.4
6-8	11.1	11.0	- 0.1
8-10	10.4	11.2	0.8
10-12am	12.6	11.8	- 0.8
12-2	12.6	12.8	0.2
2-4	13.0	14.1	1.1
4-6	14.3	15.2	0.9
6-8	18.0	15.4	- 2.6
8-10am	12.7	13.7	1.0

The differences between the actual \bar{Y}_a and the predicted \bar{Y}_p are shown graphically in Fig. 3

* Significance of coefficients shown in Table 10

Table 12. Cosine Model of Oxygen Consumption vs. Time for
24 Hour Respiration Data of Limnodrilus hoff-
meisteri, in Constant Light, L:L

Cosine Model: $F = \alpha + \beta \cos(\omega t + \phi)$

whose estimates are: $F = 11.8 + 2.4\cos(1.0t + 1.7)$

where, F = predicted mean O_2 -consumption at time t

α = 0-line

β = amplitude

ω = angular frequency

ϕ = phase (location of first peak in time units)

cycle in hours (c) = $\frac{2\pi}{\omega} = 12.3$ hours

% Variability accounted for by the cosine model = 19%

Predicted vs. Actual Results

Hour	Actual \bar{X}	Predicted \bar{F}	Deviation $(\bar{F} - \bar{X})$
10-12pm	6.5	9.5	3.0
12-2	7.2	9.8	2.6
2-4	10.8	12.0	1.2
4-6	12.4	14.0	1.6
6-8	11.1	13.8	2.7
8-10	10.4	11.7	1.3
10-12am	12.6	9.7	- 2.9
12-2	12.6	9.6	- 3.0
2-4	13.0	11.6	- 1.4
4-6	14.3	13.8	- 0.5
6-8	18.0	14.0	- 4.0
8-10am	12.7	12.1	- 0.6

The differences between the actual \bar{X} and the predicted \bar{F} are shown graphically in Fig. 3.

Table 12 (cont.). Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L

C

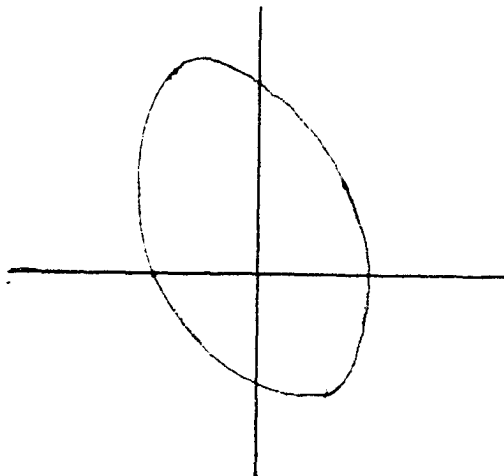
Cosine Fitness Test		
Flask*	β (radians)	ϕ (radians)
1	2.9	1.7
2	1.9	2.4
3	3.4	1.9
4	1.0	1.1

center of error ellipse (\bar{x} , \bar{y}) = (-0.6, 2.1)

angle of major axis from x-axis = 25°

length of major axis = 10.3, of minor axis = 18.4

Graphic Display of 95% Error Ellipse



The origin, (0, 0) is contained in the 95% error ellipse; the null hypothesis is accepted.

* The data from one flask was eliminated in this analysis because of exceptional values of β and ϕ .

Figure 3. Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L, Showing best-fit Quartic and Cosine Curves

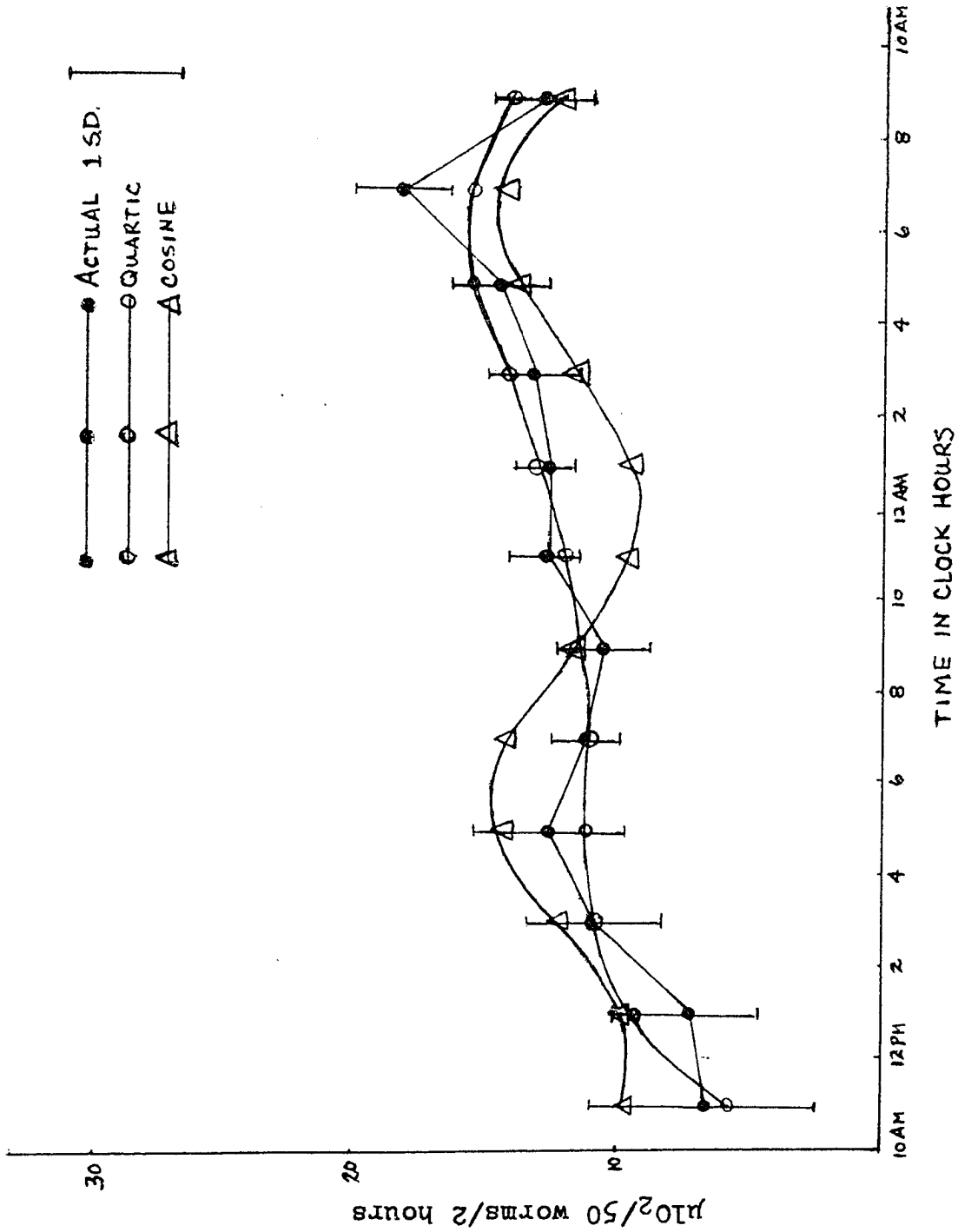


Table 13. Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of Limnodrilus hoffmeisteri, in Constant Light, LL

D

Hour	Mean μO_2 / 2hr./ 50worms	Std. Dev.	Coeff. Var.
8-10am	16.4	6.4	.39
10-12pm	24.5	4.4	.18
12-2	26.8	4.3	.16
2-4	39.8	9.2	.23
4-6	31.9	4.0	.13
6-8	30.1	3.9	.13
8-10	28.9	2.5	.09
10-12am	27.7	3.7	.13
12-2	33.0	3.7	.11
2-4	28.0	3.0	.10
4-6	28.2	3.8	.13
6-8am	31.9	2.9	.09

Table 14. One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, LL

Source	SS	DF	MS	F
Between Flasks	440.97	4		
Within Flasks	2287.27	55		
Time	1696.43	11	154.21	11.49*
Linear	293.57	1	293.57	21.86*
Quadratic	483.82	1	483.82	36.03*
Cubic	446.60	1	446.60	33.26*
Quartic	63.20	1	63.20	4.70
> Quartic	409.24	7	58.46	4.35*
Residual	590.84	44	13.43	
Total	2728.24	59		

$$F_{11,44}(\text{Time}) = \underline{2.72} \text{ at } .01 \text{ prob. level}$$

$$F_{1,44}(\text{linear-quartic trend}) = \underline{7.30} \text{ at } .01 \text{ prob. level}$$

$$F_{7,44}(>\text{quartic trend}) = \underline{3.10} \text{ at } .01 \text{ prob. level}$$

* F statistic significant

Table 15. Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L

$$\text{Quartic model: } \bar{Y}_p = +\beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \beta_4 t^4 + \epsilon$$

whose estimates are: $\bar{Y}_p = 0.8 + 20.0t + 4.1t^2 + 0.3t^3 + 0.0t^4$

where \bar{Y}_p = predicted mean O₂-consumption at time t

β_1 = partial regression coefficient*

% Variability accounted for by the quartic model = 44%

Predicted vs. Actual Results

Hour	Actual (\bar{Y}_a)	Predicted (\bar{Y}_p)	Deviation ($\bar{Y}_p - \bar{Y}_a$)
8-10am	16.4	15.5	- 0.9
10-12pm	24.5	25.5	1.0
12-2	26.8	30.8	4.0
2-4	39.8	32.8	- 7.0
4-6	31.9	32.8	0.9
6-8	30.1	31.7	1.6
8-10	28.9	30.4	1.5
10-12am	27.7	29.3	1.6
12-2	33.0	28.8	- 4.2
2-4	28.0	29.1	1.1
4-6	28.2	30.0	1.8
6-8am	31.9	31.3	- 0.6

The differences between the actual \bar{Y}_a and the predicted \bar{Y}_p are shown graphically in Fig. 4

* Significance of coefficients shown in Table 13

Table 16. Cosine Model of Oxygen Consumption vs. Time for
24 Hour Respiration Data of Limnodrilus hoff-
D meisteri, in Constant Light, L:L

$$\text{Cosine Model: } F = \alpha + \beta \cos(\omega t + \phi)$$

$$\text{whose estimates are: } F = 28.9 + 4.6\cos(1.0t + 1.9)$$

where, F = predicted mean O₂-consumption at time t

α = 0-line

β = amplitude

ω = angular frequency

ϕ = phase (location of first peak in time units)

$$\text{cycle in hours (c)} = \frac{2\pi}{\omega} = 12.6 \text{ hr.}$$

% Variability accounted for by the cosine model = 10%

Predicted vs. Actual Results

Hour	Actual \bar{X}	Predicted \bar{F}	Deviation $(\bar{F} - \bar{X})$
8-10am	16.4	24.4	8.0
10-12pm	24.5	25.7	1.2
12-2	26.8	30.0	3.2
2-4	39.8	33.3	- 6.5
4-6	31.9	32.5	0.6
6-8	30.1	28.4	- 1.7
8-10	28.9	24.7	- 4.2
10-12am	27.7	24.9	- 2.8
12-2	33.0	28.9	- 4.1
2-4	28.8	32.8	4.0
4-6	28.2	33.1	4.9
6-8am	31.9	29.5	- 2.4

The differences between the actual \bar{X} and the predicted \bar{F} are shown graphically in Fig. 4.

Table 16 (cont.). Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L

D

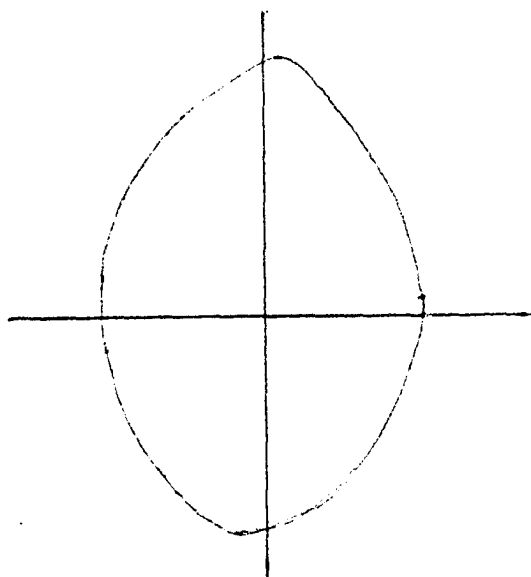
Cosine Fitness Test		
Flask*	β (radians)	ϕ (radians)
1	2.0	- 1.5
2	4.9	1.8
3	8.0	1.1
4	7.4	1.9

center of error ellipse $(\bar{x}, \bar{y}) = (0.0, 4.2)$

angle of major axis from x-axis = -5°

length of major axis = 40.6, of minor axis = 64.7

Graphic Display of 95% Error Ellipse



The origin, $(0, 0)$ is contained in the 95% error ellipse; the null hypothesis is accepted.

* The data from one flask was eliminated in this analysis because of exceptional values of β and ϕ .

Figure 4. Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L, Showing best-fit Quartic and Cosine Curves

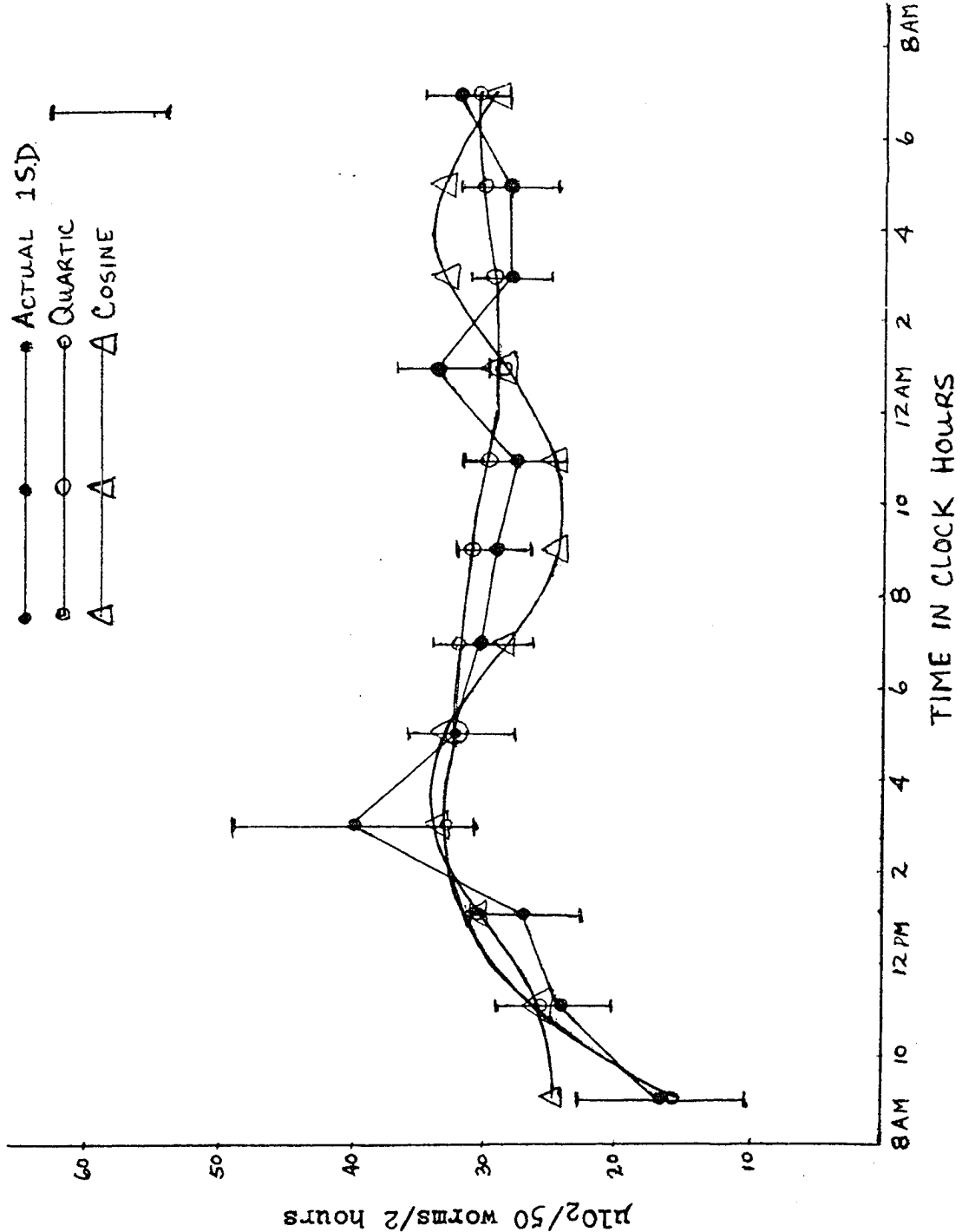


Table 17. Means, Standard Deviations, and Coefficients of Variation for 24 Hour Respiration of Limnodrilus hoffmeisteri, in Constant Light, LL
E

Hour	Mean μO_2 / 2hr./2/ 50worms	Std. Dev.	Coeff. Var.
10-12am	25.1	2.8	.11
12-2	28.1	2.3	.08
2-4	30.9	2.0	.07
4-6	30.7	1.6	.05
6-8	26.4	2.2	.08
8-10	18.8	3.9	.20
10-12pm	16.0	2.1	.13
12-2	26.1	1.5	.06
2-4	29.9	2.1	.07
4-6	28.9	3.0	.10
6-8	27.7	1.1	.04
8-10pm	28.4	2.0	.07

Table 18. One-Way Repeated Measure ANOVA and Test for Trend for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, LL

Source	SS	DF	MS	F
Between Flasks	157.30	4		
Within Flasks	1271.20	55		
Time	1165.21	11	105.93	43.98*
Linear	0.99	1	0.99	0.41
Quadratic	129.25	1	129.25	53.66*
Cubic	38.35	1	38.35	15.92*
Quartic	505.43	1	505.43	209.83*
> Quartic	491.20	7	70.17	29.13*
Residual	105.98	44	2.41	
Total	1428.50	59		

$$F_{11,44}(\text{Time}) = \underline{2.72} \text{ at } .01 \text{ prob. level}$$

$$F_{1,44}(\text{linear-quartic trend}) = \underline{7.30} \text{ at } .01 \text{ prob. level}$$

$$F_{7,44}(> \text{quartic trend}) = \underline{3.10} \text{ at } .01 \text{ prob. level}$$

* F statistic significant



Table 19. Quartic Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L

$$\text{Quartic model: } \bar{Y}_p = \alpha + \beta_1 t + \beta_2 t^2 + \beta_3 t^3 + \beta_4 t^4 + \epsilon$$

whose estimates are: $\bar{Y}_p = 7.7 + 23.0t + 7.3t^2 + 0.8t^3 + 0.0t^4$

where \bar{Y}_p = predicted mean O_2 -consumption at time t

β_1 = partial regression coefficient*

* Variability accounted for by the quartic model = 47%

Predicted vs. Actual Results

Hour	Actual (\bar{Y}_a)	Predicted (\bar{Y}_p)	Deviation ($\bar{Y}_p - \bar{Y}_a$)
10-12am	25.1	24.2	- 0.9
12-2	28.1	30.6	2.5
2-4	30.9	30.8	- 0.1
4-6	30.7	27.9	- 2.8
6-8	26.4	24.4	- 2.0
8-10	18.8	21.8	3.0
10-12pm	16.0	21.2	5.2
12-2	26.1	22.6	- 3.5
2-4	29.9	25.6	- 4.3
4-6	28.9	28.8	- 0.1
6-8	27.7	30.2	2.5
8-10pm	28.4	27.1	- 1.3

The differences between the actual \bar{Y}_a and the predicted \bar{Y}_p are shown graphically in Fig. 5

* Significance of coefficients shown in Table 18

Table 20. Cosine Model of Oxygen Consumption vs. Time for
 24 Hour Respiration Data of Limnodrilus hoff-
 E meisteri, in Constant Light, L:L

Cosine Model: $F = \alpha + \beta \cos(\omega t + \phi)$

whose estimates are: $F = 25.4 + 5.9\cos(0.9t + 3.6)$

where, F = predicted mean O_2 -consumption at time t

α = 0-line

β = amplitude

ω = angular frequency

ϕ = phase (location of first peak in time units)

cycle in hours (c) = $\frac{2\pi}{\omega} = 13.8$ hr.

% Variability accounted for by the cosine model = 58%

Predicted vs. Actual Results

Hour	Actual \bar{X}	Predicted \bar{F}	Deviation $(\bar{F} - \bar{X})$
10-12am	25.0	24.1	- 0.9
12-2	28.1	29.0	0.9
2-4	30.9	31.3	0.4
4-6	30.7	29.5	- 1.2
6-8	26.4	24.7	- 1.7
8-10	18.8	20.4	1.6
10-12pm	16.0	19.7	3.7
12-2	26.1	23.1	- 3.0
2-4	29.9	28.1	- 1.8
4-6	28.9	31.2	2.3
6-8	27.7	30.1	2.4
8-10pm	28.4	25.7	- 2.7

The differences between the actual \bar{X} and the predicted \bar{F} are shown graphically in Fig. 5.

Table 20 (cont.). Cosine Model of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnodrilus hoffmeisteri, in Constant Light, L:L

E

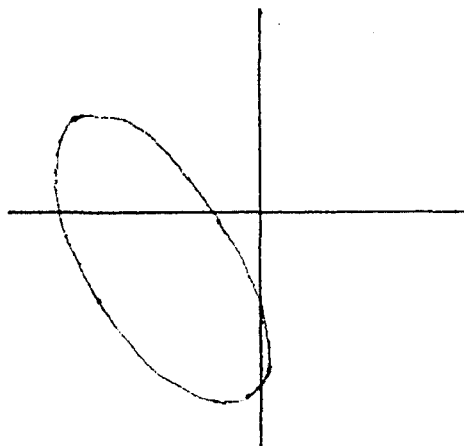
Cosine Fitness Test		
Flask	β (radians)	ϕ (radians)
1	5.4	4.1
2	6.5	3.7
3	6.5	3.6
4	6.1	3.2
5	5.3	3.5

center of error ellipse (\bar{x} , \bar{y}) = (-5.1, -2.6)

angle of major axis from x-axis = 34°

length of major axis = 7.1, of minor axis = 17.3

Graphic Display of 95% Error Ellipse



The origin, (0, 0) is not contained in the 95% error ellipse; the null hypothesis is rejected.

Figure 5. Graph of Oxygen Consumption vs. Time for 24 Hour Respiration Data of Limnocalanus hoffmeisteri, in Constant Light, L:L, Showing best-fit Quartic and Cosine Curves

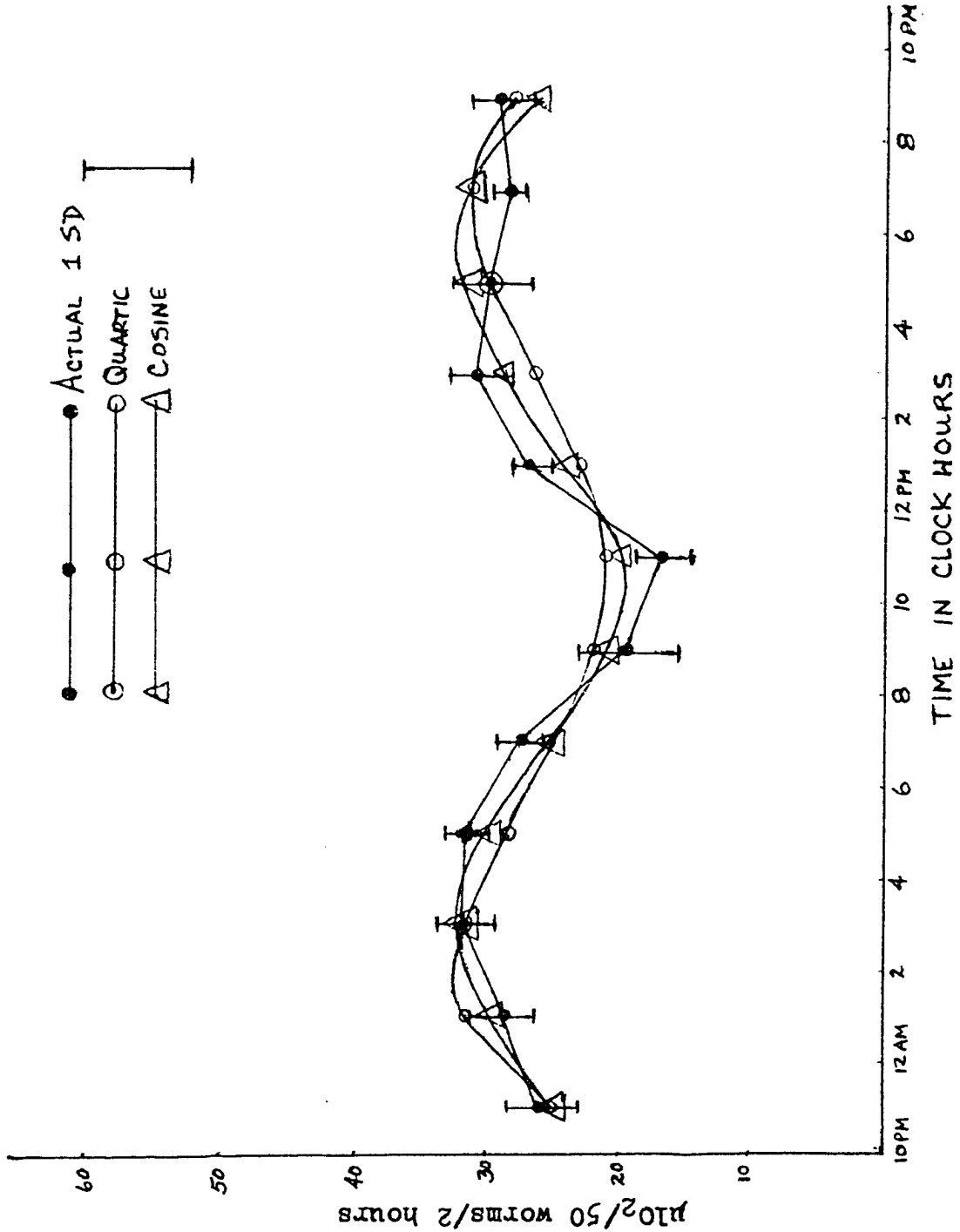
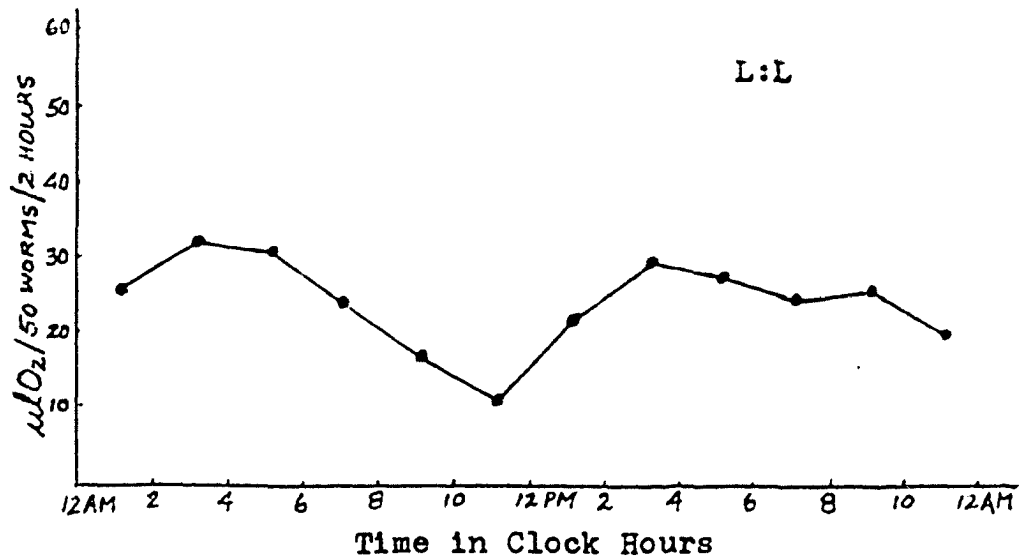
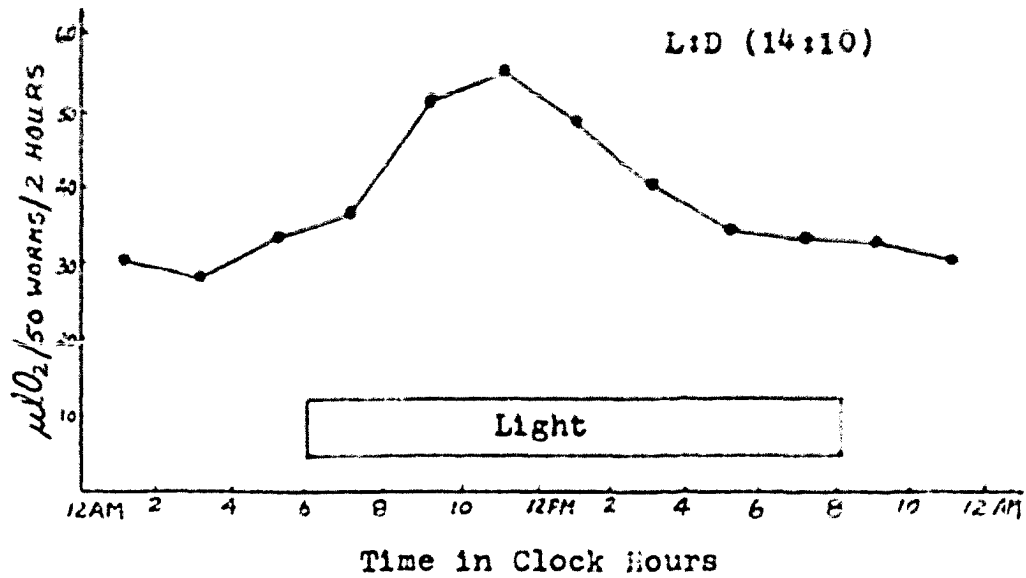


Figure 6. Graph Showing Comparison of Oxygen Consumption vs. Time for Respiration Data of Limnodrilus hoffmeisteri, in L:D (14:10) and L:L



DISCUSSION

The pattern of oxygen consumption of Limnodrilus hoffmeisteri was studied. Respiration was measured for twenty-four hour periods in conditions of constant light in autumn, and in a photoperiod of L:D (14:10) in the summer.

It has been shown that in both L:D and L:L trials, there was significant variation in the oxygen consumption of these aquatic oligochaetes over a 24 hour period; the worms did not consume the same amounts of oxygen at all times of the day.

The patterns of respiration were very different between the L:D and L:L trials. The oxygen consumption of the worms in the L:D trials was highest in the late morning hours, from 10:00 A.M. to 12:00 P.M., and declined steadily to a low around midnight. Respiration began to rise again in the early morning hours, about 3:00 A.M.

The quartic model accounted for 80% of the variation in oxygen consumption due to time in A, and 55% of this variation in B, due mostly to linear and quadratic components. The predicted mean oxygen consumption was highest in the late morning, declined steadily to a single minimum around 2:00 A.M., and increased toward the late morning peak. The best-fit quartic curves of oxygen consumption vs. time were almost identical for the two L:D trials

The cosine model of Halberg et. al. (1965) provided estimates of the amplitude, phase, and length of the cosine cycle that were extremely different between the two L:D trials. From the shapes of the best-fit cosine curves, it is apparent that the cosine model is not suited to explain significantly the relationship between oxygen consumption and time in the respiration trials conducted in the normal photoperiod.

The results of the twenty-four hour respiration studies made in a photoperiod of L:D (14:10) show that the daily pattern of oxygen consumption of L. hoffmeisteri was characterized by a single maximum which occurred in the light period, and a single minimum which occurred in the dark period.

The oxygen consumption of the worms held in constant light was highest in the mid-afternoon, around 3:00 P.M., and in the early morning, around 3:00 A.M. Lows in respiration occurred during the late morning hours, from 9:00 A.M. to 11:00 A.M., and in the evening hours, from 7:00 P.M. to 11:00 P.M.

The quartic model accounted for about 50% of the variation in oxygen consumption due to time in the L:L trials; the quartic trend was significant in two of the three trials, C and E. The best-fit quartic curves were similar in these studies, and predicted maxima in respiration

in the late afternoon and early morning hours, and minima in the late morning and late evening hours.

Even though the cosine fitness test detected a cosine rhythm in only one of the three L:L trials (E), the mean oxygen consumption patterns predicted by the cosine model were very similar. In each trial there were two cosine cycles in about 24 hours. In C, two cosine cycles occurred in 24.6 hours, in D, there were two cosine cycles in 25.2 hours, and in E, two cosine cycles occurred in 27.6 hours. Cosine predicted peaks in oxygen consumption occurred from 3:30 P.M. to 5:00 P.M. and from 3:00 A.M. to 4:00 A.M.; predicted minima occurred from 9:00 A.M. to 11:00 A.M., and from 10:00 P.M. to 12:00 A.M. in the L:L trials.

These results show that the oxygen consumption of L. hoffmeisteri, over 24 hours, and held in constant light, is different from the respiration of these worms under a photoperiod that approximates normal changes of light and dark. In constant light, the daily pattern of oxygen consumption was characterized by two maxima, which occurred in the mid-afternoon and pre-dawn hours, and two minima, which occurred in the late morning hours, and in the late evening hours.

A circadian rhythm is the daily variation of a physiological activity that exhibits a recurring pattern in

periods of about 24 hours. In the natural habitat, organismic rhythms are entrained to periods of exactly 24 hours by light and dark cycles generated by the rotation of the earth on its axis. The rhythm can persist in constant conditions, but the period becomes slightly longer or shorter than 24 hours. The length of the period can vary slightly with the intensity of the constant illumination (Brown, Palmer, and Hastings, 1970).

Investigators (Harker, 1958; Bruce and Pittendrigh, 1957) have shown that various invertebrate rhythms that existed in normal conditions of light and dark were lost when the organism was subjected to constant light. Others (Tweedy and Stephen, 1971) have shown that invertebrate rhythms persisted in constant light, but that the rates of oxygen consumption shifted under these conditions (Ulanoski and McDiffett, 1972). The daily pattern of oxygen consumption of L. hoffmeisteri, in this study, not only showed two maxima, and two minima in constant light, as opposed to one maximum and one minimum in the normal photoperiod, but also, the highs and lows in respiration occurred at different times of the day between the two experimental conditions.

Ralph (1957) showed that under constant dim illumination, the oxygen consumption rhythm of Lumbricus terrestris followed a characteristic pattern of two maxima and

two minima in a period of about twenty-four hours. Like the respiration cycles found in Lumbricus, the oxygen consumption of Limnodrilus hoffmeisteri, under constant illumination, exhibited two maxima and two minima in a period of about 24 hours.

Seasonal differences in respiration were of interest in this study. The daily pattern of respiration of L. hoffmeisteri, in the normal photoperiod, was measured in the summer, and the daily pattern of respiration, in constant light, was measured in the fall. Ralph (1957) found that maxima and minima in the daily respiration rhythm of Lumbricus varied between May and November. The circadian rhythm of oxygen consumption of the beetle, Dytiscus marginalis, was shown to be different between summer and winter by Poljakov (1938). Similarly, the differences between the respiration patterns in the two experimental conditions found in this study, could be accounted for, in part, by the times of the year when measurements of oxygen consumption were made.

Differences in the reproductive stage of the worms may help explain the differences in the observed patterns of oxygen consumption. Kennedy (1966) demonstrated that the annual cycle of reproduction of L. hoffmeisteri varied according to local conditions. Breeding took place in the winter months in the most productive habitats, and in the

summer months in the least productive habitats. In less productive habitats, breeding was probably dependent upon the increase in water temperature in spring and summer. Both mature and breeding worms were used in all respiration determinations in this study, although the proportions of mature and breeding stages were not determined. Since the worms were collected from two locations, differences in the numbers of mature and breeding worms might have caused differences in the daily patterns of oxygen consumption.

Members of the Tubificidae seem to be very sensitive to stress placed on them in the laboratory. The withdrawal and coiling responses of L. hoffmeisteri to agitation, strong light, and handling were observed in the separation and identification procedures employed in this study. Constant light was an additional stress during the L:L trials, and this added stress may have affected their respiration.

One of the unique behavioral responses of tubificids is the formation of the "ball cluster". In the absence of a burrowing substrate, the worms coil around each other, forming a ball. The worms were observed to remain in the "ball clusters" in the respirometer flasks in both L:D and L:L studies. The effects of this ball formation on respiration in Tubifex tubifex were discussed by Fowler and

Goodnight (1965). They found that oxygen consumption per individual varied with the size of the "ball cluster". Oxygen consumption per individual decreased as the size of the ball increased up to 50 worms, but more oxygen per individual was consumed in a ball of 100 worms, than in any of the smaller groups. Collier (1947) found that the respiration rate of worms in the "ball cluster" was lower than that of the same number of "dispersed" worms. Possibly the inner worms in the ball remain immobile and respire at very low rates, or they may respire anaerobically, building up large oxygen debts over extended periods of time in the "ball cluster". Alternatively, the "ball cluster" may be a dynamic aggregation, the outer worms respiring at higher rates than the inner worms, but with the worms changing positions from the center to the edge of the ball to meet individual oxygen demands. In the natural habitat of these worms, where oxygen tensions may fall to very low levels, this ball formation could be a unique survival adaptation.

Factors other than those listed above may have affected the respiration patterns reported here. The constant conditions imposed on L. hoffmeisteri in this study did not include controls of subtle environmental changes such as geomagnetism and cosmic radiation, that have been

shown to affect the biological rhythms of organisms by Brown, Palmer, and Hastings (1970).

Differences between the absolute values of oxygen consumption between the various trials, and between the flasks in a trial, can be accounted for by the process for the selection of the worms. An attempt was made to minimize the individual flask differences by selecting worms of as uniform a size as possible, and by using only those worms that were active and observed to be in good condition for respiration determinations. Differences in the size and physical condition of the worms between the various field collections were not controlled.

If the rhythms of respiration of L. hoffmeisteri, reported here are actually those that occur in the natural habitat, they would be useful in explaining the timing of certain daily events. Recurring daily changes in metabolic rate would account for variation in the rate of uptake of radionuclides such as Ca-45 or Sr-89, that have been shown to be readily taken up in the tissues of Limnodrilus by Whitten and Goodnight (1967). Rates of uptake by feeding, or by metabolically related absorption through membranes, might be shown to be dependent on these daily changes in oxygen consumption. In the natural habitat, periods of high oxygen consumption might not accompany periods of high activity, as Ralph (1957) has found to be

the case for Lumbricus. During periods of low oxygen consumption, the worms could burrow into the oxygen-poor mud and sediments, and repay an incurred oxygen debt during times of higher oxygen consumption when they returned to the mud-water interface.

SUMMARY

1. A differential respirometer was used to measure the oxygen consumption of Limnodrilus hoffmeisteri over 24 hour periods, in a photoperiod of L:D (14:10), and in constant light, L:L.

2. In the normal photoperiod, the daily pattern of oxygen consumption was characterized by a single maximum, from 10:00 A.M. to 12:00 P.M., and a single minimum, from 1:00 A.M. to 3:00 A.M.

3. The daily pattern of oxygen consumption in constant light was characterized by maxima at 3:00 P.M. and 3:00 A.M., and minima from 9:00 A.M. to 11:00 A.M., and from 7:00 P.M. to 11:00 P.M.

4. A cosine rhythm of respiration was detected in one of the L:L trials; none were found in the L:D trials.

5. Seasonal, reproductive, and behavioral effects on the patterns of oxygen consumption were discussed.

6. The significance of recurring daily patterns of oxygen consumption in Limnodrilus hoffmeisteri were discussed in relation to their general physiology, and respiratory adaptations in the natural habitat.

APPENDIX

Table A. Experimental Data of 24 Hour Respiration of L. hoffmeisteri, in a Photoperiod of L:D (14:10), July 26-27, 1972

Hour	STP Corrected µl O ₂ /2hr./50 worms					Mean µl O ₂ / 2hr./50 worms \bar{X}	Bar. Press. (CF)
	1	2	3	4	5		
8-10am	46.6	47.3	47.9	60.2	54.2	51.2	764mmHg (.93)
10-12pm	53.4	60.4	59.7	53.8	53.0	56.1	
12-2	52.4	44.5	43.4	48.7	52.9	48.4	
2-4	43.5	41.8	34.8	42.5	40.0	40.5	
4-6	37.6	36.4	34.4	37.0	32.6	35.6	
6-8	35.5	35.1	31.6	36.8	34.5	34.7	764mmHg (.93)
8-10	36.9	39.4	31.5	33.4	27.7	33.8	
10-12am	33.7	31.1	26.2	29.2	30.5	30.1	
12-2	30.8	31.8	32.8	27.4	29.7	30.5	
2-4	30.8	32.2	31.7	28.0	23.8	29.3	
4-6	34.1	36.0	39.4	29.4	31.1	34.0	
6-8am	32.9	39.7	47.1	29.7	32.0	36.3	764mmHg (.93)

Table B. Experimental Data of 24 Hour Respiration of L. hoffmeisteri, in a Photoperiod of L:D (14:10), July 28-29, 1972

Hour	STP Corrected $\mu\text{l O}_2/2\text{hr.}/50$ worms					Mean $\mu\text{l O}_2/$ 2hr./50 worms \bar{X}	Bar. Press. (CF)
	1	2	3	4	5		
10-12pm	42.3	54.0	55.6	60.4	49.6	52.4	765mmHg (.93)
12-2	49.7	54.2	45.7	54.6	41.3	49.1	
2-4	40.4	44.3	39.5	49.6	37.5	42.3	
4-6	33.6	45.1	33.6	48.0	34.2	38.9	
6-8	42.6	38.2	33.6	39.0	34.6	37.6	
8-10	27.4	46.6	30.5	46.8	20.5	34.4	
10-12am	29.0	31.6	26.5	35.9	31.7	30.9	764mmHg (.93)
12-2	26.0	27.0	19.5	28.9	18.3	23.9	
2-4	20.0	30.3	19.2	34.5	18.9	24.6	
4-6	34.0	40.3	36.6	44.5	33.1	37.7	
6-8	27.7	39.1	26.9	40.8	25.8	32.1	
8-10am	23.8	39.3	25.6	47.4	21.3	31.5	764mmHg (.93)

Table C. Experimental Data of 24 Hour Respiration of L. hoffmeisteri, in Constant Light, L:L, September 1-2, 1971

Hour	STP Corrected µl O ₂ /2hr./50 worms					Mean µl O ₂ / 2hr./50 worms \bar{X}	Bar. Press. (CF)
	1	2	3	4	5		
10-12pm	4.4	5.1	3.4	5.5	14.0	6.5	764mmHg (.93)
12-2	3.6	6.1	11.0	6.5	9.0	7.2	
2-4	8.2	12.3	9.1	10.2	14.0	10.8	
4-6	10.0	12.2	10.5	17.2	12.2	12.4	
6-8	9.6	12.8	10.6	10.4	12.0	11.1	
8-10	11.3	11.7	8.2	8.8	12.2	10.4	
10-12am	11.9	13.5	13.1	11.0	13.7	12.6	762mmHg (.93)
12-2	11.4	13.9	13.1	11.6	13.0	12.6	
2-4	11.1	14.1	13.0	11.7	15.3	13.0	
4-6	11.7	15.1	15.0	13.4	16.5	14.3	
6-8	16.0	20.6	18.8	17.4	17.1	18.0	
8-10am	11.7	14.2	13.2	10.0	14.2	12.7	762mmHg (.93)

Table D. Experimental Data of 24 Hour Respiration of L. hoffmeisteri, in Constant Light, L:L, October 21-22, 1971

Hour	1	STP Corrected $\mu\text{l O}_2/2\text{hr.}/50$ worms				5	Mean $\mu\text{l O}_2/$ 2hr./50 worms \bar{X}	Bar. Press. (CF)
		2	3	4				
8-10am	8.8	19.2	11.2	18.0	24.6	16.4	760mmHg (.92)	
10-12pm	20.2	28.0	19.2	27.6	27.4	24.5		
12-2	25.7	31.1	21.9	24.8	31.4	26.8		
2-4	47.6	51.1	37.6	32.2	30.5	39.8		
4-6	27.7	34.2	27.3	34.7	35.4	31.8		
6-8	28.6	27.8	25.8	34.1	34.3	30.1		
8-10	28.1	29.0	27.6	26.6	33.0	28.9	756mmHg (.89)	
10-12am	24.5	32.7	23.8	27.8	29.9	27.7		
12-2	31.4	38.3	30.3	29.6	35.2	33.0		
2-4	29.6	32.0	26.6	30.9	24.9	28.8		
4-6	25.2	29.6	23.9	29.1	33.4	28.2		
6-8am	29.7	35.2	29.2	30.4	34.8	31.9	755mmHg (.89)	

Table E. Experimental Data of 24 Hour Respiration of L. hoffmeisteri, in Constant Light, L:L, November 18-19, 1971

Hour	1	STP Corrected μl O ₂ /2hr./50 worms				5	Mean μl O ₂ / 2hr./50 worms \bar{X}	Bar. Press. (CF)
		2	3	4				
10-12am	28.0	22.0	22.5	25.2	27.6	25.1	760mmHg (.92)	
12-2	31.5	27.8	26.9	25.4	28.8	28.1		
2-4	34.5	29.6	30.4	30.0	30.2	30.9		
4-6	33.5	29.6	30.0	30.7	29.7	30.7		
6-8	29.7	27.7	24.6	24.5	25.6	26.4		
8-10	23.6	21.4	15.0	14.7	19.2	18.8		
10-12pm	19.6	14.4	14.6	15.3	16.3	16.0	760mmHg (.92)	
12-2	28.5	24.9	24.8	26.4	25.9	26.1		
2-4	32.1	31.5	28.4	27.0	30.4	29.9		
4-6	32.1	29.4	24.5	31.1	27.4	28.9		
6-8	28.1	28.6	27.8	25.7	28.2	27.7		
8-10pm	30.9	28.1	25.4	28.3	29.1	28.4	760mmHg (.92)	

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