

A STUDY OF OXYGEN CONSUMPTION IN
Calanus plumchrus MARUKAWA 1921
AND IMPLICATIONS ON VERTICAL MIGRATION

by

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Abstract

Oxygen consumption of the copepodid V stages of Calanus plumchrus was studied with respect to environmental and endogenous factors using standard closed chamber technique and Warburg respirometry. Specimens were collected from San Juan Channel, Washington and Indian Arm, British Columbia.

Rate of oxygen consumption of C. plumchrus (1) is significantly decreased by population densities of 5 or more copepods / ml, (2) demonstrates no regular endogenous change, (3) is not significantly affected by presence or absence of light, (4) is directly proportional to temperature (being variously linear and non-linear in response) throughout the range of 5-20° C, (5) does not vary significantly over a salinity range of 20-35 ppt (but during May 1965 increased at 10 ppt and decreased at 45 ppt), (6) decreases to a minimum below an ambient oxygen concentration of 3 cc O₂ per liter and (7) is not significantly affected by increased hydrostatic pressure corresponding to a depth of about 400 m. Response to a range of temperatures, in particular, indicates that oxygen measurements taken from different collections are statistically different and therefore not directly comparable.

In general, the data presented are consistent with McLaren's theory of energy utilization and vertical migration, although temperature does not always appear to be the most significant factor. Interaction and variation of environmental factors may explain some of the complexity of vertical migration.

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I. Introduction

Crustaceans are the predominant component of marine zooplankton, both in numbers and in species, and the calanoid copepods comprise the largest portion of this group (Raymont, 1963). Therefore, the role of marine calanoid copepods in energy utilization in the ocean is vital to our understanding of the marine ecosystem. Most studies of oxygen consumption of various marine species concern the interpretation of food requirements and feeding relationships, and involve the effect of one or at most two environmental factors. Only one reported study is concerned with an extensive survey of oxygen consumption with respect to environmental factors (Marshall, Nicholls and Orr, 1935). In addition, most of the information available is restricted to species occurring in the northern Atlantic Ocean. Hence, it is desirable to obtain more complete knowledge of oxygen consumption of various species.

In view of a hypothesis proposed by McLaren (1963), the general problem of energy utilization in zooplankton with reference to their vertical position in a water body is of interest. According to this hypothesis, zooplankton lower in a water body should use less oxygen. If temperature is the only factor considered, zooplankton in most oceanic situations would decrease energy utilization with increase in depth. However, at least two other environmental factors change in a predictable manner as a function of depth and consequently are of at least theoretical interest, viz., light and pressure.

In particular, the only published report of the effect of hydrostatic pressure on the oxygen consumption of a marine zooplankton indicates that oxygen consumption increases with increase in pressure (Napora, 1964). If Napora's observation represents a general response of zooplankton, then one would not automatically predict a decrease in energy utilization with increase in depth. Moreover, in coastal waters other water properties may vary considerably (e.g., salinity and oxygen concentration). Generalization of the effect of most environmental factors on oxygen consumption of zooplankton is impossible and therefore, in order to conclude that indeed less energy is used when deeper in a water body, several factors must be studied.

The purpose of this study is to investigate McLaren's hypothesis as it relates to energy utilization of a marine calanoid copepod by analysis of endogenous change in rate of oxygen consumption and change in rate of oxygen consumption in response to environmental factors, and to contribute to the general knowledge of oxygen consumption in copepods.

II. Materials and Methods

Copepodid V stages of Calanus plumchrus Marukawa 1921 were used throughout the study. C. plumchrus occurs in the northern Pacific Ocean (Brodsky, 1950; Mori, 1937). In the Strait of Georgia and neighboring waters the species is most abundant from February to May, but may be taken from deep waters throughout most of the year. Confusion exists on the status of C. plumchrus as a species distinct from Calanus tonsus Brady. Brodsky (1957:49) and Zenkevitch (1963) consider C. plumchrus to be C. tonsus; however, Tanaka (1956) states emphatically that both are distinct species. In view of this confusion, comparative data which was taken from the literature and which concerns C. tonsus is used in the following only when C. plumchrus was clearly the form (species) to which reference was made.

Experiments reported herein were conducted at Friday Harbor Laboratories, University of Washington (FHL) and at the Department of Zoology, University of British Columbia (UBC). Copepods used at the respective localities were collected from San Juan Channel, Washington ($48^{\circ} 44' N$ lat, $123^{\circ} 2' W$ long) and Indian Arm, British Columbia ($49^{\circ} 21' N$ lat, $122^{\circ} 54' W$ long). Specimens were collected by means of a coarse meter net using oblique and vertical tows from depths of 50-150 m. Water used in experiments at FHL was taken from the running sea water system and water used at UBC was collected with a Van Dorn bottle from a depth of 150 m at the locality where copepods were collected. After collection, copepods used for

experiments at PHL were diluted and returned to the laboratory for separation (a trip of 20 min), while copepods used at UBC were separated on ship prior to return. Material was maintained in aquaria containing unfiltered sea water at 10° - 12° C for periods varying from one day to two weeks subsequent to collection. Laboratory photoperiod to which copepods were exposed during this period was not controlled. No copepods were used for experimentation until 24 hrs after collection.

Closed chambers of 10, 35, and 50 ml and a Warburg respirometer were used to determine rate of oxygen consumption. Procedure used in closed chamber experiments is described by Conover (1956) and techniques used in the Warburg studies are described by Umbreit et al. (1959). Copepods were acclimated to the test chambers at 10° - 12° C for at least 12 hrs prior to test in all except the 10 ml chambers. Copepods tested in the 10 ml chambers were used immediately after introduction into the chambers. All rates of oxygen consumption were determined acutely and except when the effect of light was studied, tests were conducted in constant darkness. Water used in the experiments was filtered using 0.45 μ HA millipore filters. Antibiotics were not used in the experiments. In closed chamber experiments, concentration of oxygen was determined by titration using the unmodified Winkler technique and by the technique described by Pamatmat (1965) using a Beckman Oxygen Analyzer (Model 777).

Copepods which were used in the experiments were collected during different seasons (and in one set of experiments

from a different locality) and they varied significantly ($P < 0.01$) in cephalothorax length (Table I). Rates of oxygen consumption determined during these seasons were thus corrected and expressed for a standard cephalothorax length of three millimeters. Corrected and uncorrected values of rates of oxygen consumption are given in Appendices IV-VII. Data for the effect of crowding, rhythmicity and photoperiod on rate of oxygen consumption were not corrected since either the responses being studied were not a function of quantitative differences in rates of oxygen consumption, or because data being compared were collected during the same season and from the same general locality. Rates of oxygen consumption of replicate experiments within the same season were not corrected for size variation.

Although rates of oxygen consumption were determined with respect to differing localities and seasons, attention is directed particularly, first, to the general character of response of oxygen consumption to the various environmental factors and second, to the relative importance of each of these factors.

Statistical analyses used are described by Freund, Livermore, and Miller (1960) and Snedecor (1956). When analysis of variance was used to analyze more than one mean, significant differences were determined by Duncan's new multiple range comparison (Freund et al., 1960). Variances of means compared by analysis of variance and by Student's "t" test were compared by ratio of maximum variance to

minimum variance (Biometrika Tables for Statisticians, pp. 60-61 and 179). In this study, $P < 0.01$ was used as the level of significance unless otherwise stated.

In addition to general methods outlined above several specific methods were employed.

A. Crowding. The effect of crowding on rate of oxygen consumption was determined by analysis of rhythmicity data in which varying numbers of copepods were studied in a constant volume of water.

B. Rhythmicity. Hourly rates of oxygen consumption under conditions of constant exposure to light and dark, and alternating exposure to dark and light were determined in a manner similar to that described by Raymont and Gauld (1951) by means of a Warburg respirometer for periods exceeding 30 hrs. Presumably an "endogenous" rhythm in oxygen consumption

Table I. Variation in length of cephalothorax of copepodid V stages of C. plumehrus used in the oxygen consumption studies. Comparison of means by Duncan's new multiple range comparison indicates all means are significantly different from each other.

Source	Date	Sample Size	Mean Length (mm)
FHL	June 1964	36	3.24
FHL	May 1965	101	1.52
UBC	November 1965	34	3.65

should appear in constant conditions and an "exogenous" rhythm in oxygen consumption should be induced by exposure to alternating dark and light (assuming light is a primary

controlling factor). The dark-light regime corresponded to the normal photoperiod cycle. Flasks contained air as the gas and were shaken at the rate of 120 times / min. Tests conducted during 1964 (FHL) were run at 10⁰ C and varying numbers of copepods were used per flask, whereas during 1965 (UBC) tests were run at 13⁰ C and constant numbers of copepods were tested.

C. Light. The rate of oxygen consumption of copepods in the light and dark over a temperature range of 5⁰-20⁰ C was determined with a Warburg respirometer. The same copepods were used at all temperatures. Acute oxygen consumption was measured for a period of about six hrs subsequent to one hr acclimation to temperature change. The experiment reported in Section II B was also analyzed to describe any effect of constant light as opposed to dark and the effect of changes from dark to light.

D. Temperature. Standard methods described by Conover (1956) were used to determine the effect of temperature.

E. Salinity. Water of salinity lower than ambient salinity was prepared by diluting with glass distilled water and water of higher salinity was prepared by boiling the sea water and readjusting to the desired salinity with glass distilled water.

F. Oxygen concentration. Oxygen concentrations were prepared by bubbling 99% nitrogen gas or by repeated filtering with a millipore apparatus. Oxygen levels were determined by titration or by an oxygen electrode and readjusted to greater

quantities as desired by shaking or bubbling with air. Minimum oxygen concentrations tolerable were estimated from tests conducted during 1965 in which copepods reduced the oxygen to a lethal concentration.

G. Pressure. The effect of pressure was studied by placing 5-10 copepods in a 10 ml plastic syringe. Water in the syringe was slowly extruded leaving a small volume with the copepods, the plunger was removed, a siphon apparatus inserted and the syringe filled and overflowed. The plunger was inserted excluding air bubbles and the volume adjusted to 10 ml. The needle was plugged with silicon grease to stop oxygen exchange with the exterior media. Replicates and controls were placed in a pressure chamber which was pre-cooled to the desired temperature. Pressure was adjusted and the chamber placed in a refrigerator. At the end of the test period, pressure was released quickly, the syringes removed and concentration of oxygen in the test and control syringes determined by means of an oxygen electrode. Oxygen was not released from solution when the pressure was lowered. Tests at different pressures were run for approximately the same length of time and under constant darkness.

The pressure apparatus is shown in Figure 1. Three large bolts hold the ends against the pipe and a seal is effected by "O" rings. A pressure gauge was tapped into one end and a screw top sealed by an "O" ring is contained in the other end. The screw top contains a screw-piston pump which created the pressure.

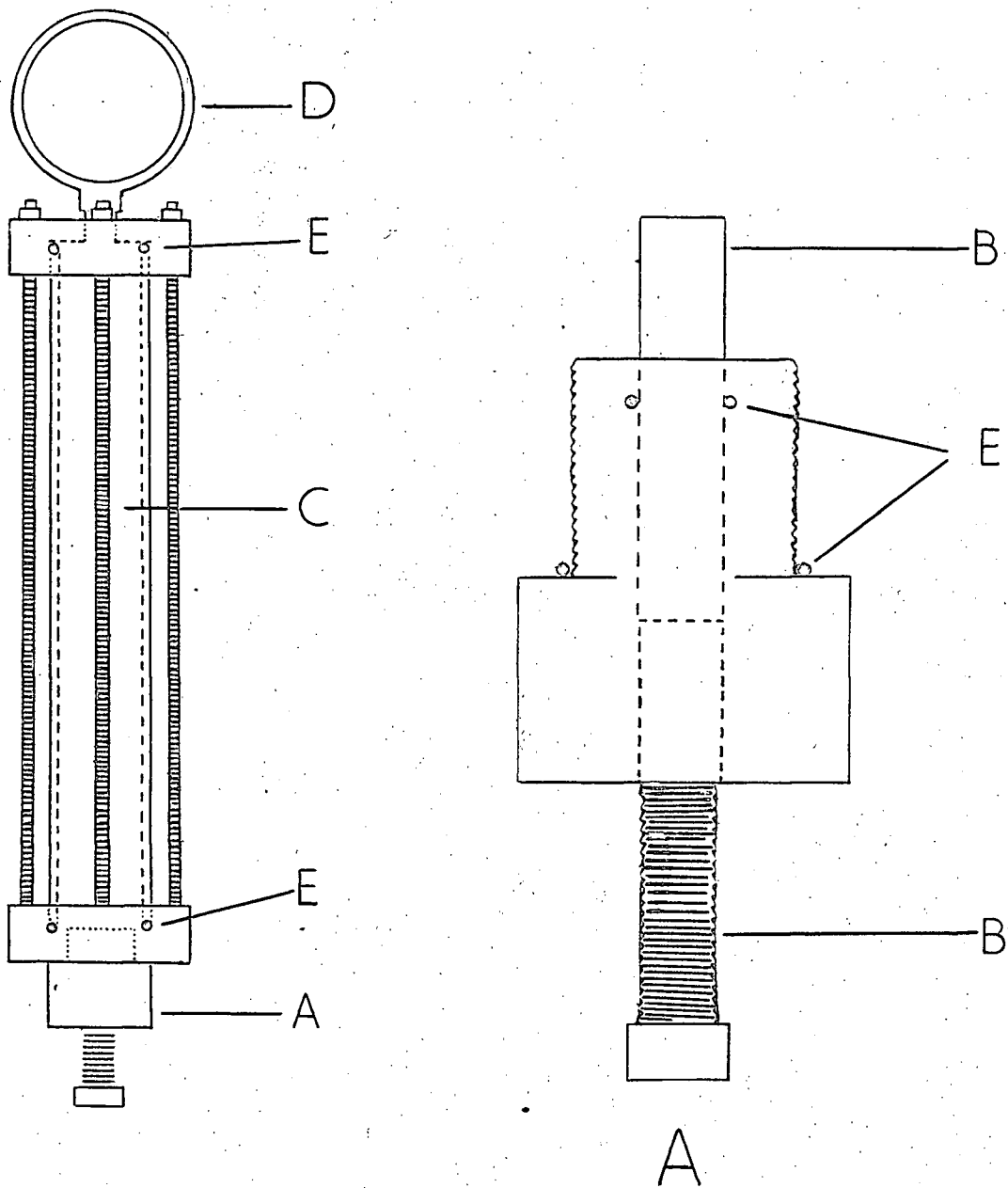


FIGURE 1. Pressure apparatus with an enlarged view of the cap (A) containing the screw-piston pump (B). The control and test chambers are introduced into the pressure chamber (C) when the cap is removed. The chamber is filled with water, the cap is screwed into place excluding air, and pressure is adjusted by means of the pump. Pressure is read from the gauge (D) and is maintained by the "O" ring seals (E).

III. Results

A. Crowding. Analysis of variance of the data collected (Figure 2 and Appendix I) indicates that rate of oxygen consumption differs significantly with respect to population density and Duncan's new multiple range comparison indicates that rate of oxygen consumption of copepods tested at the density of five copepods per ml is significantly lower than rates determined for copepods tested at lesser densities.

B. Rhythmicity. Diel changes in rate of oxygen consumption were determined for copepods acclimatized to summer and winter conditions and tested in various light regimes (Figure 3 and 4 and Appendix II). The sets of data were analyzed by analysis of variance to determine the presence of significant temporal changes in rate of oxygen consumption and those sets of data with significant changes were both transformed and analyzed by the method described by Enright (1965) which is designed to emphasize regular trends and to minimize the effect of random fluctuations in serial data. In addition, each of the sets of data were analyzed by the method described by Swed and Eisenhart (1943) to determine if rises and falls in rates of oxygen consumption occur randomly or in a non-random pattern.

Significant changes in rates of oxygen consumption were observed in curves A, B, and E. No regular trend could be associated with the changes observed in curves A and B, but the changes in rates in curve E may be at least partially associated with the change from dark to light. No significant

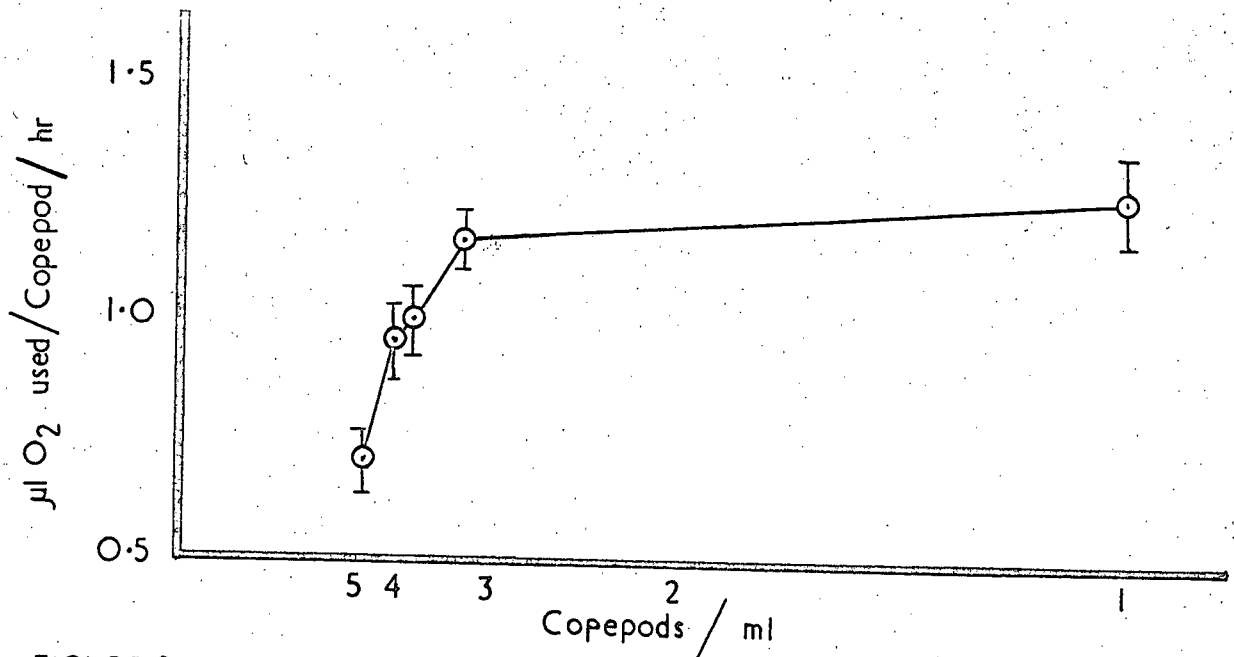


FIGURE 2. Rate of oxygen consumption (\pm one standard error) with respect to crowding. Copepods tested were taken from a single collection during June 1964 (FHL).

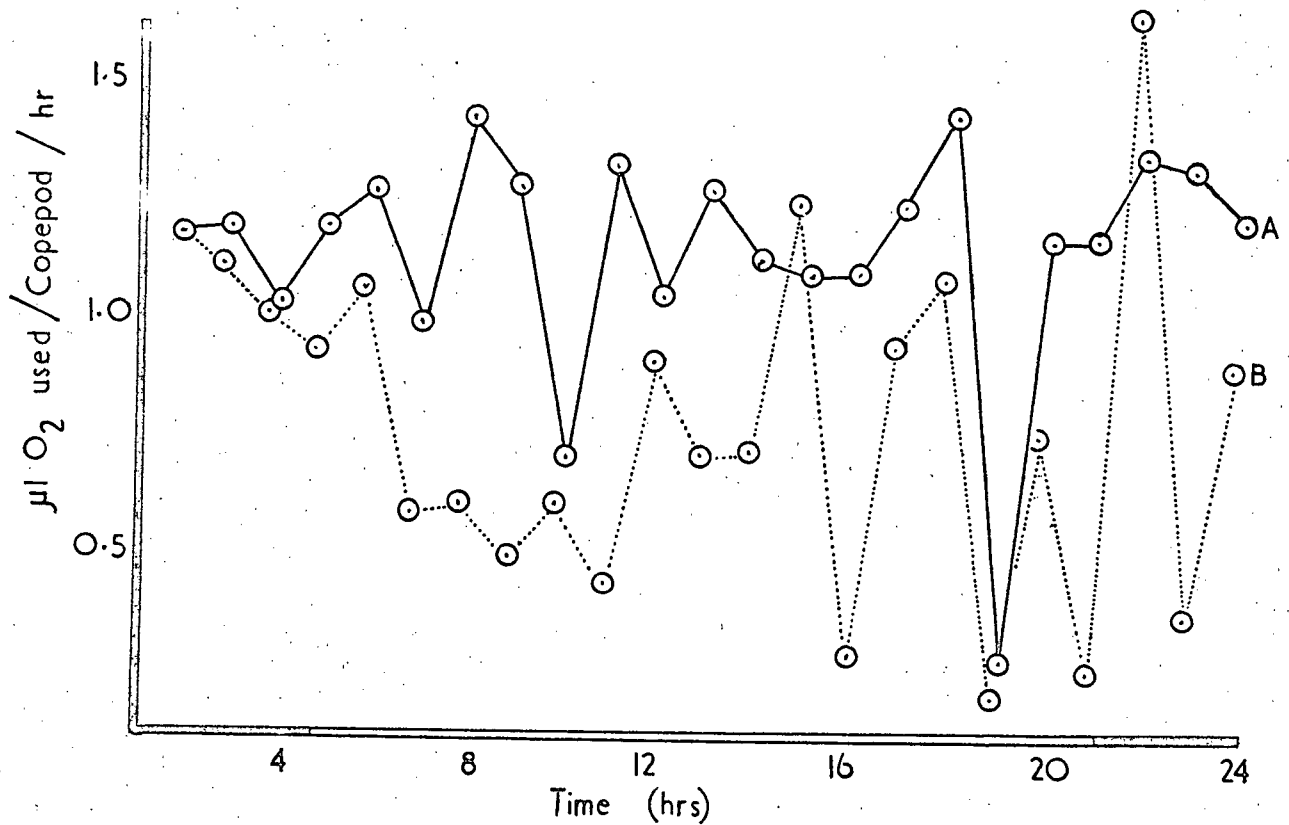


FIGURE 3. Diel changes in rate of oxygen consumption of two collections of copepods tested under constant exposure to light during June 1964 (FHL). Normal photoperiodic cycle is shown on the abscissa.

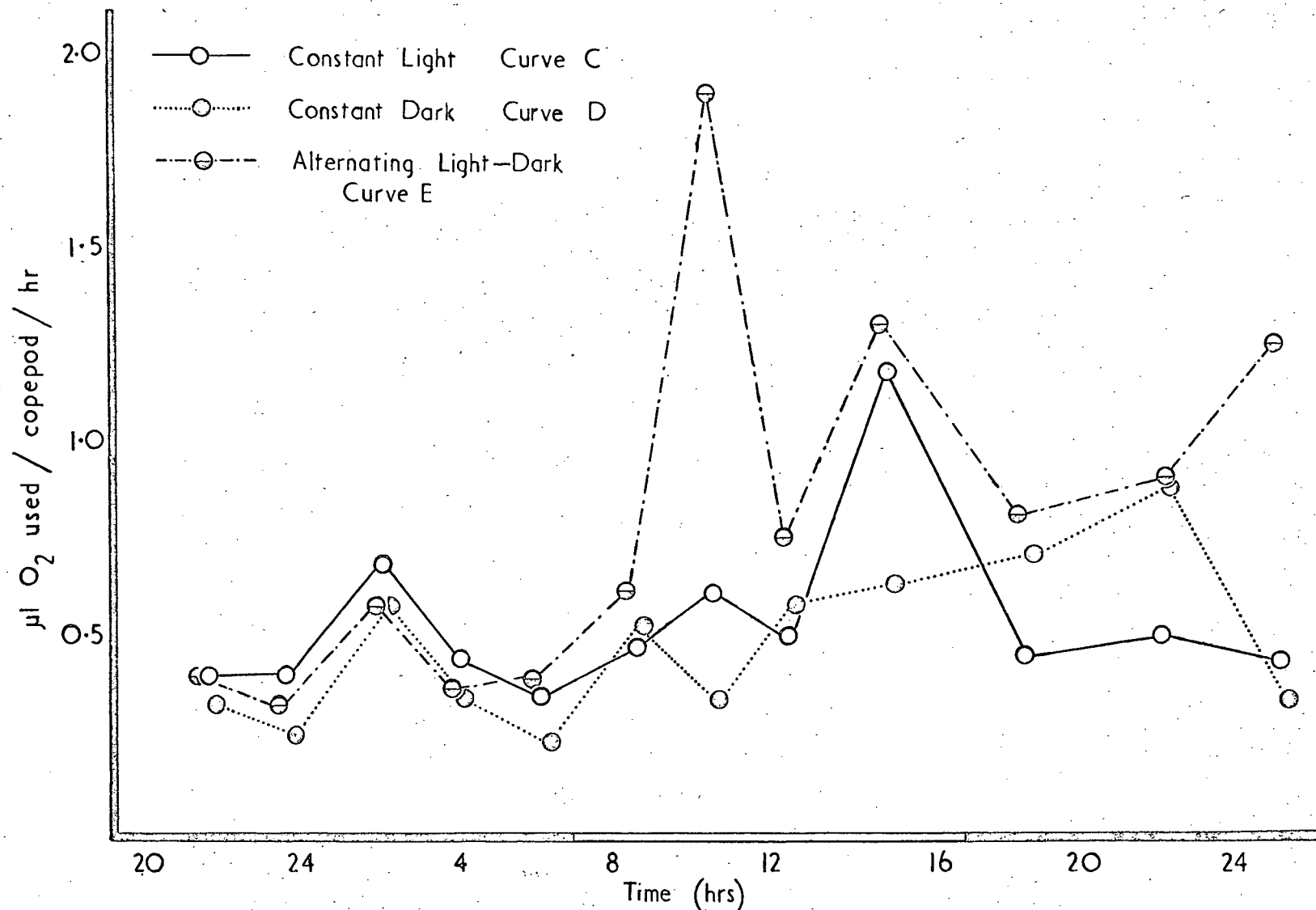


FIGURE 4. Diel changes in rate of oxygen consumption of copepods under exposure to constant light (curve C), constant dark (curve D), and alternating light-dark (curve E). Copepods tested were taken from a single collection during November 1965 (UBC). Normal photoperiodic cycle is shown on the abscissa.

temporal changes in rate of oxygen consumption were observed in curves C and D. Further, the rise and fall in rate of oxygen consumption does not occur in any significant pattern (Table II). Therefore, no rhythmic change in rate of oxygen consumption was concluded to be present.

Table II. Probability of random grouping of rise and fall in rate of oxygen consumption with respect to time in curves A-E in Figures 3 and 4.

Curve	Probability
A	0.58
B	0.99
C	0.96
D	0.73
E	0.97

C. Light. Analysis of variance indicates no significant difference between rates of oxygen consumption of copepods tested in the presence or absence of light either with respect to temperature (Figure 5 and Appendix III) or with respect to diel changes (curves C and D, Figure 4). However, a significant increase in rate of oxygen consumption was observed when copepods were switched suddenly from dark to light (curve E, Figure 4). Subsequent means (1.30 and 1.25) were also found to differ significantly from the remainder of the curve.

D. Temperature. Four examples of rate of oxygen consumption as a function of temperature are given (Figure 6 and Appendix IV). Rate of oxygen consumption increased with

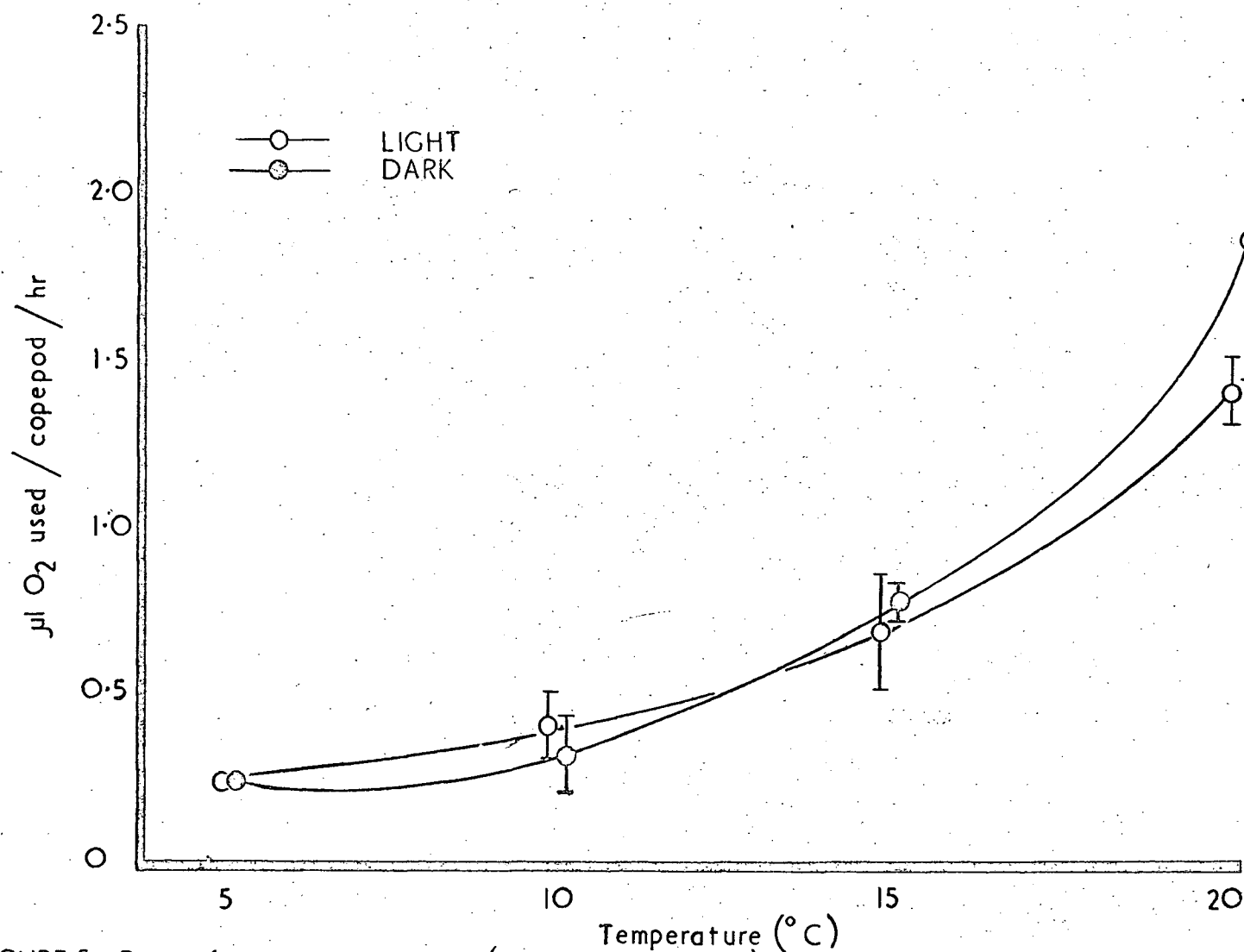


FIGURE 5. Rate of oxygen consumption (\pm one standard error) with respect exposure to light and dark at four temperatures. Copepods tested were taken from a single collection during June 1965 (UBC).

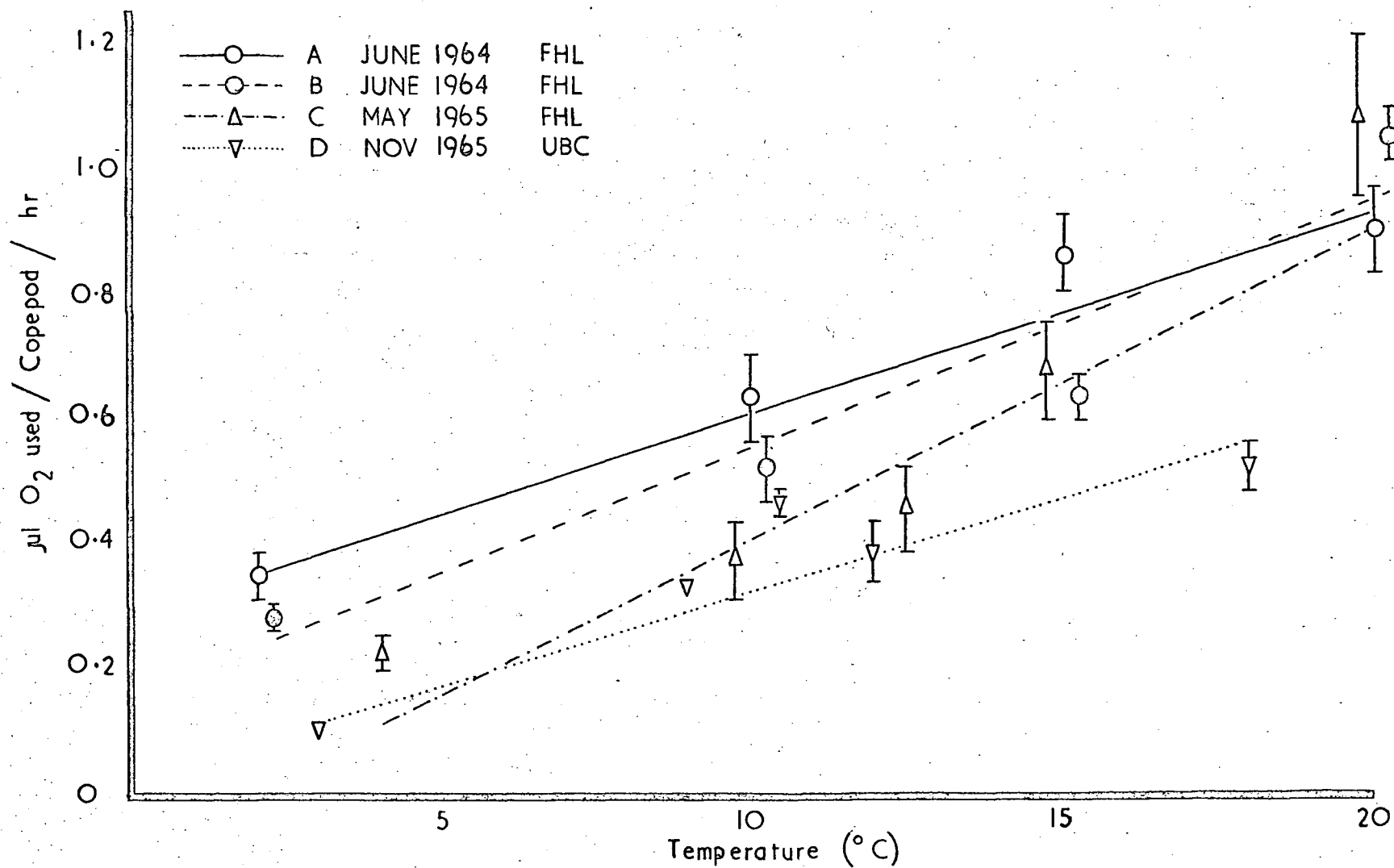


FIGURE 6. Rate of oxygen consumption (\pm one standard error) with respect to temperature. Data for each line were collected from separate collections of copepods.

temperature when tested over a range of 5²20° C. Data for line C were collected from a population statistically different from that which lines A, B, and D were collected (i.e., the sample variance of data for line C differed from the variances of data for lines A, B, and D by a ratio with $P < 0.01$) and likely were from a different population. Since the sample variance of line C could not be attributed to sampling error, the line is compared no further statistically. Analysis of co-variance of lines A, B, and D indicate the regression coefficients of the lines do not differ significantly, but elevations of the lines are significantly different. Duncan's new multiple range comparison indicates each of the means when adjusted (Table III) differ significantly from each other.

Table III. Adjusted means of lines A, B, and D, Figure 6.

Line	Mean	Adjusted Means
A	0.67	0.69
B	0.62	0.64
C	0.30	0.24

Mortality occurred in tests of rate of oxygen consumption at 20° C during May 1965 and at 18° C during November 1965, indicating the upper lethal temperature was approached. No mortality was observed at 20° C during June 1964.

E. Salinity. Analysis of variance of change in rate of oxygen consumption with respect to salinity (Figure 7 and

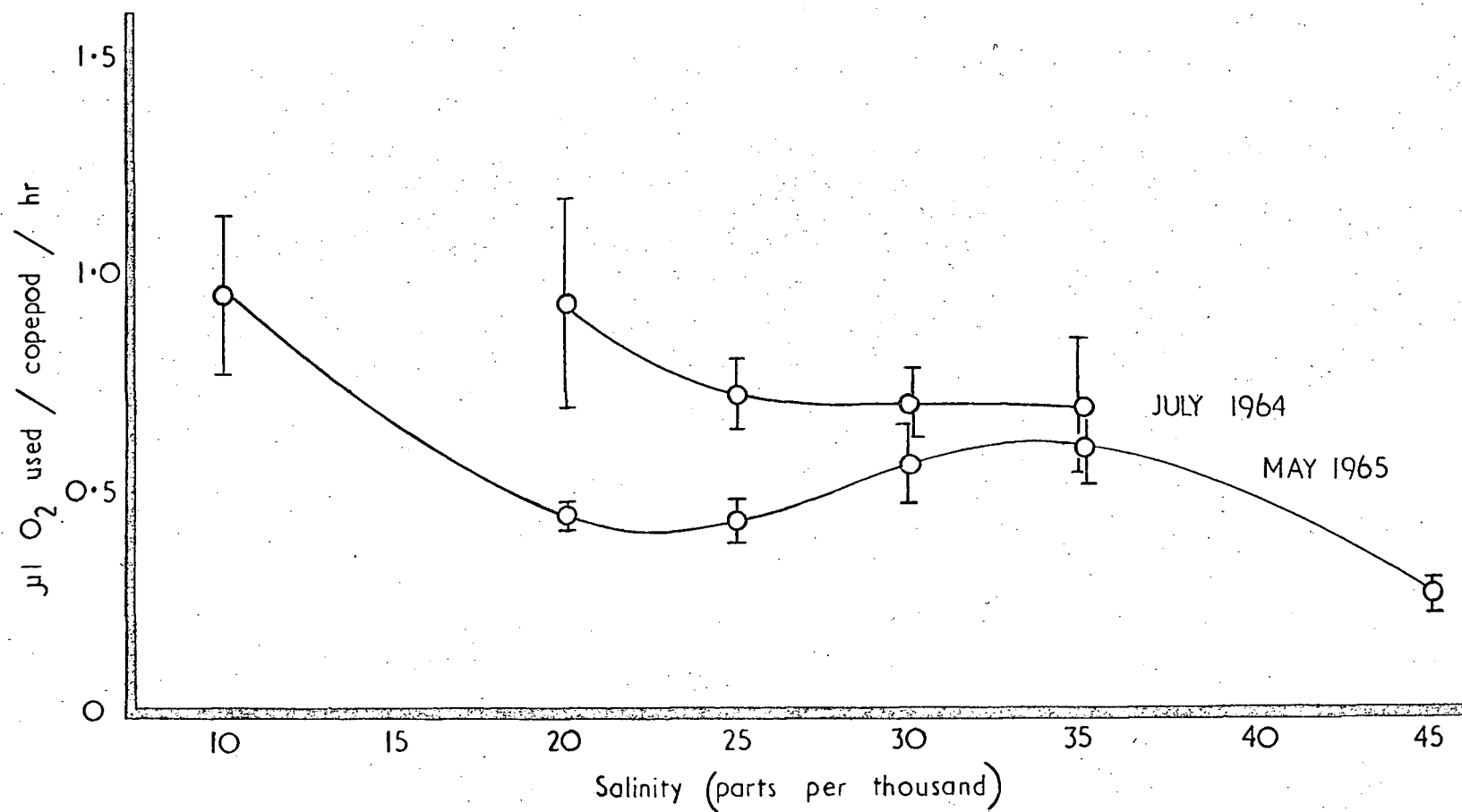


FIGURE 7. Rate of oxygen consumption (\pm one standard error) with respect to salinity of two collections of copepods (FHL).

Appendix V) indicates no significant difference in 1964 data, but indicates significant variation in the 1965 data. Duncan's new multiple range comparison indicates rate is significantly higher at 10 parts per thousand (ppt) and significantly lower at 45 ppt during 1965. Two-way analysis of variance of rates tested at 20, 25, 30, and 35 ppt during 1964 and 1965 indicates no significant difference with salinity or time.

F. Oxygen concentration. Significant change in rate of oxygen consumption with respect to oxygen concentration and temperature was not observed during May 1965, but was observed at 3° C and 18° C during November 1965 (Figure 8 and Appendix VI). At both temperatures during November 1965, rate of oxygen consumption was restricted by concentrations of oxygen less than approximately 3 cc oxygen per liter. Minimal lethal oxygen concentrations at three temperatures were determined during November 1965 (Table IV). Lethal minimum oxygen concentrations did not differ with temperature.

Table IV. Minimal oxygen concentrations (cc O₂/l) tolerable at three temperatures for copepods taken from Indian Arm, B.C.

	3° C	12° C	18° C
	0.80	0.80	0.85
	0.70	0.70	0.85
		0.65	0.90
		1.22	1.00
			0.87
Mean	0.75	0.84	0.89

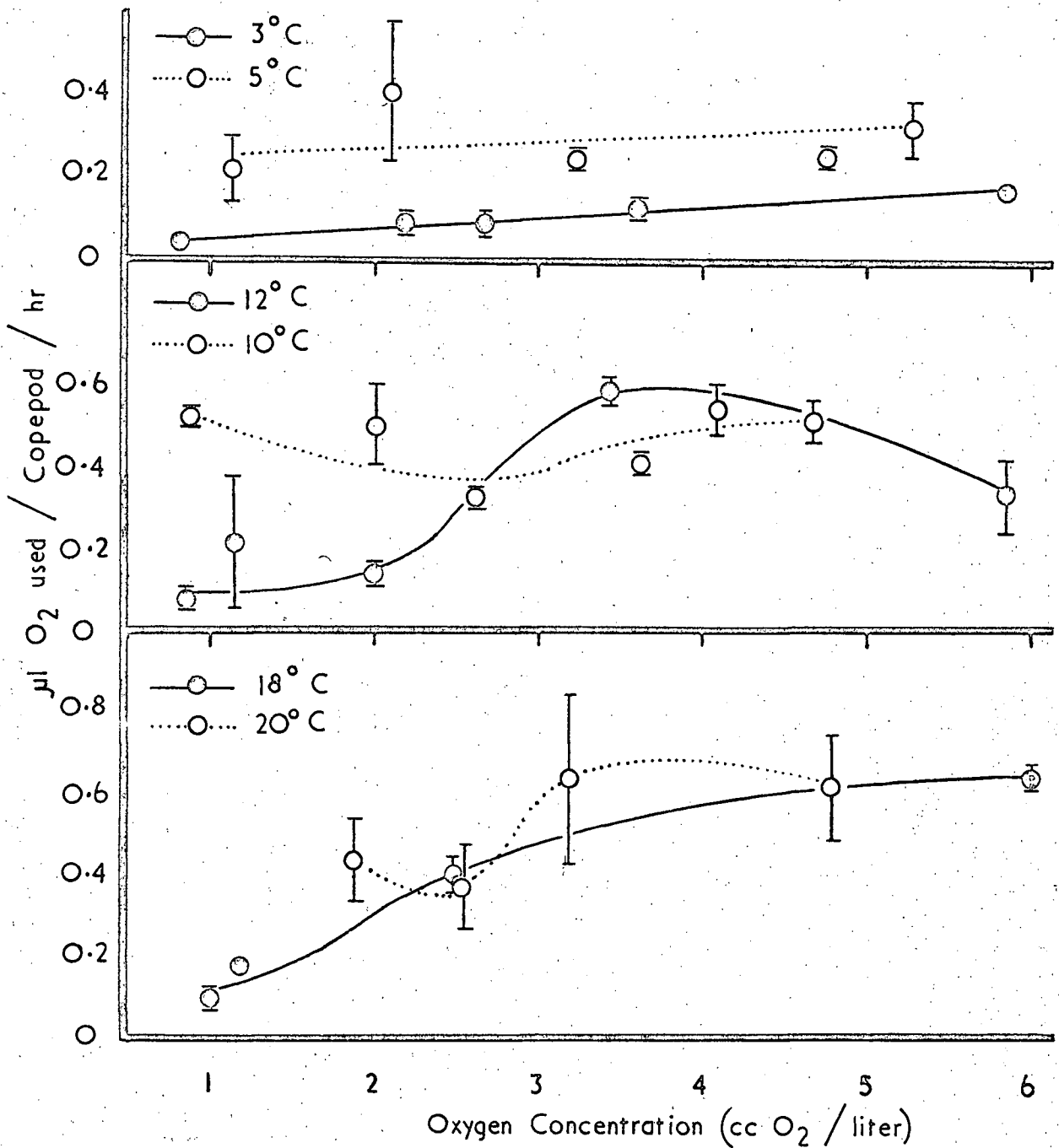


FIGURE 8. Rate of oxygen consumption (\pm one standard error) with respect to oxygen concentration and temperature. Copepods tested at 5°, 10°, 20° C. were taken from a single collection during May 1965 (FHL) and copepods tested at 3°, 12°, and 18° C. were taken from a single collection during November 1965 (UBC).

G. Pressure. Rate of oxygen consumption was not significantly changed by pressures equivalent to a depth of 400 m at 10° or 12° C (Figure 9 and Appendix VII). Further, the data were not linearly correlated at 10° C and the probability of linear correlation at 12° C was $P < 0.05$ (shown by a dashed line in Figure 9). However, in all experiments rate of oxygen consumption decreased with increased pressure and for that reason the dashed line was fitted to the 12° C data by least squares.

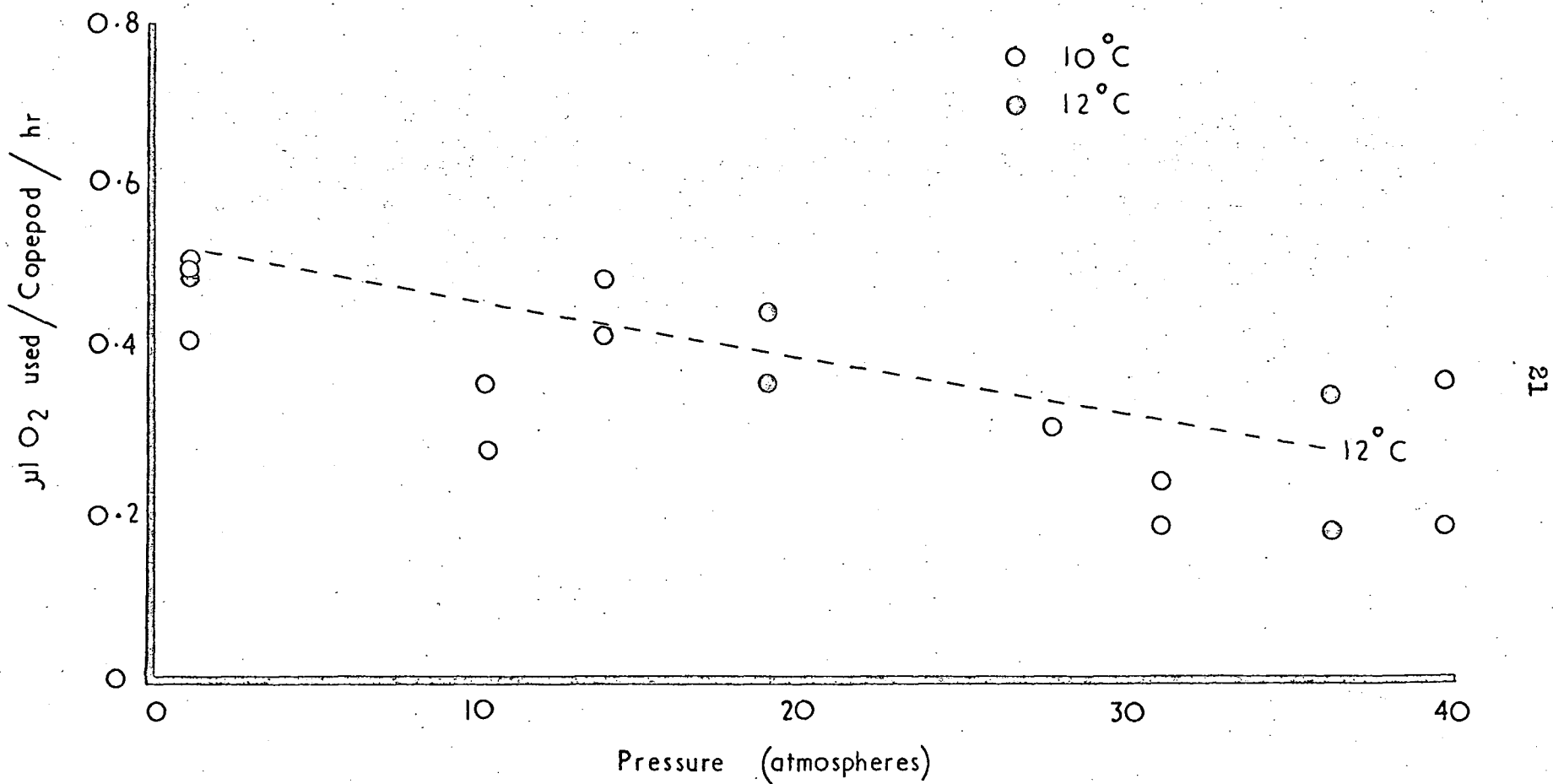


FIGURE 9. Rate of oxygen consumption with respect to pressure. Copepods tested at the two temperatures were taken from a single collection during November 1965 (UBC).

IV. Discussion

Consideration of the variations in rate of oxygen consumption to endogenous and environmental factors is important (1) in order to evaluate the effect of techniques used on the data obtained, (2) in order to evaluate the effect and to interpret the mechanism of action of particular factors on oxygen consumption and (3) in order to evaluate oxygen utilization and requirements in the natural environment.

A. Considerations of technique. The effect of temperature, salinity, light and density of experimental animals, on rate of oxygen consumption is generally appreciated (Prosser and Brown, 1961). In addition, if regular endogenous changes in rate of oxygen consumption are present, data which are to be compared must be selected from the same stage of the rhythm. Irregular endogenous changes may be removed by continuous monitoring and selection of comparable data, when the necessary equipment is available.

Pressure is ordinarily maintained at one atmosphere. However, when pressure is tested as the variable affecting rate of oxygen consumption of aquatic organisms and the experimental chamber must be removed from under pressure to analyze oxygen, caution must be employed to ensure oxygen is not released from solution due to temperature change as pressure is released. Control experiments indicated that sudden release of pressure equivalent to 40 atm together with the change from 10° C to about 20° C during analysis

(encompassing a time period of about 10 min) did not result in release of oxygen from solution. Two solutions of equal oxygen concentration, one kept at one atm and one kept at 40 atm for two hrs followed by sudden release of pressure, agreed within one per cent (100% saturation \pm full scale) on a Beckman Oxygen Analyzer, Model 777. Therefore, under the experimental conditions used, release of oxygen from solution did not prove to be a problem.

The importance of the effect of the excitatory state of copepods on rate of oxygen consumption (Berner, 1962:636) can not be emphasized too strongly if reproducible results are to be obtained. Excitation of the copepods by transfer or by direct exposure to an environmental variable, may result in a quantitatively unpredictable overshoot response of oxygen consumption (Halorow, 1963; Grainger, 1958). If the experiment is of short duration and the chamber volume is small in relation to the number of copepods tested, rate of oxygen consumption during overshoot may strongly bias the resultant rate. In contrast, when experiments are long in duration (achieved in closed systems by having a large ratio of water and hence oxygen to organism tested) time spent in overshoot is short in comparison to the total length of time and consequently the measured rate of oxygen consumption is less biased by change in rate during overshoot. Some of the overshoot response may be minimized by acclimating the copepods to the test chambers prior to test (Conover, 1956; Berner, 1962).

In the present study the effects of the variables on rates measured were minimized by maintaining the variables constant or not allowing them to vary to levels at which oxygen consumption is affected. Irregular endogenous changes could not be removed since in most cases rate of oxygen consumption was not monitored continuously.

The desire for better reproducibility of results and the ability to correct spurious changes in rates will necessitate, in subsequent investigations, continuous monitoring of oxygen consumption (Teal and Halcrow, 1962 and Halcrow, 1963).

B. Effect of factors. The effect of population density and light were studied primarily to determine if either significantly affects rate of oxygen consumption. The presence of endogenous rhythms was investigated to determine if measurements should be restricted to a particular time period and to test if an endogenous rhythm in locomotor activity was present. Salinity, oxygen concentration, pressure, and temperature were investigated in order to describe how these major environmental variables affect oxygen consumption.

Increase in population density results in decrease in rate of oxygen consumption of C. plumchrus as is reported for Calanus finmarchicus (Gunner) (Zeiss, 1963). However, C. plumchrus is generally less sensitive to crowding. Zeiss stated the decrease in oxygen consumption of C. finmarchicus indicated the influence of some other factor and suggested increased metabolite concentration. The data

presented in Figure 2 might appear to agree with this hypothesis; however, if metabolite accumulation is the significant factor decreasing rate of oxygen consumption, one would expect a decrease in rate of oxygen consumption with time at each density tested (or allowing for a threshold effect, one should observe a decrease in rate above a particular density). But, analysis of the 24 hourly measurements of rate of oxygen consumption at each density indicates that at densities of 4-5 copepods / ml rate did not differ significantly with time (i.e., rates measured during the first 12 hrs were the same as measured during the second 12 hrs) and copepods tested at a concentration of less than 4 copepods / ml increased rate of oxygen consumption with time (i.e., rate during the second 12 hrs was significantly higher than the first 12 hrs). One might also argue that the increase in rate with time represents an endogenous increase in rate of oxygen consumption and consequently maintenance of a constant rate is actually a relative decrease, but it should be observed that three densities tested in the 4-5 copepods / ml density range did not differ significantly from the density of less than 4 copepods / ml. Hence, maintenance of a constant rate cannot be interpreted as a relative decrease. The data presented in this study for the effect of population density on rate of oxygen consumption of C. plumehrus, therefore, do not support the hypothesis that metabolite accumulation is responsible for decrease in rate of oxygen consumption. At present, I can offer no

alternative explanation for the observed decrease.

The effect of light was in part investigated with the analysis for endogenous changes in oxygen consumption. No difference in rate of oxygen consumption between copepods kept in continuous light and continuous dark could be demonstrated. However, in one set of experiments change from dark to light did result in a significant increase in rate of oxygen consumption. Conover (1956) reported no effect of presence or absence of light on rate of oxygen consumption of Acartia clausi Giesbrecht and Acartia tonsa Dana and in contrast Marshall et al. (1935) report a marked effect of light on rate of oxygen consumption of C. finmarchicus. Marshall et al. (1935) eliminated UV light as the factor causing significant increase in rate of oxygen consumption, although exposure to UV light did result in increased mortality. Also, Ringleberg (1961, 1964) has demonstrated that different light intensities and rates of change of intensity of laboratory illumination can affect locomotory activity of the cladoceran Daphnia magna Straus. In most studies of oxygen consumption, the effect of light or dark is not determined, but rather experimental conditions are maintained uniform. Since light is correlated with so many responses (e.g., Cushing, 1951; Lewis, 1959; Moore and O'Berry, 1957; Ringleberg, 1961, 1964) more investigation concerning its physiological effect is warranted.

Harris (1963) reported experimental demonstration of an intrinsic rhythm in locomotor activity in the zooplanktors

D. magna and C. finmarchicus and correlated these circadian rhythms with vertical migration. Moore and O'Berry (1957) also argue for the presence of an endogenous rhythm in association with vertical migration. If an endogenous rhythm related to vertical migration is a general phenomenon of zooplankton and if the rhythm is related to locomotor activity, its presence should be testable by measurement of oxygen consumption. However, C. plumchrus demonstrates no regular significant change in rate of oxygen consumption. Therefore, one might conclude that C. plumchrus does not migrate, but Zenkevitch (1963:793) reports Calanus tonsus (C. plumchrus) to be both a daily and seasonal vertical migrator. Alternative explanations of the absence of a rhythm cannot be excluded. Other possible explanations are (1) the populations of copepods tested may have been asynchronous, (2) the rhythm may be ephemereral and hence disappears when the copepods are taken into the laboratory and tested in the Warburg respirometer, (3) the rhythm might not be related to locomotor activity, (4) active vertical migration might only be a function of less than half of the population (Moore, 1955; Moore and O'Berry, 1957) and therefore animals tested might have been selected from non-migrators present in the same populations or (5) the method of monitoring oxygen consumption may not be sensitive enough. However, it should be kept in mind that the response measured by Harris was at least a "semi-persistent" population phenomena measured under laboratory conditions. Still another possibility is that Harris

actually measured an endogenous rhythm in maintenance of vertical position rather than a locomotor rhythm since downward movement (especially in nature) need not necessarily imply only passive sinking.

Thus, with reference to the effect of the remaining environmental factors studied on measurement of oxygen consumption, crowding is a significant factor only at concentrations when density is equal to or greater than five copepods / ml, neither light nor dark cause difference unless the copepods are changed from one to the other during or just prior to the tests, and tests need not be restricted to a particular time of the day. However, during the summer random significant increases in rate of oxygen consumption do occur. Since oxygen consumption was calculated from measurements taken at the termination of the test, rates could not be corrected for these changes. Furthermore, it is not known if these changes are "real" or if they merely reflect the experimental technique. Hence, the data must be considered a maximum estimate of oxygen consumption.

Response to temperature is enlightening and consideration of this response is important to the remainder of the data. Rate of oxygen consumption increased both linearly (Figure 6) and non-linearly (Figure 5) over a temperature range of 5° - 20° C when plotted identically and as well, the linear response differed by sample variance and by elevation difference. One of the lines differing by elevation was derived from copepods collected from a different season and locality,

disallowing the conclusion that a quantitative difference indicates a biological difference (this being due to seasonal adaptation, see Conover (1959) and Marshall and Orr (1958)). However, the difference in the locality strongly implies that the populations are biologically distinct.

Thus, various aspects of each of the sets of oxygen consumption data suggest that each of the sets of data is collected from a different biological population. In addition, each of the sets of temperature data indicates a different history of thermal acclimatization in each of the populations studied. The slopes of the lines however, do not vary, thereby indicating that Q_{10} does not change with season or population tested. The general response of non-proportional increase or decrease of rate of oxygen consumption at 20° C indicates that this temperature is near the upper lethal limit.

The effect of salinity on rate of oxygen consumption of copepods is variable. Marshall et al. (1935) and Anraku (1964) report rate of oxygen consumption of C. finmarchicus decreases with decrease in salinity. A similar decrease is reported for Centropages hamatus (Lilljeborg) (Anraku, 1964). In contrast, Lance (1965) reports a consistent increase in rate of oxygen consumption of A. tonsa with decrease in salinity. C. plumchrus appears to respond in an intermediate fashion with no significant change in rate of oxygen consumption over a range of 20-35 ppt with a significant increase at 10 ppt and a significant decrease at 45 ppt. Comparative data from Calanus spp. and Pleurobrachia bachei Agassiz indicate

a constant or slightly increasing rate of oxygen consumption over a range of 20-35 ppt and a marked increase in rate at lower or higher salinities (Topping, unpublished data).

Sample variance of the two sets of data presented were unequal indicating that the population from which the data were collected are biologically different. However, the two responses are not quantitatively different.

The manner in which salinity affects oxygen consumption of crustaceans is still disputed (e.g., Schlieper, 1929; Flemister and Flemister, 1951; Lofts, 1956; Gross, 1957; Potts and Parry, 1964). Lance (1965) reported maintenance of slight hypertonicity with decrease in salinity in A. tonsa and in view of considerations reported by Potts (1954), this response should require little energy utilization. The technique employed is difficult and the data require verification. It remains possible that increased rate may be due to initial bursts of locomotor activity.

That oxygen concentration may act to regulate rate of oxygen consumption is well known (Prosser and Brown, 1961). However, evidence that oxygen concentration may act to affect oxygen consumption of copepods is restricted to one report (Marshall et al., 1935). The present study indicates that rate of oxygen consumption of C. plumchrus is restricted by concentrations less than 3 cc oxygen per liter and that the minimum lethal concentration is about one cc oxygen per liter. The 1964 data do not clearly demonstrate this observation because rate of oxygen consumption of these copepods was at a

minimal level and the difference in rate due to oxygen concentration was small. Also, when the rates of the copepods tested in May 1965 were corrected for size difference, experimental error as well as rate of oxygen consumption was multiplied by the same factor and hence no difference in rate was shown. Since the rates of winter acclimatized animals is lower than summer animals, the above considerations imply that there would be a correspondingly smaller effect of oxygen concentration. Comparative data for Calanus spp. and P. bachei indicate a response similar to that reported for C. plumchrus. However, rates of both planktors at 5° C are not reduced until oxygen concentrations are reduced to 1-2 cc oxygen per liter. The data may be interpreted to indicate that oxygen concentration is essentially unimportant in most of the open ocean, but the factor may be important in coastal areas and certainly should be considered in fresh-water environments.

Pressure has been proposed to be a factor of importance to marine zooplankton (Moore and Corwin, 1956; Moore and O'Berry, 1957), but again evidence of the effect of pressure on oxygen consumption is restricted to one study (Napora, 1964). Napora reported that rate of oxygen consumption of the prawn Stellaspis debilis M.-Edwards increased with increase in hydrostatic pressure and that at about 900 m the effect of pressure is equal and opposite to that of temperature. Teal (in manuscript) has studied the effect of pressure on rate of oxygen consumption of various larger

crustaceans and euphausiids and has obtained variable results (e.g., Thysanopoda monocantha Ortman increases rate of oxygen consumption with increase in pressure, T. tricuspidata M. Edw. undergoes essentially no change in rate and T. obtusifrons Sars decreases rate). The rate of oxygen consumption of C. plumchrus was not significantly changed with increase in pressure equal to about 40 atm (or equivalent to about 400m). I observed a similar response in a fresh-water daphnid Daphnia spp. which was collected from a lake with a maximum depth not exceeding 15 m. In view of the results thus far obtained and because of the paucity of taxa tested, generalization of the effect of pressure on oxygen consumption of planktonic crustaceans is impossible. However, effect of pressure on oxygen consumption of C. plumchrus may have been masked by the technique employed (see Sec. II G and Sec. IV A). If the effect of pressure is hidden, then the regression line shown in Figure 9 for the 12° C data (significant at $P < 0.05$) may give a better interpretation of the effect of pressure. In this case, the data presented give an interpretation of the minimum effect of pressure.

C. Oxygen utilization in nature. Initially, the temperature data indicate that data collected from copepods taken from different collections (in this study equal different sample dates) may be from different biological populations. Since the effect of all factors were not determined on a single collection of copepods, the separate responses may not be grouped and argued to represent the response of a single

population of C. plumchrus. Rather, a generalized response of C. plumchrus, not specific to a particular population, to the factors tested is obtained. This is in no way detrimental to interpretation of the general picture of energy utilization of C. plumchrus in nature.

The environmental factors considered in this study (e.g., salinity, temperature or oxygen concentration may vary according to the particular part of the marine environment studied. Light and pressure will always decrease and increase respectively with increase in depth. Temperature will also generally decrease with increase in depth, except in areas of extreme mixing wherein temperature may remain essentially constant or with a thermal inversion. Salinity and oxygen concentration will remain essentially constant with increase in depth in the open ocean, but in coastal situations, particularly fjords and inlets, salinity may increase and oxygen concentration decrease markedly with depth. Therefore, according to variance of the environmental factors considered, oxygen consumption might vary with depth according to the environment. Comparison of the two localities from which C. plumchrus were collected for this study are given in Table V.

Zenkevitch (1963) reported that Calanus tonsus (Calanus plumchrus) occurring in the north Pacific migrates vertically both seasonally and diurnally. Therefore, McLaren's (1963) hypothesis that the adaptive value of vertical migration of zooplankton is to (1) be present in or near the euphotic

Table V. Summary of water properties with depth and season in San Juan Channel and Indian Arm (Tulley and Dodimead, 1957; Waldichuk, 1957; Gilmartin, 1962).

Depth (m)	Temperature (° C)		Salinity (ppt)		Oxygen (ccO ₂ /l)	
<hr/>						
<hr/>						
San Juan Channel:						
	June '50	Winter	June '50	Feb. '50	June '31	Feb. '31
0	13	6	24.0	28.5	8.2	7.5
150	8	6	30.6	30.4	6.0	6.0
Indian Arm:						
	June '59	Nov. '57	Sum. Max	Win. Min	May '59	Oct. '59
0	15	8	15	16	9.0	6.0
100	7	7	28	27	4.5	3.0
200	7	7	28	28	2.0	2.2

zone to feed and yet (2) to sink lower in the water body where, primarily due to lower temperature, less energy will be used and (3) the energy is shunted to growth of larger more fecund plankton, which in turn (4) produce more offspring allowing selection for vertically migrating forms, is of special significance. Migration to or through discontinuity layers will presumably result in exposure of the plankton to wide ranges of environmental factors and overshoot in physiological adjustment to these properties should result. Therefore, overshoot in rate of oxygen consumption (although maximizing the quantity of response to a particular factor) is considered part of the normal response and hence, is not removed from the results presented in this study.

Due to lack of better data, responses to the environmental factors are considered to be additive (for limits to this assumption see Kinne (1964) and Napora (1964)).

C. plumchrus in the open ocean should decrease oxygen utilization with depth primarily due to temperature. In a coastal situation such as San Juan Channel, rate of oxygen consumption may decrease slightly during summer with depth due to decrease in temperature, but should undergo essentially no change during most of the year. Consideration of temperature, salinity and oxygen concentration in another coastal body of water (Indian Arm, a British Columbia fjord) also indicates rate of oxygen consumption should decrease with depth. However, in this case oxygen consumption should decrease primarily because of oxygen concentration since higher temperatures are above the halocline and the surface salinities reported should be lethal to C. plumchrus. Below the halocline, temperature decreases little. Therefore, in each of the situations described the copepod C. plumchrus should decrease rate of oxygen consumption with depth, except possibly when passing through a thermal inversion in the water body. Consequently, change in rate of oxygen consumption with depth is in general agreement with McLaren's hypothesis of energy utilization. However, in particular coastal situations oxygen concentration or salinity might act in concert with temperature to decrease energy utilization. This conclusion might appear to be a "truism", but in view of Napora's (1964) report and Teal's tentative results, such a conclusion is not axiomatic and cannot therefore necessarily be generalized to other species of zooplankton.

Various peculiarities of vertical migration observed

both in the field and in the laboratory (Hansen, 1951; Harder, 1957; Lance, 1962; Moore and O'Berry, 1957), together with the report that zooplankton will tend to regulate their distribution such that energy utilization is maintained at a constant rate (Bishop, 1964) suggest that interpretation of specific patterns of plankton distribution and movement in terms of physiology and particularly energy utilization may be fruitful.

V. Summary

1. Oxygen consumption of C. plumchrus with respect to environmental and endogenous changes was studied. Copepodid V stages were used throughout the study and specimens were collected from San Juan Channel, Washington and Indian Arm, British Columbia.
2. Rates of oxygen consumption were determined using standard closed chamber technique described by Conover (1956) and Warburg respirometry.
3. Rate of oxygen consumption of C. plumchrus is limited by extreme population densities and this limitation is not attributable to metabolite accumulation.
4. The relation between volume of sample chamber and duration of test is important in evaluating rate of oxygen consumption.
5. Temperature data indicate that for various measurements of oxygen consumption to be comparable, rates must be measured on the same collection of copepods. Either due to the real distribution of copepods or due to the lack of reproducibility of measuring rate of oxygen consumption of copepods, data from subsequent collections within the same season and from the same locality are statistically different.
6. Changes in rate of oxygen consumption of C. plumchrus in response to environmental and endogenous factors may be grossly summarized as follows:
 - a. Rate of oxygen consumption is significantly decreased

- by population densities of 5 or more copepods / ml.
- b. Regular endogenous changes in rate of oxygen consumption do not appear to occur.
 - c. The presence or absence of light does not significantly affect rate of oxygen consumption, but change from one condition to the other may cause a significant increase.
 - d. Rate of oxygen consumption may be described as directly proportional (being variously linear and non-linear) to temperature throughout the range of 5°-20° C.
 - e. Rate of oxygen consumption does not vary significantly over a salinity range of 20-35 ppt, but increased at 10 ppt and decreased at 45 ppt.
 - f. Rate of oxygen consumption decreases to a minimal rate below an oxygen concentration of three cc oxygen per liter.
 - g. Rate of oxygen consumption is not significantly affected by increased hydrostatic pressure corresponding to a depth of about 400 m.
7. In general, the data are consistent with McLaren's (1963) theory of energy utilization and vertical migration, although temperature does not appear to always be the most significant factor. Interaction and variation of environmental factors may explain some of the complexity of vertical distribution.

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Appendix I. Replicate measurements of rate of oxygen consumption (ul O₂ used / copepod / hr) with respect to crowding. Data were collected during June 1964 at FHL

	Crowding (copepods / ml)				
	1.0	3.3	4.0	4.3	5.0
	1.06	0.81	0.45	0.78	1.09
	0.28	1.21	1.08	1.37	1.00
	2.10	1.41	1.05	1.37	0.92
	0.29	1.21	0.91	1.17	1.05
	0.89	1.21	1.17	1.17	0.58
	0.88	1.41	1.11	1.56	0.60
	0.65	1.01	1.16	0.39	0.49
	0.65	1.62	1.22	1.76	0.60
	0.78	1.62	1.25	0.98	0.43
	0.60	1.62	0.50	0.39	0.90
	2.45	1.82	1.19	1.17	0.70
	1.26	1.41	0.87	1.17	0.71
	2.03	1.62	1.17	1.17	1.23
	0.43	1.62	1.00	0.98	0.28
	1.94	1.62	1.00	0.78	0.94
	1.72	1.62	0.88	1.17	1.07
	0.60	1.62	1.18	0.98	0.19
		2.22	1.43	0.59	0.75
		0.81	0.18	0.00	0.25
		1.82	1.00	0.98	1.63
		1.82	1.06	0.78	0.37
		2.42	1.24	0.59	0.89
		2.02	1.13	1.17	0.33
		1.82	0.77	0.39	0.51
Mean	1.24	1.15	1.00	0.95	0.70
S.E.	0.14	0.08	0.06	0.08	0.07

Appendix II. Diel measurement of rate of oxygen consumption (ul O₂ used / copepod / hr). Mean values of replicate determinations are listed with standard error given below in parenthesis. Data for columns A and B were collected during June 1964 at FHL and data for columns C, D, and E were collected during November 1965 at UBC. Data listed under columns A, B, etc., correspond to the curves designated by the same letters in Figures 3 and 4 of the text.

Curves shown in Figs. 3 and 4

	A	B	C	D	E
# of replicates	5	4	3	3	3
Copepods / replicate	8-13	15	5	5	5
Determinations:					
Time					
10:00 pm			0.40 (0.21)	0.33 (0.12)	0.40 (0.23)
1:00 am	0.58 (0.13)		0.40 (0.10)	0.25 (0.08)	0.32 (0.04)
2:00	1.17 (0.13)				
3:00	1.18 (0.12)	1.09 (0.16)	0.69 (0.14)	0.56 (0.17)	0.57 (0.21)
4:00	1.02 (0.10)	1.00 (0.94)			
5:00	1.18 (0.08)	0.92 (0.31)	0.46 (0.01)	0.34 (0.03)	0.36 (0.03)
6:00	1.26 (0.11)	1.05 (0.02)			
7:00	0.98 (0.26)	0.58 (0.04)			
7:15			0.35 (0.02)	0.23 (0.04)	0.39 (0.13)
8:00	1.41 (0.18)	0.60 (0.06)			
9:00	1.27 (0.15)	0.49 (0.04)			
10:00	0.70 (0.25)	0.60 (0.08)	0.47 (0.10)	0.53 (0.06)	0.62 (0.04)
11:00	1.31 (0.15)	0.43 (0.04)	0.60 (0.11)	0.33 (0.13)	1.88 (0.46)
12:00	1.04 (0.14)	0.90 (0.02)			

Appendix II (Cont'd.)

Curves shown in Figs. 3 and 4

	A	B	C	D	E
1:00 pm	1.26 (0.12)	0.70 (0.04)			
2:00	1.12 (0.15)	0.71 (0.03)	0.49 (0.12)	0.58 (0.13)	0.75 (0.25)
3:00	1.08 (0.17)	1.23 (0.05)			
4:00	1.09 (0.18)	0.28 (0.17)	1.17 (0.33)	0.63 (0.09)	1.70 (0.55)
5:00	1.23 (0.11)	0.94 (0.14)			
6:00	1.42 (0.28)	1.07 (0.05)			
7:00	0.27 (0.15)	0.19 (0.04)			
8:00	1.16 (0.19)	0.75 (0.05)			
9:00	1.16 (0.19)	0.25 (0.06)	0.45 (0.04)	0.71 (0.17)	0.81 (0.38)
10:00	1.34 (0.32)	1.63 (0.21)			
11:00	1.31 (0.19)	0.37 (0.06)	0.46 (0.05)	0.78 (0.18)	0.88 (0.48)
12:00	1.10 (0.27)	0.89 (0.40)			
1:00 am	1.12 (0.29)	0.33 (0.04)			
2:00		0.51 (0.16)			
3:00			0.43 (0.10)	0.34 (0.15)	1.25 (0.50)

Appendix III. Comparison of rate of oxygen consumption (ul O₂ used / copepod / hr) with respect to light and dark at four temperatures. Standard errors are listed in parentheses below the means. Copepods were tested at a concentration of five per three ml of sea water. Tests were run during June 1965 at UBC.

Temperature (° C)	Duration of test (hrs)	Light	Dark
5	7.13	0.24 0.25 \bar{x} 0.24 (0.01)	0.23 0.25 0.24 (0.02)
10	7.17	0.45 0.51 0.27 \bar{x} 0.41 (0.10)	0.15 0.38 0.53 0.32 (0.12)
15	4.03	0.42 0.65 0.99 \bar{x} 0.69 (0.17)	0.72 0.84 0.78 (0.06)
20	6.53	1.56 1.25 1.41 \bar{x} 1.41 (0.10)	1.41 2.31 1.86 (0.42)

Appendix IV. Rate of oxygen consumption ($\mu\text{l O}_2$ used / copepod / hr) with respect to temperature. Standard errors are listed in parentheses beside the values. Data presented for 1964 and May 1965 were collected at FHL, whereas data presented for November 1965 were collected at UBC. In the table, X denotes uncorrected raw data and X' denotes data corrected for seasonal size variation.

Temp. (° C)	Duration of tests (hrs)	Test Vol. (ml)	# copepods used/test; # of tests	$\mu\text{l O}_2$ used/cop./hr	
				X	X'

Date: 4/VI/64

2	12.0	50	6-8; 5	0.38 (0.04)	0.35 (0.04)
10	11.9	50	6-9; 5	0.69 (0.08)	0.63 (0.07)
15	12.0	50	6-9; 4	0.94 (0.07)	0.86 (0.06)
20	12.0	50	7-8; 5	0.96 (0.08)	0.90 (0.07)

Date: 9-10/VI/64

2	12.0	50	9-10; 10	0.30 (0.02)	0.28 (0.02)
10	11.5	50	11-15; 10	0.57 (0.07)	0.52 (0.07)
15	12.0	50	9-11; 10	0.68 (0.04)	0.63 (0.04)
20	12.0	50	10-13; 10	1.14 (0.04)	1.05 (0.04)

Date: 19 /V/65

4	36.9	35	5; 10	0.12 (0.01)	0.22 (0.03)
10	36.6	35	3-5; 10	0.20 (0.03)	0.37 (0.06)
12.5	35.5	35	1-5; 10	0.24 (0.04)	0.45 (0.07)
15	37.1	35	2-5; 10	0.35 (0.04)	0.67 (0.08)
20	15.3	35	5; 5	0.58 (0.07)	1.08 (0.13)

Date: 11/XI/65

3	26.5	35	5; 6	0.13 (0.02)	0.10 (0.01)
8	15.0	35	5; 1	0.39	0.30
10	3.0	10	5-8; 2	0.58 (0.06)	0.46 (0.02)
12	17.0	35	5; 9	0.50 (0.06)	0.38 (0.05)
18	3.0	35	5; 2	0.67 (0.05)	0.52 (0.04)

Appendix V. Rate of oxygen consumption (ul O₂ used / copepod / hr) with respect to salinity. Standard errors are listed in parentheses beside the values. Experiments reported for July 1964 were conducted in 50 ml chambers and experiments reported for May 1965 were conducted in 35 ml chambers. The experiments were conducted at FHL and all tests were run at 10° C. In the table, X denotes uncorrected raw data and X' denotes data corrected for seasonal size variation.

Salinity of tests (ppt)	Duration of tests (hrs)	Test Vol. (ml)	# copepods used/test; # of tests	ul O ₂ used/cep./hr	
				X	X'
Date: 26/VII/64					
20	13.5	50	4; 3	1.02 (0.26)	0.93 (0.24)
25	12.8	50	4; 3	0.78 (0.09)	0.72 (0.08)
30	12.5	50	6-10; 10	0.76 (0.09)	0.70 (0.08)
35	12.3	50	4; 3	0.76 (0.18)	0.69 (0.16)
Date: 18/V/65					
10	35	35	5-6; 4	0.56 (0.11)	0.95 (0.18)
20	35	35	5; 5	0.22 (0.01)	0.42 (0.03)
25	48	35	5; 5	0.23 (0.03)	0.43 (0.05)
30	35	35	4-5; 5	0.24 (0.05)	0.44 (0.09)
35	35	35	3-5; 5	0.32 (0.05)	0.60 (0.09)
45	35	35	5; 4	0.13 (0.02)	0.26 (0.04)

Appendix VI. Rate of oxygen consumption (ul O₂ used/coepod/hr) with respect to oxygen concentration and temperature. Standard errors are listed in parentheses beside the values. Data reported for May 1965 were collected at FHL and data reported for November 1965 were collected at UBC. All tests were conducted in 35 ml test chambers. In the table, X denotes uncorrected raw data and X' denotes data corrected for seasonal size variation.

Temp. (°C): O ₂ conc. (% O ₂ /l)	Duration of tests (hrs)	# copepods used/test; # of tests	ul O ₂ used / cop. / hr	
			X	X'
Date: 24-26/V/65				
5: 1.12	30	5; 3	0.11 (0.04)	0.21 (0.08)
2.10	48	4-5; 3	0.21 (0.08)	0.40 (0.17)
3.24	53	5; 3	0.13 (0.01)	0.24 (0.02)
4.75	47	5; 3	0.14 (0.01)	0.25 (0.02)
5.28	46	4-5; 3	0.17 (0.04)	0.32 (0.07)
10: 0.88	17	5; 3	0.28 (0.01)	0.51 (0.01)
2.02	13	5; 3	0.25 (0.04)	0.49 (0.10)
2.63	20	5; 3	0.18 (0.01)	0.32 (0.01)
3.64	20	4-5; 2	0.22 (0.01)	0.40 (0.01)
4.10	19	5; 3	0.28 (0.03)	0.53 (0.06)
4.68	20	5; 3	0.26 (0.02)	0.50 (0.05)
15: 1.87	12	5; 3	0.23 (0.06)	0.44 (0.10)
2.53	12	5; 3	0.19 (0.06)	0.37 (0.10)
3.16	15	5; 3	0.33 (0.12)	0.63 (0.21)
4.78	15	5; 3	0.33 (0.09)	0.61 (0.13)
Date: 11-12/XI/65				
3: 0.80	26	4; 1	0.03	0.03
2.17	26	4; 2	0.09 (0.02)	0.08 (0.02)
2.67	27	4; 2	0.09 (0.01)	0.08 (0.01)
3.60	27	4; 2	0.13 (0.01)	0.12 (0.01)
5.85	26	4; 2	0.19 (0.01)	0.17
12: 0.85	15	4; 2	0.07 (0.02)	0.07 (0.02)
1.15	15.5	4; 2	0.23 (0.18)	0.21 (0.16)
2.00	17	4; 2	0.14 (0.03)	0.13 (0.03)
3.43	17	4; 2	0.63 (0.03)	0.58 (0.03)
5.85	17	4; 2	0.36 (0.10)	0.33 (0.09)
18: 1.00	10	4; 4	0.10 (0.01)	0.09 (0.01)
1.17	12	2; 4	0.18	0.17
2.50	12	2; 4	0.48 (0.04)	0.44 (0.04)
6.00	14	4; 2	0.70 (0.03)	0.64 (0.03)

Appendix VII. Rate of oxygen consumption (ul O₂ used/copepod/hr) with respect to hydrostatic pressure. Standard errors are listed in parentheses beside the values. All experiments were conducted during November 1965 at UBC. Test chambers were 10 ml. In the table, X denotes uncorrected raw data and X' denotes data corrected for seasonal size variation.

Pressure (atm)	Duration of tests (hrs)	# copepods used/test; # of tests	ul O ₂ used / cop. / hr	
			X	X'

Temperature: 10° C

1.00	2.90	5,8; 2	0.58 (0.06)	0.46 (0.05)
10.35	1.40	5,9; 2	0.41 (0.05)	0.32 (0.05)
13.80	3.15	5,8; 2	0.55 (0.01)	0.43 (0.01)
27.60	9.30	8; 1	0.40	0.31
31.00	1.70	5,9; 2	0.28 (0.04)	0.21 (0.03)
39.70	3.25	5,8; 2	0.36 (0.11)	0.28 (0.09)

Temperature: 12° C

1.00	1.65	5,9; 2	0.65 (0.03)	0.50 (0.02)
19.00	1.75	9,11; 2	0.52 (0.06)	0.41 (0.05)
36.20	7.30	11; 2	0.34 (0.11)	0.27 (0.09)
