STUDIES ON RESPIRATION OF THE OMMASTREPHID SQUID ILLEX ILLECEBROSUS (LESUEUR, 1821)

MING-HSIUNG KAO, B.Sc.

STUDIES ON RESPIRATION OF THE OMMASTREPHID SQUID *ILLEX ILLECEBROSUS* (LESUEUR, 1821)

by

© Ming-hsiung Kao, B.Sc.

A Thesis submitted in partial fulfilment
of the requirements for the degree of
Master of Science

Department of Biology
and
Marine Sciences Research Laboratory
Memorial University of Newfoundland

St. John's Newfoundland

March 1970

Find out the cause of this effect . . .

Shakespeare

Abstract

The oxygen consumption of the ommastrephid squid *Illex illecebrosus* from Newfoundland waters was determined by the flowing water method and the hyponomal tube implanation method. Using statistical analysis, the total oxygen consumption per hour was found to be related to body weight, but with no significant difference between the sexes. This relationship was found to be similar to that reported for *Octopus cyanea* and *Sepia officinalis*. The rate of oxygen consumption does not show a significant relationship to body weight.

Illex illecebrosus shows no evidence of diurnal rhythm with regard to oxygen consumption under laboratory conditions. There is a significant decrease in oxygen consumption with decreasing salinity values.

The effect of either raising or lowering the pH of the environmental medium causes irregular responses in oxygen consumption. These are discussed in detail, showing the sequence of alteration in the consumption through different ranges of pH. All of the increases in oxygen consumption may be explained as secondary effects of either physical (chemical) stimulation, or osmotic stress due to the addition of pH-altering chemicals (NaOH and HCl) or their dissociation products. Decreases in oxygen consumption with change of pH may be due to

lessened oxygen-carrying capacity of haemocyanin, the Bohr Effect, or to acute shock when nearing the lethal ranges of pH.

The absolute utilization of oxygen for *I. illecebrosus* was found not to be correlated with the body weight. The mean absolute oxygen utilization coefficient value was calculated to be 19.53%. No correlation was found between body weight and volume of exhalent water per unit time. The mean exhalent water volume was found to be 35.62 litres per hour.

All experiments were performed in the range of water temperatures 10-12°C.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The candidate wishes to express his gratitude to Professor F. A. Aldrich who provided invaluable encouragement and considerable guidance throughout the course of this study. The research was supported by National Research Council of Canada Grant-in-aid-of Research No. A-1368 to Dr. Aldrich. The candidate also wishes to acknowledge with gratitude the award of a Memorial University of Newfoundland University Fellowship through the Department of Biology.

The candidate is greatly indebted to Drs. G. I. McT. Cowan and C. C. Davis for their helpful suggestions, advice and criticism throughout the several phases of this study. Appreciation is also expressed to Miss Lillian M. Sullivan for the typing of the manuscript.

Grateful acknowledgement is due to Messrs. C. C. Lu, M. J. Mercer, T. J. Stevenson, M. Hickey and R. P. Scaplen of the Marine Sciences Research Laboratory, and to Miss Helen E. Bradbury and Mrs. M. M. Aldrich, as well as Mr. S. J. Hwang, for their most valuable help in the collecting of the specimens of squid used in the study herein reported.

LIST OF FIGURES

INTRODUCTION

Many of the investigations conducted on squid, have been physiological studies. However, relatively few of them have been concerned with oxygen consumption. Montouri (1913) worked on the oxygen consumption of several species of cephalopods (the octopods, *Octopus vulgaris* Lamarck,1798; *Eledone moschata* (Lamarck,1798);and the sepioid, *Sepia officinalis* Linnaeus, 1761). His published data, however, did not provide sufficient information with which to confirm the relationship between body weight and oxygen consumption of the animals.

Winterstein (1925) reported 70% oxygen utilization by the octopus, *O. vulgaris*. On the basis of his published data, it remains unclear as to the conditions under which the oxygen consumption of those animals was measured. More recently, Maginniss and Wells (1969), reported that the relationship between body weight and oxygen consumption of *O. cyanea* Gray, 1849 was in agreement with that given by Zeuthen (1947), the slope of the regression line being b = 0.833. However, the majority of published data on the oxygen consumption of cephalopods have been restricted largely to representatives of the genera *Octopus* and *Sepia*. Octopods and sepioids have rather different living habits than do the pelagic squids. The latter form living much more actively than the former two. Furthermore, the squid is not as easy as the octopods and the sepioids to maintain under laboratory conditions. For this reason, experimental studies involving squid are scarce.

It is well known that many environmental factors influence the oxygen consumption of aquatic animals. The salinity and pH of the media may be conceded as the main environmental factors, in addition to the temperature, and to the partial pressures of oxygen and carbon dioxide.

Some ichthyologists have pointed out the existence of diurnal fluctuation in oxygen consumption of some species of fish (e.g., Graham, 1949; Clausen, 1936). It was thus deemed necessary to perform some experiments in order to determine whether or not the *Illex illecebrosus* (Lesueur, 1821) also exhibits this diurnal fluctuation.

In general, there are three methods which have been used widely in determining the rate of oxygen consumption of aquatic animals. These are (1) the flowing water method, (2) manometric method, and (3) the sealed chamber method. Each of these methods has certain advantages and disadvantages, and they are not always applicable in all situations. For example, the flowing water method, is unsuitable for animals which naturally inhabit stagnant water, while the manometric method is only suitable for studying small animals such as guppies or small crustaceans. Therefore, in selecting a method for the present respiratory studies, the physiological and ecological characteristics of the squid had to be taken into consideration.

Briefly, the three methods and the principles behind their use may be described as follows:

A - The sealed chamber method

The animal is placed in a measured volume of water which is sealed from atmospheric air. Over a given period of time, the difference between the initial and final oxygen content of the water is considered to represent the amount of oxygen taken up by the animal. The major advantage of the sealed chamber method is that it can be performed under a wide range of conditions and is especially suitable for field work in that it demands less elaborate equipment. However, the main disadvantages of this method are:

(1) It is not suitable for actively moving animals, which in general have a high oxygen consumption rate,and requires a considerable space for their movement. Fry and Hart (1948) were able to overcome this difficulty. However, there are other problems presented by this method.

(2) When the animal is placed in the sealed chamber for diurnal rhythm experiments, a large quantity of water is required. It builds up the stratification of oxygen concentration in the whole chamber (Keys, 1930). If a magnetic stirrer is used on the bottom of the chamber, this factor can be reduced, but the stirring movement

causes the animal to become excited, which may affect the animal's rate of oxygen consumption.

(3) Carbon dioxide and feces accumulating in the chamber may affect the metabolic rate of the animal and the accuracy of the chemical determination of oxygen content.

B - The manometric method

This method is based on the physical properties of gaseous media. One can calculate the rate of oxygen consumption from pressure changes in a closed container in which the experimental animal is held at a constant temperature. The Warburg respirometer is the most widely used apparatus for this method. This method is most suited for small organisms and has the same weak points as mentioned above for the sealed chamber method. Oxygen can not be supplied continuously during the experiment, thereby obviating experiments of long duration.

C - The flowing water method

This method is used most extensively in studies involving larger aquatic animals. It is basically a simple method, and is one in which an animal is placed in a respiratory chamber through which the water flows constantly. The difference between oxygen content of inflowing and outflowing water represents the amount of oxygen taken up by the animal over a given period of time. This method has proved to be best suited for the purpose of studying aquatic animals which necessitate running water. Eliminated wastes, including carbon dioxide, are excluded easily by the continuous running water. Oxygen is continuously replenished in this method. However, it also has some imperfections. Differences in the absolute values of oxygen content between inflowing and outflowing are small. Therefore, considerable error can be introduced, when calculating the rate of oxygen consumption (Winberg, 1956). The rate of water flow and size of the respirometer must be considered in experimental design for the particular organism under study.

Taking all these aforementioned factors into consideration, it was decided that the most generally suitable method for studies involving *I. illecebrosus* was the flowing water method.

The main objective of this study is to gain some knowledge of oxygen consumption of the squid in order possibly to understand better its habits and distribution in relation to salinity and pH. It is furthermore hoped that this work will provide some of the much needed information on squid metabolism in general.

MATERIALS AND METHODS

Method of Capture and Maintenance of Squid

The squid used in this series of experiments were caught by: (1) the use of conventional Neyle patent hand jiggers, (2) a mechanized Japanese jigging machine (Quigley, 1964), or (3) a standard squid trap.

All specimens were captured at Portugal Cove, Conception Bay, Newfoundland, between July and November in 1968 and 1969. Upon capture, a maximum of eight squid were immediately introduced into each of several sea water filled plastic buckets and rapidly trucked to the Marine Sciences Research Laboratory at Logy Bay. Each bucket contained approximately 16 gallons of sea water. The trip could normally be completed in 30 minutes. Usually, some of the squid thus transported died enroute to the Laboratory.

Upon arrival at the Marine Sciences Research Laboratory, the squid were immediately transferred to 275-gallon circular tanks provided with continuous running sea water at a temperature of 10 - 12°C. This temperature closely approximated the water temperature of the squid jigging ground during the period of the study. An acclimation period of at least one day under laboratory conditions was permitted prior to experimentation. It was found that stocks of squid could be maintained at the laboratory in these circular tanks for a number of weeks, if the squid were adequately fed.

The squid were fed with dead capelin [*Mallotus villosus* (Müller 1776)] which were suspended in the tank by a very fine monofilament nylon line as described by Bradbury & Aldrich (1969a). Occasionally it was found that squid would feed on living material such as small Atlantic cod [*Gadus morhua* L.], small tom cod [*Microgadus tomcod* (Walbaum)] or three spined stickleback [*Gasterosteus aculeatus* L.]. After some time in captivity a number of individuals developed lesions and other abrasions. Whenever possible, these damaged individuals were not utilized for experimentation and only specimens in good physical condition were used.

The experiments reported herein involved the use of 60 squid, which ranged in wet weight from 89.5 grams to 473.3 grams, with only two squid weighing less than 100 grams. Prior to experimentation, the selected squid were starved for a period of at least 12 hours. At the completion of metabolic studies, the squid were immediately weighed and dissected to determine sex and content of digestive tract, if any. Records were also kept with respect to internal parasites and morphological anomalies that were encountered.

Chemical Determination of Dissolved Oxygen Content

Since the water of Logy Bay is not polluted, the dissolved oxygen concentration could be determined by the Unmodified Winkler method (Barnes, 1959). Standardization of sodium thiosulphate solution was performed every two days. A 10 ml automatic burette was used for titrations.

Determination of Basal Metabolism

A. Tube implantation method

Twelve squid were studied for oxygen consumption using a tube fixed in the hyponome (or funnel) (Fig. 1) in such a way as to permit collection of all water expelled from the mantle cavity in the exhalent phase of a hydrojet cycle (Zuev, 1970).

The implantation of surgical gum rubber tube followed, in principle, the procedures used by Johansen (1965) and Johansen and Lenfant (1966) in *Octopus dofleini* (Wülker, 1910).

After 12 hours of starvation, the squid were immersed in -1°C or -2°C sea water for anesthesia during the implantation of the rubber tube. The tube, with an internal diameter of 9 mm, measured 15 cms in length. It was inserted into the hyponome and fixed there by means of sutures. After this procedure, the squid were returned to sea water of normal temperature. Such animals were not able to maintain normal position and always turned upside down (i.e., ventrum uppermost); nevertheless, respiration still continued unobstructed.

The squid were then placed into a respirometer and allowed to further recuperate; usually the squid recovered quickly (in about 20 minutes) and could live at least two days without feeding, although maintaining their upside down posture.

From the respirometer, the distal end of the tube was inserted into a graduated container and it was thus possible to obtain

Figure 1. Drawing of head of *Illex illecebrosus* indicating the method of implantation of tube into the hyponome and suturing it into place.

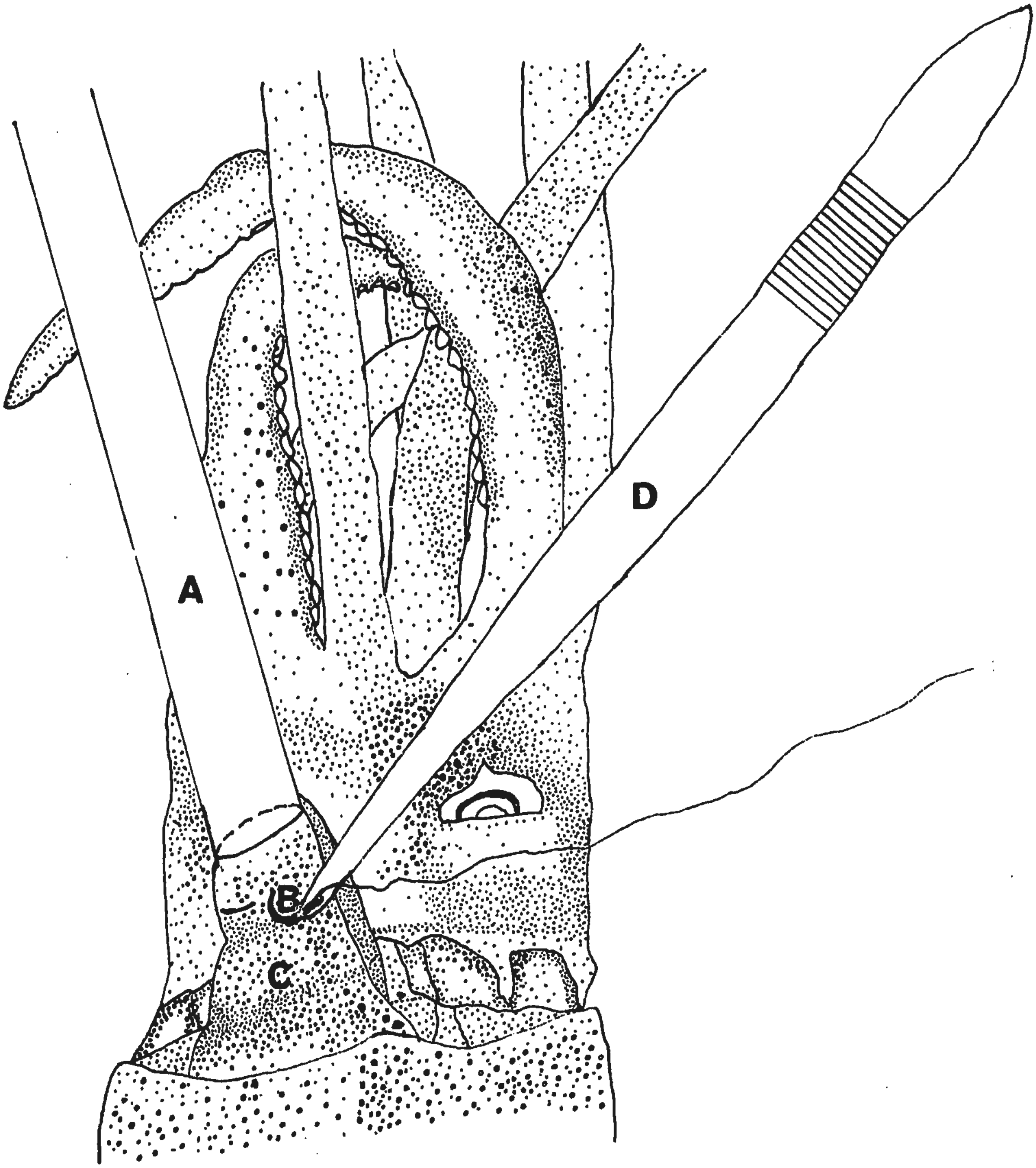
Legend:

A - Rubber Tube

B - Curved Needle with sterile silk thread

C - Hyponome or Funnel

D - Forceps


A
B
C
D

data on the total volume of exhalent water. Oxygen consumption rates were obtained by a comparison of simultaneous dissolved oxygen determinations on (a) reservoir water samples, and (b) exhalent water samples delivered directly into B.O.D. bottles, again using the Unmodified Winkler method.

B. Flowing water method

(I) A description of the apparatus

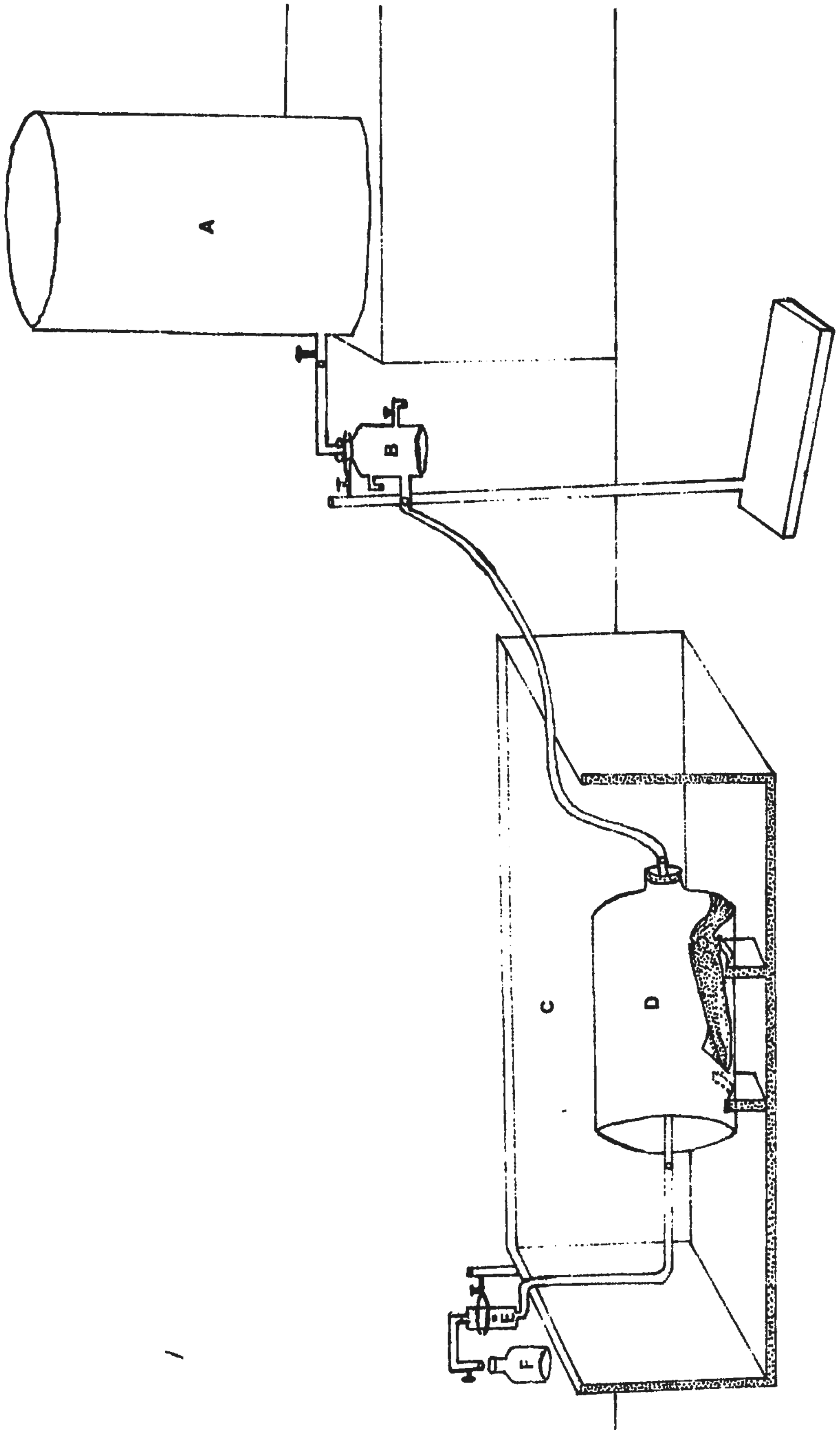
The respirometer (Fig. 2) employed in this study closely followed that used by Keys (1930) but with one major modification. This was the incorporation of a magnetic stirrer at the bottom of the 16-gallon water reservoir which prevented oxygen stratification of the water either before or during the period of experimentation. The water from the reservoir was carried through transparent plastic tubing to the constant-level bottle which functioned to maintain a constant flow rate throughout the system. The water from the constant-level bottle flowed into one or both of the two respirometers. The respirometers were completely immersed in a sea water bath to maintain constant temperature. The tube entering the respirometer was wrapped with gauze, inducing an even exchange of water within the apparatus.

Cole-Parmer Company flow meters were attached to the outlet of each respirometer so that the rate of flow of the water could be adjusted either by means of a clamp between the respirometer and the

Figure 2. Diagram of respirometer and associated apparatus used in measuring oxygen consumption by *Illex illecebrosus*.

Legend:

A - Sea Water Reservoir
B - Constant-Level Water Bottle
C - Water Bath
D - Respirometer with Squid in Place
E - Flow Meter
F - Source of Water Samples for Subsequent Oxygen Analyses.


A
B
C
D
E
F

flow meter, or by the constant-level water bottle.

Water samples were taken simultaneously from the constant-level bottle and flow meter outlet, using standard 250 ml B.O.D. bottles, following procedures recommended by Strickland and Parsons (1968).

The respirometers were constructed of transparent glass, and were placed in a water bath which was completely covered by black plastic screens to prevent visual excitation of the squid during an experiment.

(II) Experimental method

In this series of experiments, 48 squid were studied. The sea water temperature of the respirometer was maintained at 11°C ± 1°C by adding either hot or ice water to the water bath. The sea water entering the reservoir was first filtered through gauze to prevent any debris or particulate matter from entering the system. The rate of water flowing through the respirometer was maintained at between 40 and 50 liters per hour.

It proved necessary to wait for five hours after the transfer of the squid to a respirometer for the squid to attain a state of basal metabolism (i.e., resting state). Therefore, the actual observations on oxygen consumption of the squid began when the repeated samples showed a fairly constant level (Fry, 1957).

In the respirometer, the squid was oriented so that the anterior end was placed facing the incoming water current. If the water current was adjusted properly, the squid would become quiescent, and assume the resting position as described by Bradbury & Aldrich (1969b).

Determination of Effect of Salinity on Basal Metabolism

The effect of salinity on the basal metabolism was determined for eight squid.

After measuring the basal metabolic rate using the flowing water method, the salinity was gradually decreased by the introduction of distilled water into the water reservoir of the apparatus. The altered salinity ranged from $16^{o}/_{oo}$ to $31.18\ ^{o}/_{oo}$. Water sampling procedures were the same as those described for ascertaining basal metabolic rate. Salinity and oxygen consumption rate of the squid were determined simultaneously at hourly intervals until the death of the squid.

The salinity of the samples was determined using the silver nitrate technique as described by Smith & Kammer (1968). The silver nitrate solution was standardized against "Eau de Mer Normale".

Determination of Effect of pH on Basal Metabolism

As in the studies involving salinity variation, a series of experiments were conducted on 31 squid for which the basal metabolic rates were known to determine the effects of altered pH on oxygen consumption. The pH of the water in the respirometer was altered by the addition of controlled amounts of either hydrochloric acid or sodium hydroxide solution. The latter gave rise to a certain amount of trouble in that precipitation of magnesium hydroxide occured at pH value's approaching 10. When this happened, the magnesium hydroxide precipitate was filtered out in another water reservoir prior to the water entering the experimental water reservoir and the respirometer. One further cause of trouble was the precipitation of calcium hydroxide at pH 12. However, most experimental squid died before the pH value approached 12.

The method of acidification and basification of sea water followed the techniques recommended by Kukubo (1962). In all instances, the pH of sea water was measured using a Corning pH meter (Model 7), compensated for temperature.

RESULTS

(I) The Effect of Sex and Size on Oxygen Consumption

The oxygen consumption data were plotted arithmetically against body weight and were found to be of a curvilinear (parabolic) nature. It was subsequently found necessary to use logarithmic transformations for all analyses. The complete data are presented in Appendix A.

The logarithmic relationship between body weight and oxygen consumption was determined by linear regression, the equation for the line being $Y = a + bx$ (Figure 3). The relationship was calculated for each sex as well as for combined sexes, and is presented in Appendix A.

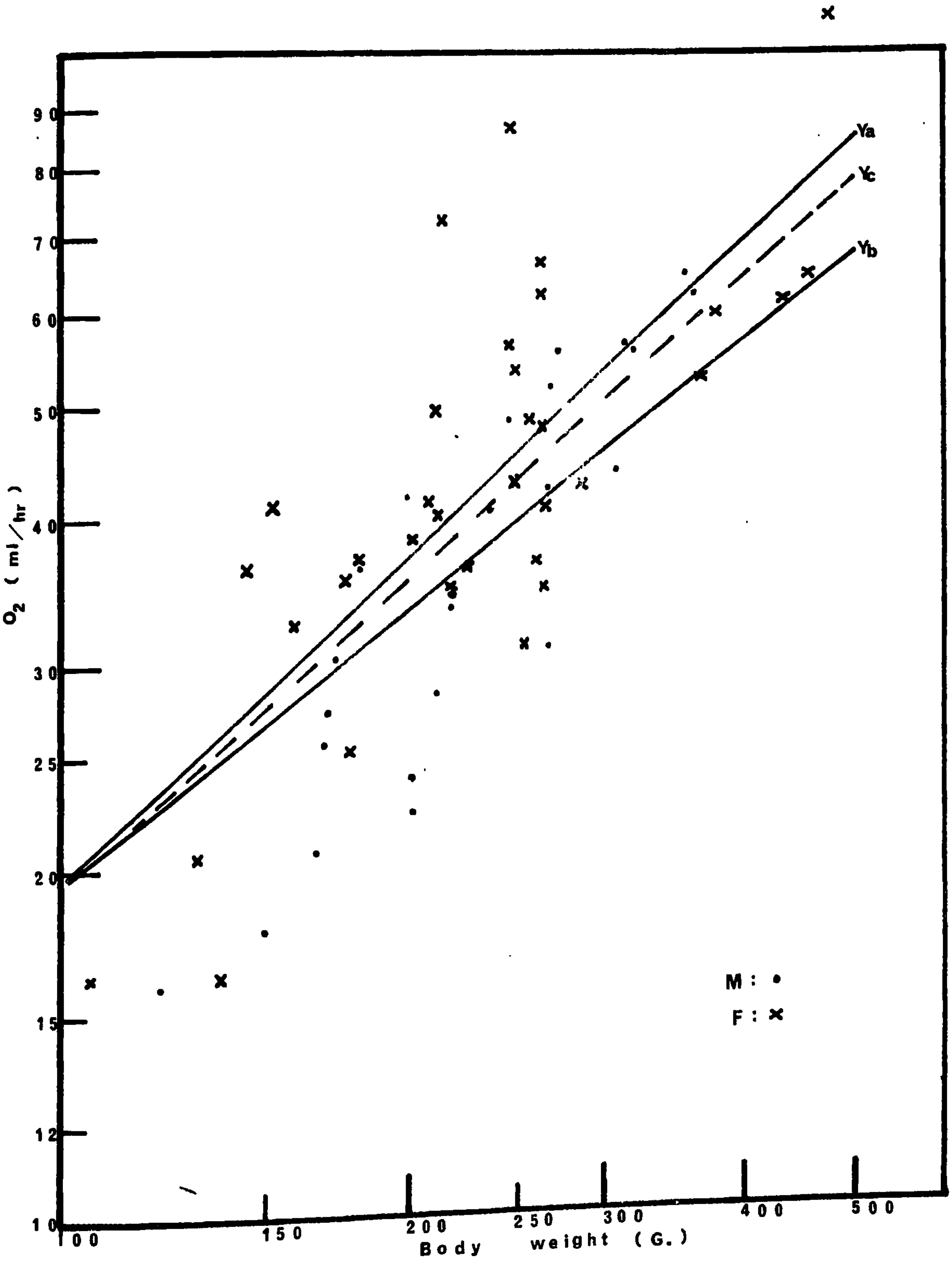
In all cases the correlation coefficients were found to be significant ($P \geq 0.05$).

In order to compare the correlation coefficients of the male and female lines, it was necessary to transform the coefficients using Fisher's Z transformation $\{Z = \frac{1}{2} [\log_e (1 + r) - \log_e (1 - r)]\}$. The Student's T test was then used to determine the significance of the two Z values. $T_{z_1 - z_2}$ was 0.2427 which was found not to be significant at 0.05 level. There is, therefore, no significant difference between the sexes in the relationship of body weight to oxygen consumption. Similarly, no significant difference

Figure 3. Relationship between oxygen consumption and body weight of *Illex illecebrosus* (Temperature 10-12°C).

Legend:

Ya - regression line for ♀ n = 34
Yb - regression line for ♂ n = 26
Yc - regression line for combined sexes n = 60


90
80
70
60
50
40
30
25
20
15
12
10
O2 (ml/hr)
100
150
200
250
300
400
500
Body weight (G.)
Ya
Yc
Yb
M : ●
F : ×

was found between the regression coefficients (r) of the male and female samples. It was thus justified to combine the samples. The regression line was calculated for the combined sexes and found to be: $\log Y = 0.8738 \log X - 0.4584$ ($n = 60$). The correlation coefficient ($r = 0.7524$) was tested and found to be significant ($P \geq 0.05$).

The relationship between the rate of oxygen consumption (ml/g/hr) and body weight (Figure 4) was also tested for the two sexes. The correlation coefficients (♀:$r = -0.05838$; ♂:$r = -0.2723$) were tested and surprisingly neither was found to be significant ($P \geq 0.05$). Thus there is no correlation between metabolic rate and body weight. Complete data are presented in Appendix A.

(II) Diurnal Rhythm in Oxygen Consumption

In an attempt to determine whether an endogenous cycle in oxygen consumption exists, five squid were maintained in the respirometer over 24-hour periods. Oxygen consumption values were obtained every hour over the entire 24-hours.

The results are presented in Figure 5.

At times, the experimental squid became active and swam about the respirometer. These periods of activity were reflected by marked increases (peaks) in the oxygen consumption and are identified by asterisks in Figure 5. Values obtained in this series of experiments are presented in Appendix B.

Figure 4. Relationship between rate of oxygen consumption to body weight of *Illex illecebrosus* (temperature 10°-12°C.)

Legend:

Ya - regression line for ♀ n = 34

Yb - regression line for ♂ n = 26

Yc - regression line for combined sexes n = 60

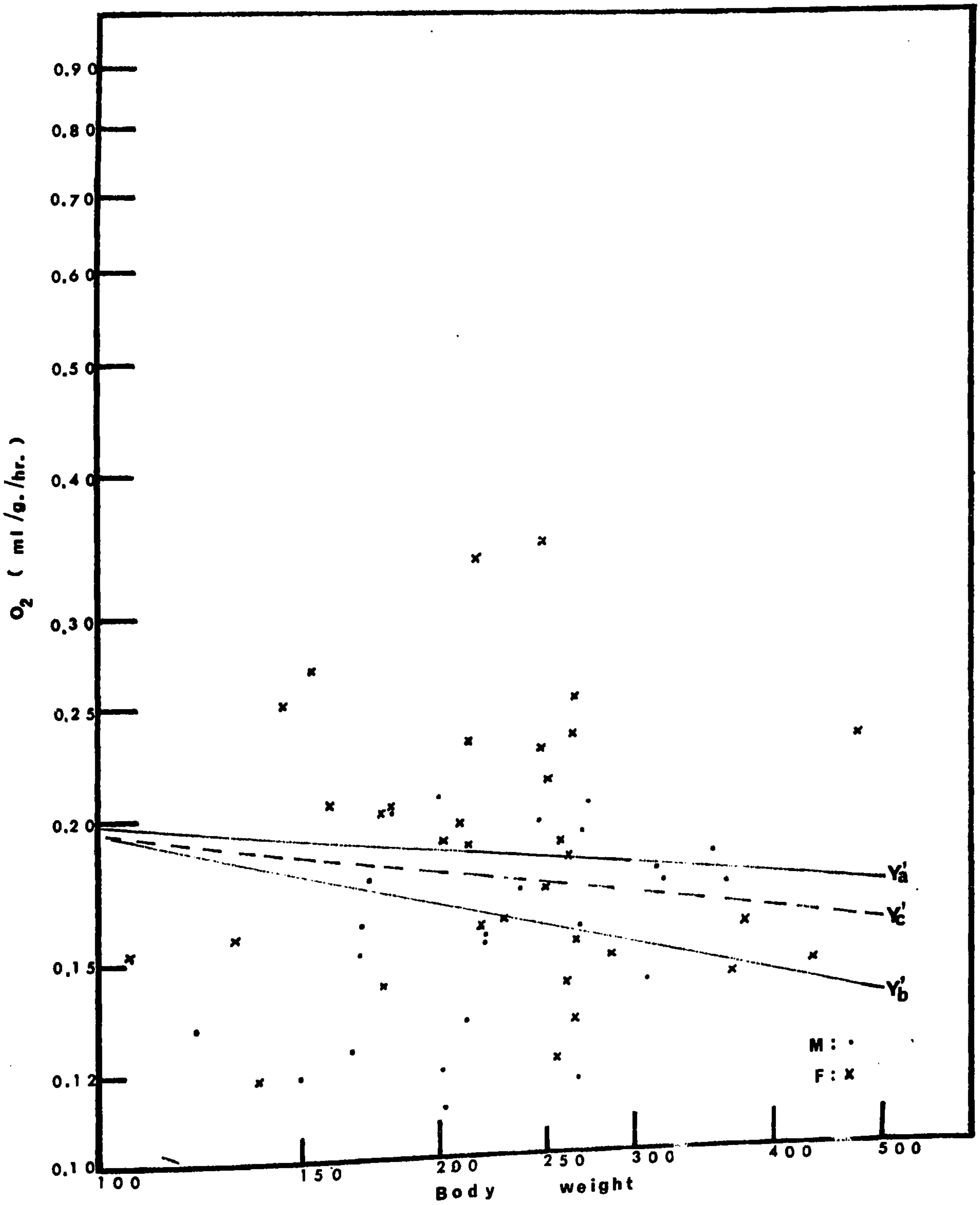
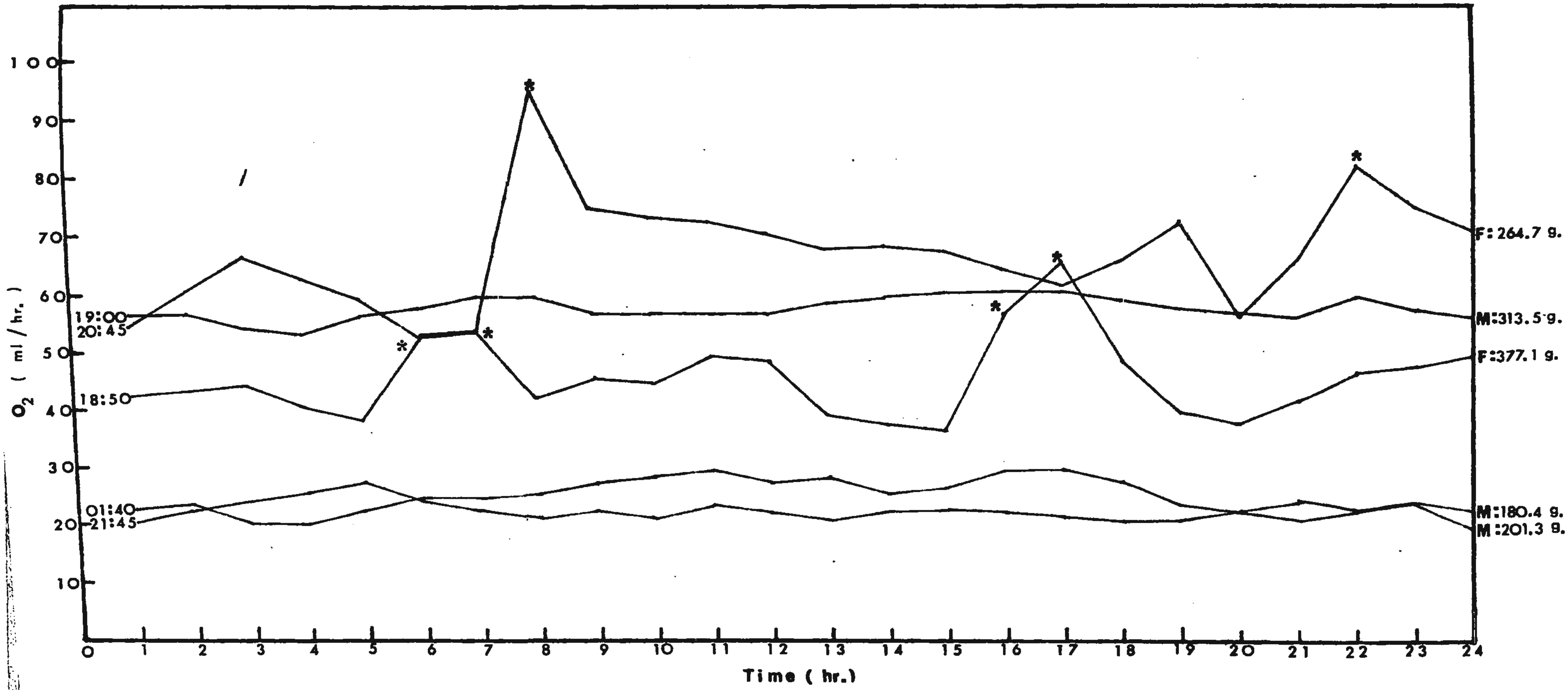

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.25
0.20
0.15
0.12
0.10
O₂ (ml /g./hr.)
100
150
200
250
300
400
500
Body weight
Y'a
Y'c
Y'b
M : •
F : x

Figure 5. Relationship between oxygen consumption by *Illex illecebrosus* and time elapsed. (Temperature 10 - 12°C).


1 0 0
9 0
8 0
7 0
6 0
5 0
4 0
3 0
2 0
1 0
O_2 (ml / hr.)
19:00
20:45
18:50
01:40
21:45
F: 264.7 g.
M:313.5 g.
F:377.1 g.
M:180.4 g.
M:201.3 g.
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Time (hr.)

The plotted data reveal no apparent trends.

(III) The Effect of Salinity on Oxygen Consumption

Regression lines were calculated for the relationship between oxygen consumption and salinity separately for each squid studied. These relationships are presented in Figure 6. The sample size, body weights, salinity regression lines and correlation coefficients are tabulated in Appendix C.

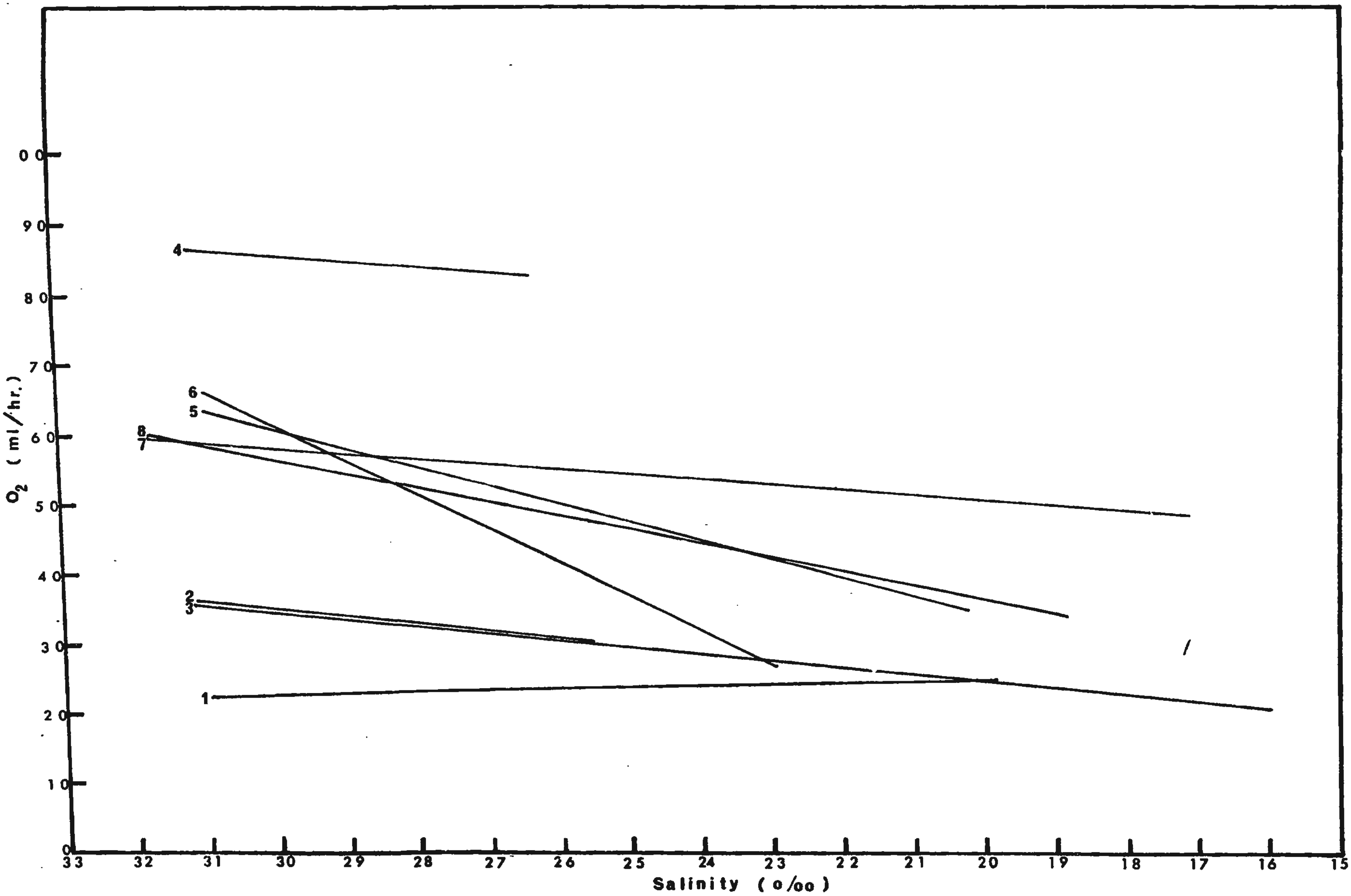
The correlation coefficients of the regression lines were tested for significance and it was found that in all but two individuals (numbers 1 and 2), they were significant at the 0.05 level. These data indicate a definite trend toward the reduction of oxygen consumption as the salinity of the sea water decreases.

(IV) The Effect of Sea Water pH on Oxygen Consumption

In Figures 7 through 10 are presented graphically the results of the pH experiments. The pH of the sea water was either gradually decreased from a pH of 8 (normal) to a terminal pH of 4.7 (Figures 7 and 8) or increased to a terminal pH of 10.2 (Figures 9 and 10).

In general, the oxygen consumption tended to decrease initially as the pH of the sea water decreased, with a further pH decrease the consumption tending to increase to a maximal level between pH 5.0 and 6.7. After the point of maximal oxygen consumption had

Figure 6. Relationship between oxygen consumption and decreasing salinity.(Temperature 10-12°C). (Each regression line represents an individual specimen of *Illex illecebrosus*.



00
90
80
70
60
50
40
30
20
10
0
O2 (ml/hr.)
4
6
5
8
7
2
3
1
33
32
31
30
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
Salinity (o/oo)

Figure 7. Relationship between oxygen consumption by male *Illex illecebrosus* and decreasing pH of the sea water. (Temperature 10-12°C). (Each line represents a single individual).

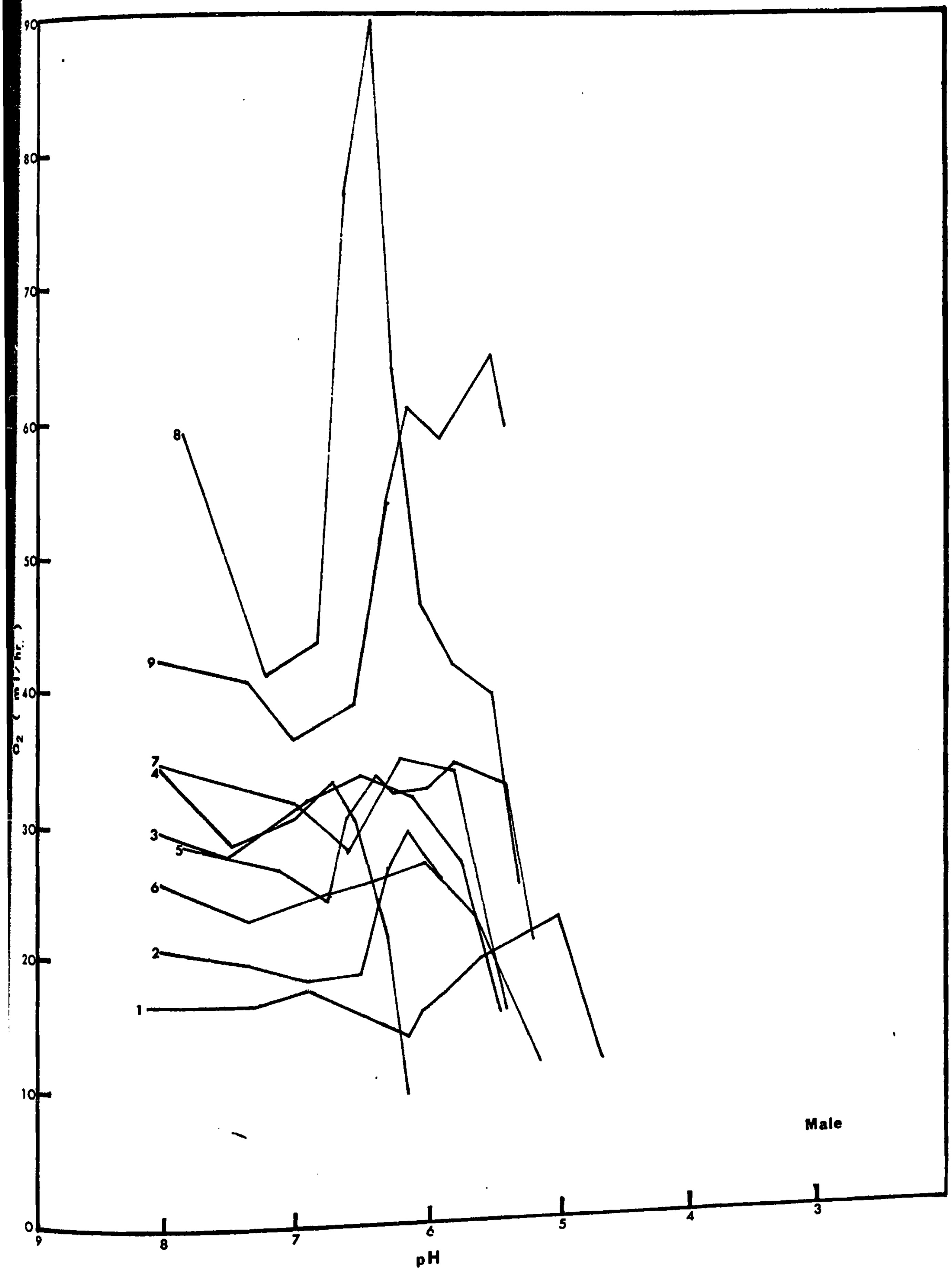

90
80
70
60
50
40
30
20
10
0
O2 (ml / hr.)
9
8
7
6
5
4
3
pH
1
2
3
4
5
6
7
8
9
Male

Figure 8. Relationship between oxygen consumption by female *Illex illecebrosus* and decreasing pH of the sea water. (Temperature 10-12°C). (Each line represents a single individual.)

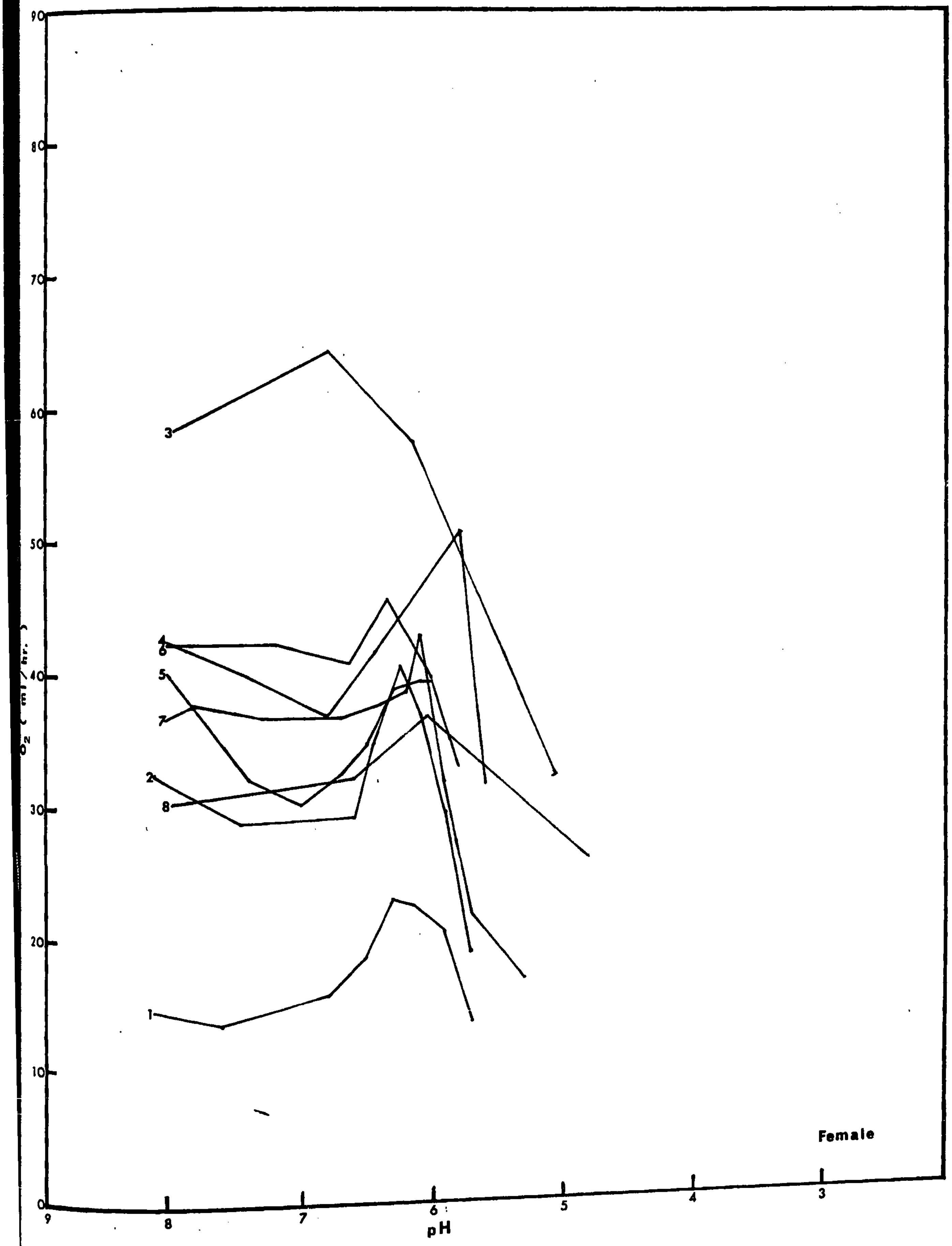

90
80
70
60
50
40
30
20
10
0
O2 (ml / hr.)
9
8
7
6
5
4
3
pH
1
2
3
4
5
6
7
8
Female

Figure 9. Relationship between oxygen consumption by male *Illex illecebrosus* and increasing pH of the sea water. (Temperature 10-12°C). (Each line represents a single individual).

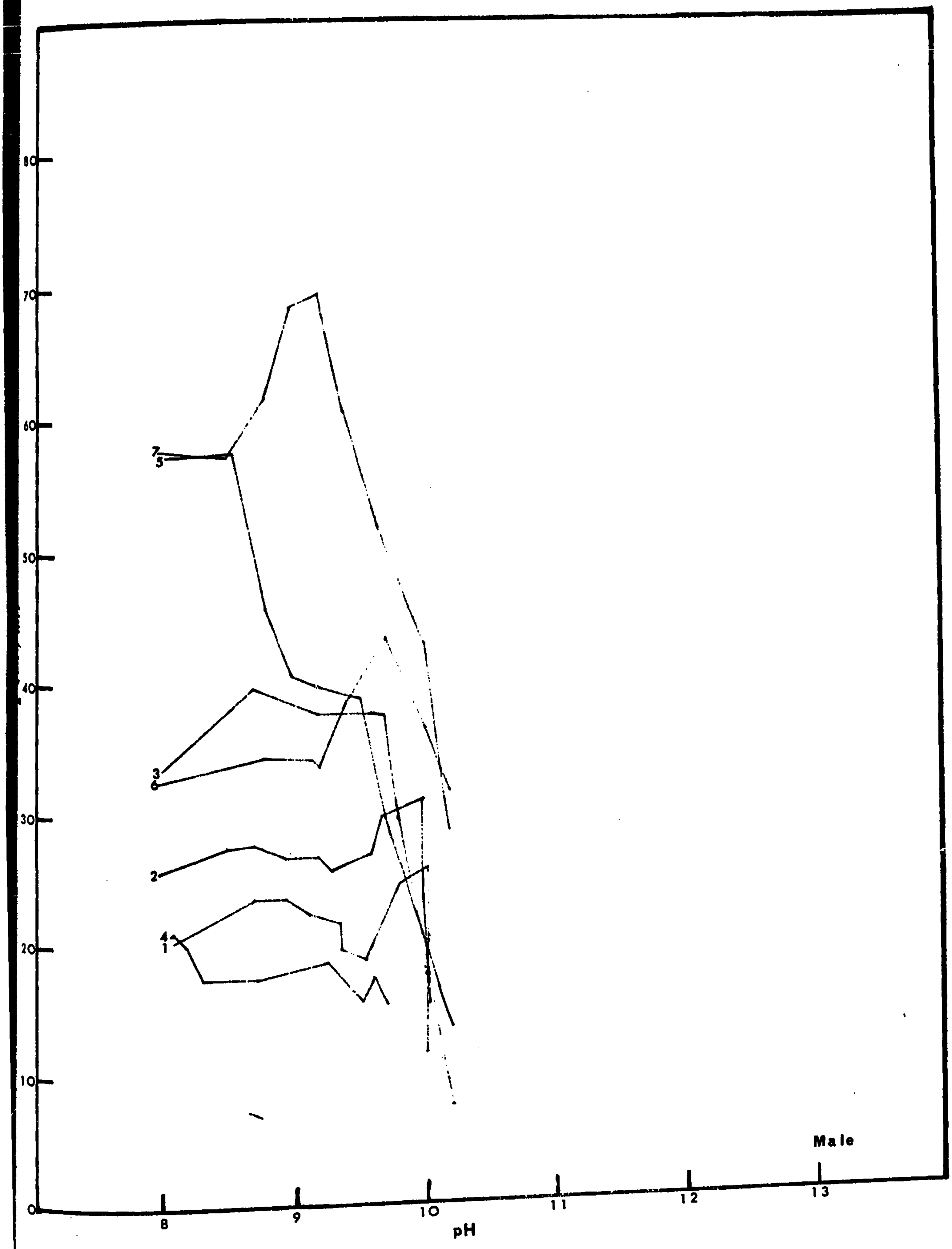
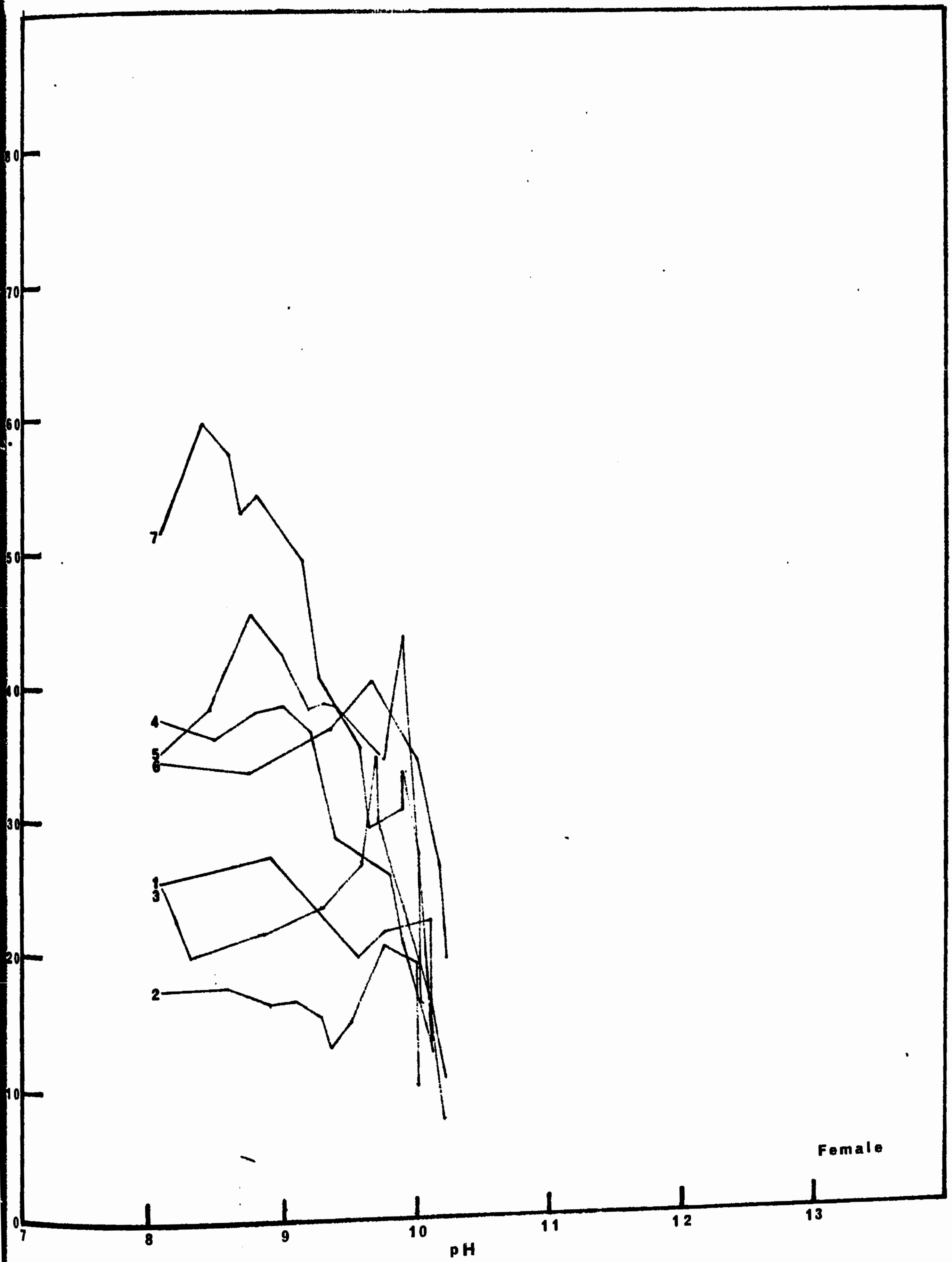

80
70
60
50
40
30
20
10
0
7
5
3
6
2
4
1
Male
8
9
10
11
12
13
pH

Figure 10. Relationship between oxygen consumption by female *Illex illecebrosus* and increasing pH of the sea water. (Temperature 10-12°C). (Each line represents a single individual.)


80
70
60
50
40
30
20
10
0
7
8
9
10
11
12
13
pH
7
4
5
6
1
3
2
Female

been reached, a further decrease in the pH resulted in a sharp decrease in consumption until death occurred.

When the pH was increased above 8 (normal) the oxygen consumption tended to increase up to a pH of approximately 9. It then declined between pH 9 and 9.5, again increasing between pH 9.5 and 10. Thereafter it decreased sharply until death occurred at pH values between 10 and 10.2.

Data on the pH experimentation is tabulated in Appendix D.

(V) The Absolute Utilization of Oxygen

The absolute utilization of oxygen (Winterstein, 1925) was determined by calculating the difference in oxygen content between the inhalent and exhalent water. This was done for the ten squid studied by the tube implantation method.

The results are presented in Appendix E.

The relationship between the absolute utilization coefficient of oxygen and body weight was calculated. The correlation coefficient, however, indicated that no significant relationship existed at the 0.05 level. The mean absolute oxygen utilization coefficient value was calculated to be 19.53% ± 5.66%.

(VI) Volume of Exhalent Water

Absolute values for the volume of exhalent water were determined for the ten squid studied by the tube implantation method.

The values obtained indicated no apparent correlation between body weight and the volume of water expelled over one-hour periods. The mean volume of exhalent water was calculated to be 35.62 litres per hour. The complete data are tabulated in Appendix F.

DISCUSSION

The apparent parabolic relationship found between oxygen consumption and body weight conforms well to the views of Zeuthen (1947) who described this relationship as a general rule in aquatic animals. Maginniss & Wells (1969) using a sealed chamber method reported a parabolic respiration relationship for *Octopus cyanea*. They reported a slope of 0.833 for a temperature range of 24.5°C - 27.5°C. The slope obtained in the present work for combined sexes is relatively similar (0.874) to that of Maginniss & Wells. However, the validity of this comparison could be rather dubious when the temperatures of the two experiments are taken into consideration. The temperatures for the experiments with *O. cyanea* were 24.5°C - 27.5°C, while those for *I. illecebrosus* were 10°C - 12°C. The effect of temperature on the slope of the line for oxygen consumption and body weight for cephalopods is not known.

For fish, however, there are conflicting views. Job (1954) reported that the relationship of body weight to standard metabolism could be discussed independently of temperature. On the other hand, Prosser & Brown (1961) state that in the tropical fish *Etroplus* sp. the slope of the line is higher at low temperatures ($b = 1$ at 0°C) and lower at high temperatures ($b = 0.67$ at 35°C).

One surprising fact which has emerged from this study is the fact that there is no correlation between total metabolic rate and body weight which is rather significant a departure from the expected inverse relationship. Maginniss & Wells (1969) reported a slope of 0.167 and a highly significant correlation coefficient of -0.888 in this relationship for *O cyanea*. Further analysis of data presented by Montuori (1913) dealing with *Sepia officinalis* revealed a similar situation to that found in the present study in that the correlation between total metabolic rate and body weight was also not significant ($P \geq 0.05$).

In both the present work and from the further analysis of data from Montouri (1913), although the regression lines are not significantly different from zero, the calculated slopes both tend to be negative.

Up to the present time, no truly acceptable explanation has been brought forward to explain the general phenomenon of decreasing metabolic rate with increased body weight (Prosser & Brown, 1961). An explanation seemingly fitting one group of animals is not supported by that of another group, and thus several explanations have been put forward to explain this trend. Zeuthen (1953) states that animals are of two types:

(1) animals can be small, grow rapidly and live intensely; or

(2) they can be large, grow slowly and metabolize slowly.

Another explanation is that as an animal becomes larger, there is a disproportionate increase in the tissues with a low metabolic rate. Nevertheless, the most probable explanation to this observed lack of correlation is that the individual variation in metabolic rate of these squid is sufficiently large so as to effectively mask any real correlation with body weight.

In order to properly study this relationship, it would be necessary to examine the rates of oxygen consumption of individual squid over a significant portion of their life cycle. Thus it would be possible to examine the true relationship between body weight and rate of oxygen consumption. At the present time, however, this is impractical since it has not been possible to date or maintain *I. illecebrosus* over sufficiently long periods of time to attempt such a study.

Within the invertebrates, the molluscs may be regarded as more diverse than others with regard to metabolic rate. Even within a single species it is known to vary greatly, especially in intertidal bivalves such as *Mytilus californianus* Conrad in which metabolic rate has been demonstrated to be correlated to tidal rhythms, even when maintained under constant laboratory conditions throughout an experiment (Rao, 1953). *Illex illecebrosus* in fact probably like most cephalopods, is not influenced by this tidal factor. However, they are most likely influenced by other factors such as light, temperature, salinity, pH and other physical-chemical factors.

Clausen (1936) stated that the fish with higher metabolic rates living in fast currents do not exhibit diurnal rhythms and, conversely, those exhibiting diurnal rhythms in oxygen consumption are not found in fast currents. The situation found in *I. illecebrosus* could thus be interpreted as analogous to this.

It is very difficult to compare the salinity-oxygen consumption relationships found in this study with those reported in the literature for other animals, due to the different methods employed.

The majority of previous studies (Job, 1969; McLusky, 1969) have been concerned with the effect of salinity on oxygen consumption, however the animals were acclimated at each salinity level. This would be extremely difficult to accomplish with this squid due to its high oxygen consumption plus the problem of keeping salinity constant during the acclimation of an extremely active pelagic species like *I. illecebrosus*.

Raffy & Ricart (1939) found that *S. officinalis* and *O. vulgaris* apparently had no osmoregulatory mechanism and that the oxygen consumption rate was constant in both high and low salinities. Unfortunately, their experimental method and the physical conditions under which their studies were conducted were not reported. The duration of their experiments was six days and thus some acclimation would probably be involved, hence the validity of comparison with the results reported herein would be at best dubious.

Kawamoto (1969) investigated 14 species of fish and concluded that on the basis of oxygen consumption in relation to salinity, the fish, could be divided into two major groups: one euryhaline, the other stenohaline. The euryhaline forms were those inhabiting brackish or coastal waters, or were anadromous or catadromous. The stenohaline forms were those which in general are totally oceanic throughout their life cycle. He also pointed out that the oxygen consumption of oceanic species would decrease after exposure to less saline water for varying periods of time, whereas in coastal forms oxygen consumption would decrease during the reduction of salinity. There was, however, no effect on brackish water forms. Maloeuf (1937) reported that the oxygen consumption of stenohaline animals decreases with an abrupt decrease in salinity.

In general, cephalopods are regarded as being strictly stenohaline forms, the majority of which are nektonic and generally highly mobile, thus having the ability to avoid areas or zones that have unfavourable salinity conditions. *Illex illecebrosus*, however, presents a somewhat different picture in that presumably it migrates from oceanic areas to coastal areas, especially around insular Newfoundland (Squires, 1957, 1959). This area has a relatively lower salinity (31 - 32 $^{o}/_{oo}$), when the squid are in Newfoundland embayments than adjacent oceanic areas (33 - 34$^{o}/_{oo}$). They could thus be regarded as an intermediate form between the truly oceanic (stenohaline) and coastal (euryhaline) animals. Bearing this in mind, the results of this study indicate a directly proportional relationship between oxygen consumption and salinity. Those results would thus support the findings of Kawamoto (1969) and Maloeuf (1937).

It is nevertheless still difficult to explain the decrease in oxygen consumption with decrease in salinity. As has previously been mentioned, the squid have little osomoregulatory ability. Therefore, it would seem probable that, if the salinity is decreased this would result in some form of osmotic stress to the animals, hence causing a degree of excitation and thus increase oxygen consumption. If, however, an osmotic stress had a narcotizing effect, oxygen consumption would be predictably reduced. This situation would, upon cursory examination, appear to put the animal in a very unfavourable position from the point of view of survival and natural selection.

It would thus appear obvious that much work has yet to be accomplished in order to glean an adequate understanding of both the mechanisms involved in regulating metabolism in relation to salinity change and also the energetics of ion transportation and the physiological effect of osmotic change in forms which are capable of only very limited osmoregulation.

Kawamoto (1969) reported the effect of pH on the respiration of various species of fish. He found that oxygen consumption exhibited irregular patterns with increasing pH, a finding similar to that of this study. He concluded that there was no specific pattern or trend relating oxygen consumption to increasing pH. It must be pointed out at this juncture that he increased the pH by adding ammonium hydroxide solution to the sea water. The addition of this chemical solution, which has a low coefficient of dissociation, would act as an irritant to the experimental subject and be a causal factor in the initiation of numerous abnormal behavioural or physiological responses resulting in the irregular patterns reported.

In the results reported herein, there was an initial period of increase in oxygen consumption with increasing pH and an initial period of decrease with declining pH. The initial increase with increasing pH could be explained by the fact that the sodium ion concentration was increasing due to the addition of the sodium hydroxide solution. This could be a cause of osmotic stress which might serve to excite the squid and thus cause an increase in oxygen consumption. However, as the pH increases, the oxygen-carrying capacity of the squid's hemocyanin would be reduced.

Since Robertson (1964) considers that the integument of cephalopods is probably permeable to water to a considerable degree, it is not beyond speculation that changes in the external pH would bring about changes in the pH of the internal circulating medium. It must be pointed out that to date, no experimentation on this aspect has been undertaken. However, such a mechanism would be of great significance, since according to Redfield & Goodkind (1929) haemocyanin is so sensitive to pH change that an increase in acidity of 0.13 pH units causes the release of from one-quarter to one-third of the total oxygen carried by the pigment in *Loligo pealei* Lesueur.

If the foregoing can be considered to be valid, such an increase in pH would tend to lower the oxygen consumption, unless there was a corresponding increase in the squid's ventilation rate. The latter would require more energy and could thus, in fact, increase the oxygen consumption. This initial increase is followed by a general decrease and then by another peak, followed by a sharp decline. The second peak corresponds rather closely to the point during the experiments at which magnesium hydroxide was observed to be precipitated. This could possibly account for the final decrease due to osmotic stress caused by the removal of Mg^{++} from the squid's environmental media.

When pH was decreased the general initial decrease in oxygen consumption might be expected also on the basis of the altered oxygen-carrying capacity of the haemocyanin, again referring to the work of Redfield & Goodkind (1929) on *L. pealei*. The increase may be either a mechanical (chemical) response to the hydrochloric acid or, possibly as osmotic response to increasing Cl^- concentrations, both of which could cause excitation and a subsequent increase in oxygen consumption. The final rapid decline leading to death could have been caused when the pH reached, or was near, the lethal point. This would be supported by observations on the ventilation rate, which became increasingly rapid during the period of oxygen consumption increase and was maintained at that rate until immediately prior to death.

Another explanation might be advanced to explain the initial decrease and the following rise with decreasing pH. When the H^+ concentration in the blood was increased, oxygen consumption would initially decrease due to the Bohr Effect. During this period there would be an accumulation of CO_2 which would stimulate respiratory centres and cause an increase in oxygen consumption. This explanation would, however, necessitate a threshold stimulus level of sensitivity of the respiratory centre and not a continuously compensating system as is found in vetebrates. The existence of such respiratory centres in the central nervous system of cephalopods has not as yet been demonstrated. However, in octopods there is a centre controlling inspiratory and expiratory phases of the mantle in the visceral ganglion (Hanstrom, 1928; Kuhlenbeck, 1967) and in the motor fibres of the magnocellular lobe of the central nervous systems in *Loligo vulgaris* Lamarck (Young, 1936, 1938, 1939).

The utilization coefficient of oxygen of *I. illecebrosus* is much lower than the values given by other authors working on octopods. Winterstein (1925) reported a mean value for *Octopus vulgaris* of 70%, whereas Hazelhoff (1939) found that the same species had values of at least 50%, but could exceed 80% in case of partial asphyxiation. Johanson (1965) reported a value of 35% for *O. dofleini* (Wülker, 1910), but in 1966 he reported for the same species an average utilization coefficient of 27%. He indicated that the lower oxygen extraction could most likely be related to a difference in temperature.

The temperatures at which the present experiments were performed were in the same ranges as those used by Johanson. The squid are, however, generally much more active animals than are octopods and thus it might be expected that the absolute oxygen utilization would be higher. The lower value obtained is probably due to a much more rapid ventilation rate in the squid than in the octopods, enabling the maintenance of a relatively higher activity.

CONCLUSIONS

1. The oxygen consumption of *Illex illecebrosus* in captivity (10 - 12°C) is significantly proportional to body weight of the squid.

2. There is no significant difference between the sexes in regard to oxygen consumption and body weight relationships.

3. The rate of oxygen consumption is not significantly correlated with body weight.

4. There is no evidence of a diurnal rhythm in oxygen consumption.

5. There is proportional relationship between oxygen consumption and decreasing salinity values.

6. Increased pH values cause either directly or indirectly, an initial increase in oxygen consumption.

7. Decreasing pH values cause either directly or indirectly,an initial decrease in oxygen consumption.

8. The pH values of 5 and 10 appear to be lethal.

9. The relationships between body weight, muscular development of the mantle, the ventilation rate and the oxygen-carrying capacity of haemocyanin and oxygen consumption shoudl be further researched.

10. Although the mean volume of exhalent water was calculated to be 32.62 litres per hour, the squid apparently utilizes only 19.53% of the available dissolved oxygen in the sea water.

REFERENCES CITED

BARNES, H. 1959. Apparatus and Methods of Oceanography, Part One: Chemical. George Allen and Unwin Ltd., London. 341 pages.

BRADBURY, Helen E. and F. A. ALDRICH. 1969a. Observations on locomotion of the short-finned squid, *Illex illecebrosus illecebrosus* (Lesueur, 1821), in captivity. Can. J. Zool., *47*(5): 741-744.

_____. 1969b. Observations on feeding of the squid, *Illex illecebrosus illecebrosus* (Lesueur, 1821), in captivity. Can. J. Zool., *47*(5): 913-915.

CLAUSEN, R. G. 1936. Oxygen consumption in fresh water fishes. Ecol., *17*: 216-226.

FRY, F. E. J. 1957. The aquatic respiration of fish. In: Physiology of Fishes (edited by Brown, M. E.), Academic Press Inc., New York. Vol. 1, 407 pages.

_____ and J. S. HART. 1948. Cruising speed of goldfish in relation to water temperature. J. Fish. Res. Bd. Can., *7*: 169-175.

GRAHAM, J. M. 1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, *Salvelinus fontinalis*. Can. J. Research Sec., D 27: 270-288.

HANSTRÖM, B. 1928. Vergleichende Anatomie des Nervensystems der wirbellosen Tiere unter Berücksichtigung seiner Funktion. Springer, Berlin. (Original not seen. Quoted from Kuhlenbeck, 1967).

HAZELHOFF, E. H. 1939. Über die Ausnutzung des Sauerstoffs bei verschiedenen Wassertieren. Z. vergl. Physiol., *26*: 306-327.

JOB, S. V. 1954. The oxygen consumption of the speckled trout (*Salvelinus fontinalis*). Ph.D. Thesis, University of Toronto (Department of Zoology). (Interlibrary loan).

_____. 1969. The respiratory metabolism of *Tilapia mossambica* (Teleostei). I. The effect of size, temperature and salinity. Mar. Biol., *2*: 121-126.

JOHANSEN, K. 1965. An apparatus for continuous recording of respiratory exchange in a cephalopod. Comp. Biochem. Physiol., 14: 377-381.

_____, and C. LENFANT. 1966. Gas exchange in the cephalopod, Octopus dofleini. Am. J. Physiol., 210(4): 910-918.

KAWAMOTO, N. Y. 1969. Physiology and Ecology of Fishes (Revised edition). Koseisya Publ. Ltd., Toyko, Japan. 374 pages. (Translated from the Japanese by M. Kao, 1969).

KEYS, A. B. 1930. The measurement of the respiratory exchange of aquatic animals. Biol. Bull., 59: 187-198.

KUHLENBECK, H. 1967. Invertebrates and Origin of Vertebrates. Vol. 2 of: The Central Nervous System of Vertebrates. Academic Press Inc., New York. 364 pages.

KUKUBO, S. 1962. Marine Biology. Koseisya Publ. Ltd., Toyko, Japan. 394 pages. (Translated from the Japanese by M. Kao, 1969).

MAGINNISS, L. A. and M. J. WELLS. 1969. The oxygen consumption of Octopus cyanea. J. exp. Biol., 51: 607-613.

MALOEUF, N. S. R. 1937. Studies on the respiration of animals. II. Aquatic animals having in their internal environment an oxygen carrier. Z. vergl. Physiol., 25: 29-42.

MCLUSKY, D. S. 1969. The oxygen consumption of Corophium volutator in relation to salinity. Comp. Biochem. Physiol., 29: 743-754.

MONTUORI, A. 1913. Les processus oxydatifs chez les animaux marins en rapport avec la loi de superficie. Arch. ital. biol., 59: 213-234.

PROSSER, C. L. and F. A. BROWN, Jr. 1961. Comparative Animal Physiology. 2nd ed. W. B. Saunders Co., Philadelphia, Pa. 688 pages.

QUIGLEY, J. J. 1964. Mechanized squid jigger. Trade News, Ottawa, 17(5): 3-5.

RAFFY, A. and R. RICART. 1939. Influence des variations de salinité sur la consommation d'oxygene par les Céphalopodes. C. R. Séanc. hebd. Acad. Sci. Paris, 208: 671-673.

RAO, K. P. 1953. Rate of water propulsion in Mytilus californianus as a function of latitude. Biol. Bull., 104: 171-181.

REDFIELD, A. C. and R. GOODKIND. 1929. The significance of the Bohr effect in the respiration and asphyxiation of the squid, Loligo pealei. J. exp. Biol., 6: 340-349.

ROBERTSON, J. D. 1964. Osmotic and Ionic Regulation. In: Physiology of Mollusca. (Edited by Wilbur, K. M. and C. M. Yonge). Vol. 1. Academic Press, Inc., New York. pp. 283-308.

SQUIRES, H. J. 1957. Squid, Illex illecebrosus (Lesueur) in the Newfoundland fishing area. J. Fish. Res. Bd. Can., 14(5): 693-728.

_____. 1959. Squid inshore in Newfoundland and on the Grand Banks, 1953 to 1958. Progress Reports, Atlantic Coastal Stations, Fish. Res. Bd. Can., 72: 23-26.

STRICKLAND, J. D. H. and T. R. PARSONS. 1968. A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Bd. Can., No. 167, 311 pages.

SMITH, R. I. and A. E. KAMMER. 1968. Laboratory Exercises in Invertebrate Physiology, 3rd ed. Burgess Publ. Co., Minneapolis, Minn. 219 pages.

WINBERG, G. G. 1956. (Rate of metabolism and food requirements of fishes). Nauchu. Tr. Belorussk. Gos. Univ. Lenina, Minskl. 253 pages. (Fish. Res. Bd. Can., Transl. Ser. No. 194).

WINTERSTEIN, H. 1925. Über die chemische Regulierung der Atmung bei der Cephalopoden. Z. vergl. Physiol., 2: 315-328.

YOUNG, J. Z. 1936. The giant-nerve fibres and epistellar body of cephalopods. Quart. J. Micr. Sci., 78: 367-386.

_____. 1938. The functioning of the giant nerve fibres in the squid. J. exp. Biol., 15: 170-185.

_____. 1939. Fused neurons and synaptic contacts in the giant nerve fibres of cephalopods. Phil. Trans. R. Soc. B 229: 465-503.

ZEUTHEN, E. 1947. Body size and metabolic rate in the animal kingdom. Compt. rend. lab. Carlsberg. Ser. Chim., 26: 17-161.

_____. 1953. Oxygen uptake as related to body size in organisms. Quart. Rev. Biol., 28: 1-12.

ZUEV, G. V. 1970. Squid locomotion. Manuscript of chapter for book, Biology of Squid. Edited by F. A. Aldrich. John Wiley & Sons, Inc., New York. In preparation.

APPENDIX A

Oxygen Consumption, Oxygen Consumption Rate, and Body Weight (♀)

Oxygen Consumption (Y'b) (ml/hr.)	Oxygen Consumption Rate (Y'a) (ml/g/hr.)	Body Weight (X) (g.)
16.3068	0.1527	106.80
20.4949	0.1573	130.30
16.1944	0.1178	137.50
36.2029	0.2502	144.70
41.0429	0.2690	152.60
32.4107	0.2046	158.40
35.3068	0.2006	176.00
25.2857	0.1422	177.80
36.9143	0.2045	180.50
38.0919	0.1889	201.55
41.0305	0.1965	208.80
40.0000	0.1889	212.99
49.3772	0.2318	213.00
72.1458	0.3355	215.05
34.5555	0.1592	217.00
36.5983	0.1614	226.75
56.1689	0.2279	246.50
86.2456	0.3478	247.95
42.7187	0.1711	249.70
53.2721	0.2131	250.00
31.0925	0.1216	255.60
48.4507	0.1883	257.35
36.7669	0.1411	260.55
47.7884	0.1817	263.00
62.0687	0.2347	264.50
66.4494	0.2510	264.70
34.6359	0.1307	265.05
40.7129	0.1532	265.70
42.6467	0.1495	285.30
52.5711	0.1431	367.50
59.7229	0.1584	377.10
61.0416	0.1456	431.20
64.1611	0.1408	455.80
108.9232	0.2301	473.30

Regression Line: Log Ya = 0.9136 Log X -0.5312 r=0.7650

Log Y'a=-0.0820 Log X -0.5414 r=-0.05838

Regression Line for combining sexes: Log Yc = 0.8738 Log X -0.4584 r = 0.7524

Log Y'c= 0.1238 Log X -0.4640

APPENDIX A

Oxygen Consumption, Oxygen Consumption Rate, and Body Weight (♂)

Oxygen Consumption (Yb) (ml/hr.)	Oxygen Consumption Rate (Y'b) (ml/g/hr.)	Body Weight (X) (g.)
24.6869	0.2758	89.50
36.8379	0.3991	92.30
15.8209	0.1311	120.70
17.7557	0.1188	149.40
20.6054	0.1245	165.45
25.5404	0.1527	167.30
27.1722	0.1604	169.40
30.4246	0.1760	172.90
36.2388	0.2009	180.40
41.6075	0.2088	199.30
23.8913	0.1190	200.70
22.3482	0.1110	201.30
28.0477	0.1326	211.60
33.5657	0.1546	217.50
34.1864	0.1558	219.40
40.6168	0.1727	235.20
48.2487	0.1969	245.00
30.9134	0.1162	266.10
42.1067	0.1577	267.00
51.8862	0.1926	269.40
55.5242	0.2041	272.00
43.7736	0.1423	307.55
56.0223	0.1787	313.50
55.4035	0.1737	319.00
64.6135	0.1830	353.15
62.0749	0.1723	360.20

Regression Line: $\log Y_b = 0.7755 \log X - 0.2582$ $r = 0.7358$

$\log Y'_b = -0.2243 \log X - 0.2587$ $r = 0.2723$

APPENDIX B

Diurnal Rhythm Data

Body Weight	Sex	Time	Oxygen Consumption (ml/hr.)
180.40 g.	M.	00:40	
		01:40	22.8877
		02:40	23.8413
		03:40	20.0267
		04:40	20.0267
		05:40	22.8877
		06:40	24.7949
		07:40	24.2228
		08:40	25.1764
		09:40	27.3614
		10:40	28.6096
		11:40	29.5632
		12:40	27.3614
		13:40	28.9136
		14:40	25.1764
		15:40	26.3412
		16:40	29.5632
		17:40	29.5632
		18:40	27.6559
		19:40	23.2691
		20:40	22.1247
		21:40	20.7896
		22:40	22.1247
		23:40	23.8758
		00:40	22.8877
201.30 g.	M.	20:45	
		21:45	20.3081
		22:45	22.5646
		23:45	24.0689
		00:45	25.3072
		01:45	27.8297
		02:45	24.0689
		03:45	22.5646
		04:45	21.6244
		05:45	22.5646
		06:45	21.2484
		07:45	23.1287
		08:45	22.0005
		09:45	20.6842
		10:45	22.1885
		11:45	22.5646
		12:45	22.0005

APPENDIX B (continued)

Body Weight	Sex	Time	Oxygen Consumption (ml/hr.)
		12:45	22.0005
		13:45	21.4364
		14:45	20.3081
		15:45	20.3081
		16:45	22.1885
		17:45	24.0689
		18:45	22.5646
		19:45	23.5048
		20:45	19.7440
313.50 g.	M.	18:00	
		19:00	56.0761
		20:00	56.8098
		21:00	54.0938
		22:00	53.4607
		23:00	56.4253
		24:00	57.6915
		01:00	59.8927
		02:00	59.1590
		03:00	56.9933
		04:00	56.4961
		05:00	56.4961
		06:00	56.4961
		07:00	58.6973
		08:00	59.8927
		09:00	60.1647
		10:00	60.5315
		11:00	60.5315
		12:00	58.8807
		13:00	57.4133
		14:00	56.8630
		15:00	55.9458
		16:00	59.2476
		17:00	57.0522
		18:00	55.7505
264.70 g.	F.	19:45	
		20:45	54.3067
		21:45	60.2862
		22:45	66.3803

APPENDIX B (continued)

Body Weight	Sex	Time	Oxygen Consumption (ml/hr.)
		22:45	66.3803
		23:45	62.6169
		00:45	59.1709
		01:45	52.6632
		02:45	53.3693
		03:45	94.9129 *
		04:45	74.9389
		05:45	73.0916
		06:45	72.7410
		07:45	70.5242
		08:45	67.8979
		09:45	68.0332
		10:45	67.2971
		11:45	64.2559
		12:45	61.5799
		13:45	65.6846
		14:45	72.4523
		15:45	55.7258
		16:45	66.0335
		17:45	81.8351 *
		18:45	74.5832
		19:45	70.4551
377.10 g.	F.	17:50	
		18:50	42.2346
		19:50	43.1332
		20:50	44.3912
		21:50	40.4373
		22:50	38.2807
		23:50	53.0178 *
		00:50	53.9165 *
		01:50	42.2346
		02:50	45.8290
		03:50	44.7507
		04:50	49.6031
		05:50	48.8842
		06:50	39.5387
		07:50	37.2024
		08:50	36.3037
		09:50	56.6123 *
		10:50	65.2389 *

APPENDIX B (continued)

Body Weight	Sex	Time	Oxygen Consumption (ml/hr.)
		11:50	48.5248
		12:50	39.8982
		13:50	37.3821
		14:50	41.8751
		15:50	46.7276
		16:50	47.6262
		17:50	49.4234

* Denotes that the squid was moving in the respirometer during this period.

APPENDIX C

Salinity Of Sea Water, Body Weight and Oxygen Consumption

No. of Squid	Body Weight (g.)	Sex	Oxygen Consumption (Y) (ml/hr.)	Salinity (X) $^{o}/_{oo}$
1.	200.70	M.	20.5779	30.95
			21.4498	29.47
			21.4498	27.92
			27.0303	25.93
			30.3437	22.48
			19.0084	19.89
2.	212.99	F.	34.3115	31.11
			35.7562	29.00
			33.7698	27.48
			28.3522	25.50
3.	235.20	M.	37.9090	31.18
			34.2986	30.93
			33.3960	30.68
			32.4934	30.25
			34.2986	30.25
			35.2012	29.99
			33.3960	29.47
			33.3960	28.10
			32.4934	25.07
			28.3415	20.75
			17.5103	16.00
4.	247.95	M.	85.7487	31.11
			85.3876	29.00
			83.4019	27.48
			82.4992	26.36
5.	264.50	F.	57.8907	30.95
			58.7925	29.47
			58.7925	27.92
			53.7428	25.93
			46.8897	22.48
			27.0518	20.23
6.	319.00	M.	58.4337	30.95
			62.9565	29.81
			60.6047	28.10
			47.9410	27.23
			23.5182	22.92

APPENDIX C (continued)

No. of Squid	Body Weight (g.)	Sex	Oxygen Consumption (Y) (ml/hr.)	Salinity (X) $^{o}/_{oo}$
7.	431.20	F.	58.6966	31.73
			60.4229	29.38
			56.1070	27.92
			53.5175	25.41
			52.6543	23.51
			50.9279	20.93
			50.9279	18.86
			47.4752	17.12
8.	455.80	F.	56.4546	31.73
			55.5585	29.38
			51.0779	27.92
			49.2858	25.41
			48.3897	23.51
			42.1169	20.93
			25.9870	18.86

The regression line and correlation coefficients for oxygen consumption and salinity of sea water.

No. of Squid	Regression Line	Correlation Coefficient (r)($p \geq 0.05$)
1.	$Y=29.7818-0.2479\ X$	0.2396
2.	$Y= 3.9231+1.0655\ X$	0.7803
3.	$Y= 4.6943+0.9948\ X$	0.9212
4.	$Y=63.8339+0.7170\ X$	0.9434
5.	$Y=-19.3476+2.6707\ X$	0.8939
6.	$Y=-84.8460+4.875\ X$	0.9277
7.	$Y= 34.4965+0.7942\ X$	0.9524
8.	$Y=-4.5250+2.0285\ X$	0.9009

APPENDIX D

The Decreasing pH Of The Sea Water and Oxygen Consumption

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
1.	120.70	M.	16.7904	8.10
			16.7904	7.30
			17.5450	6.90
			14.9319	6.35
			13.9986	6.15
			15.6705	6.08
			17.1717	5.89
			19.5981	5.60
			22.3979	5.00
			11.7581	4.69
2.	149.40	M.	20.8565	8.00
			19.5645	7.35
			18.0879	6.90
			18.4571	6.50
			26.3936	6.30
			29.1621	6.15
			25.8399	5.90
3.	167.30	M.	29.2230	8.00
			27.7975	7.50
			31.8958	6.90
			33.4995	6.50
			31.7176	6.10
			26.7284	5.75
			15.1461	5.45
4.	199.30	M.	34.3021	8.00
			28.3688	7.48
			30.2229	7.00
			33.0042	6.70
			30.0376	6.55
			21.1375	6.30
			09.6417	6.15

APPENDIX D (continued)

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
5.	211.60	M.	28.6924	7.80
			26.6909	7.10
			24.0290	6.75
			30.2507	6.60
			33.4499	6.40
			32.2166	6.25
			32.3956	6.00
			34.1746	5.80
			32.5726	5.40
			20.8251	5.20
6.	217.05	M.	25.8884	8.00
			22.8532	7.35
			24.6387	6.75
			25.5314	6.40
			26.9597	6.00
			22.8532	5.65
			11.6052	5.15
7.	219.40	M.	34.6679	8.00
			31.7789	7.00
			27.9269	6.60
			34.6679	6.20
			33.7049	5.80
			15.4080	5.40
8.	269.40	M.	59.1628	7.80
			41.1339	7.20
			43.4094	6.80
			76.6666	6.60
			89.2694	6.40
			63.8889	6.25
			46.2100	6.05
			41.8341	5.80
			39.2085	5.50
			25.0304	5.30

APPENDIX D (continued)

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
9.	307.55	M.	42.1784	8.00
			40.7606	7.35
			36.3301	7.00
			38.9884	6.55
			53.6977	6.30
			60.7865	6.15
			58.4826	5.90
			64.6853	5.50
			59.3687	5.40

APPENDIX D

The Decreasing pH Of The Sea Water and Oxygen Consumption

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
1.	106.80	F.	14.7769	8.10
			13.5600	7.60
			15.6461	6.80
			18.2538	6.50
			22.9477	6.30
			22.2523	6.15
			20.1661	5.90
			14.7769	5.70
2.	176.00	F.	32.4621	8.10
			28.7092	7.45
			29.0845	6.60
			34.7138	6.45
			40.3430	6.25
			36.7778	6.10
			29.2722	5.90
			18.7642	5.70
3.	215.05	F.	58.2476	7.95
			64.0723	6.80
			57.2768	6.15
			32.0362	5.05
4.	226.75	F.	42.2678	8.00
			39.9831	7.40
			36.7463	6.80
			41.5062	6.45
			50.4548	5.80
			31.4153	5.60
5.	249.70	F.	40.2402	8.00
			32.0755	7.40
			30.1315	7.00
			32.4643	6.70
			34.7971	6.50
			38.6850	6.30
			39.2682	6.10
			39.2682	6.00
			32.8531	5.80
			16.9125	5.30

APPENDIX D (continued)

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
6.	257.35	F.	42.1331	8.00
			42.1331	7.20
			40.8781	6.65
			45.3603	6.35
			39.4437	6.00
			17.0330	5.70
7.	260.55	F.	36.9089	8.00
			37.8553	7.80
			36.9089	7.30
			36.9089	6.70
			37.8553	6.40
			38.4231	6.20
			42.7765	6.10
			31.7985	5.90
			21.7668	5.70
8.	265.05	F.	30.2606	7.95
			32.0946	6.60
			36.6795	6.05
			25.6757	4.80

APPENDIX D

The Increasing pH Of The Sea Water and Oxygen Consumption

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
1.	165.45	M.	20.1349	8.10
			23.5221	8.70
			23.5221	8.90
			22.5812	9.10
			21.6400	9.35
			19.7585	9.35
			18.8177	9.55
			24.4629	9.80
			25.4038	10.00
			20.6994	10.00
			17.3122	10.00
			11.6669	10.20
2.	169.40	M.	25.9778	8.00
			27.2319	8.50
			27.7694	8.70
			26.8736	8.93
			26.8736	9.20
			25.4404	9.30
			26.8736	9.60
			29.7401	9.68
			30.8151	9.90
			23.2905	9.90
			15.2284	10.00
3.	172.90	M.	33.6981	8.00
			39.7069	8.70
			37.5493	9.20
			37.5493	9.60
			37.3567	9.70
			29.6543	9.80
			17.3304	10.00
			07.3173	10.20

APPENDIX D (continued)

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
4.	201.30	M.	21.0325	8.10
			20.0936	8.20
			17.6524	8.30
			17.6524	8.70
			18.7791	9.25
			15.9622	9.50
			17.2768	9.60
			15.3989	9.70
5.	245.00	M.	57.2764	7.95
			57.6557	8.55
			45.8969	8.80
			40.8142	9.00
			39.8279	9.20
			38.8797	9.50
			29.7761	9.70
			16.5002	10.10
			13.2760	10.20
6.	266.10	M.	32.5853	8.00
			34.2611	8.70
			34.0749	9.15
			33.5163	9.20
			38.1713	9.40
			43.1907	9.70
			36.3093	10.00
			31.5000	10.20
7.	353.15	M.	57.9829	8.00
			57.4342	8.50
			61.6412	8.80
			68.5918	9.00
			69.5064	9.20
			60.3608	9.40
			51.9469	9.65
			42.8013	10.00
			28.3513	10.20

APPENDIX D

The Increasing pH Of The Sea Water and Oxygen Consumption

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
1.	130.30	F.	25.5006	8.10
			27.3895	8.90
			19.8338	9.55
			21.7227	9.75
			22.2894	10.10
			15.6781	10.10
			07.5557	10.20
2.	137.50	F.	17.5668	8.10
			17.7498	8.60
			16.4689	8.90
			16.8348	9.10
			15.3710	9.30
			13.1751	9.35
			15.0050	9.50
			20.4947	9.75
			19.2137	10.00
			10.0640	10.00
3.	177.80	F.	25.2857	8.10
			22.4123	8.20
			20.1136	8.30
			21.8377	8.85
			23.7532	9.30
			26.8182	9.60
			34.8636	9.70
			30.0747	9.70
			16.6660	10.10
			10.9188	10.20
4.	201.55	F.	37.6217	8.10
			36.1168	8.50
			37.9979	8.80
			38.5622	9.00
			36.6812	9.20
			32.5428	9.30
			28.7806	9.40
			25.9589	9.80
			20.8800	9.90
			12.4152	10.15

APPENDIX D (continued)

No.	Body Weight (g.)	Sex	Oxygen Consumption (ml/hr.)	pH
5.	208.80	F.	35.1433	8.10
			38.2071	8.45
			45.4159	8.75
			42.3522	8.99
			38.2071	9.20
			38.7477	9.30
			38.3873	9.40
			34.7828	9.75
			43.4335	9.90
			13.6969	10.10
6.	265.70	F.	34.6493	8.10
			33.7374	8.75
			36.8376	9.35
			40.1202	9.65
			34.2845	10.00
			26.4429	10.15
			19.5130	10.20
7.	367.50	F.	51.3403	8.10
			59.7798	8.40
			57.3183	8.60
			53.0985	8.70
			54.1535	8.80
			49.5821	9.15
			40.9668	9.28
			35.1646	9.58
			29.3624	9.62
			30.7690	9.90
			33.4064	9.90
			27.2526	10.00
			15.9999	10.00

APPENDIX E

Utilization Coefficient and Body Weight

	Utilization Coefficient (Y) (%)	Body Weight (X) (g.)
	11.31	158.40
	26.03	263.00
	23.94	273.00
	19.21	267.00
	12.27	217.30
	18.07	144.70
	23.88	285.30
	27.18	250.00
	19.02	152.60
	14.35	246.50
average	19.53 ± 5.66	225.78

Correlation coefficient between body weight and utilization coefficient r=0.559 (n=10)

APPENDIX F

Volume Of Exhalent Water and Body Weight

	Body Weight (g.)	Volume (l/hr.)
	144.70	29.0200
	152.60	34.5140
	158.40	40.9230
	217.30	39.8600
	246.50	53.4360
	250.00	32.0008
	263.00	29.9220
	267.00	34.8600
	272.00	34.2500
	285.30	27.4300
average	225.68	35.62158