On the Biology of *Calanus finmarchicus*. Part VI. Oxygen Consumption in Relation to Environmental Conditions.

By

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With 7 Figures in the Text.

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INTRODUCTION.

VERY few observations have hitherto been made on the respiration of marine copepods and apart from the work by Pütter (1909, 1922, 1923, 1924–25) and a single observation by Ostenfeld (1913) little is known. Pütter used mixed plankton catches containing several species of copepods and other organisms in addition to phytoplankton and bacteria. By comparing the results of a number of experiments with different quantities of plankton he was able to calculate the oxygen requirements of the copepods which he divided into three groups, small, medium and large. Subsequent work, however, has shown that his results are too high.

The object of the experiments recorded in the present paper was primarily to find the food requirements of *Calanus finmarchicus*. In addition, the influence on respiration was investigated of those conditions, which, in the sea, are subject to seasonal change. As a rule, the range of variation studied was not much beyond that which actually occurs in the sea.

Where Calanus is mentioned in this paper it should be understood that *C. finmarchicus* is referred to.

METHODS.

The Calanus were taken, usually in deep water, off Garroch Head by a stramin net and the catch, suitably diluted, brought back to the laboratory in large glass jars. They were usually picked out at once, separated into males, females and Stage V and put into sea-water filtered through a coarse sintered glass filter. Males were sometimes scarce and could not always be got in sufficient numbers. No work was done on younger stages. The three classes can be distinguished by the naked eye and for the majority of the experiments they were picked out by eye examination and checked with the aid of a lens.

As a rule 120 Calanus were put in a bottle of about 170 ml. capacity and this was fitted with a two-holed rubber stopper. Through one hole a glass tube passed which projected just below the stopper; this was connected to a large reservoir of filtered sea-water, the physical or chemical condition of which was adjusted according to the experiment. The outlet tube passed through the other hole in the stopper to near the bottom of the bottle where it was covered with bolting silk to prevent the escape of the Calanus. The bottle was filled and washed through with eight to nine times its own volume of water and then samples for oxygen determination were drawn off into bottles of about 50 ml. capacity. The rubber stopper was removed and a well-ground glass stopper inserted, care being taken to exclude air bubbles. The bottle was enclosed in a dark cloth bag and submerged for a suitable time, usually four hours, in a tank kept at constant temperature by means of a thermostat. At the end of the experiment the bottle was well shaken and samples for oxygen determination withdrawn by a siphon, the inlet of which was covered with bolting silk. Oxygen determinations were made, usually in duplicate, by Winkler's method, using for the titration N/200 sodium thiosulphate. The oxygen consumed during the experiment is in all cases expressed as the amount of oxygen used by 1000 Calanus in one hour.

In a few of the earliest experiments, the method adopted was to put ten or twenty Calanus in a bottle of about 50 ml. capacity filled with sea-water, to expose this to the experimental conditions along with a control bottle without Calanus and, after a suitable period, to estimate the oxygen in these bottles. This method was discontinued because when the small bottles were used the Calanus had to be killed after each experiment.

The technique was altered to make it possible to use the same Calanus several times. In addition, by using a larger number of animals, the experimental error was reduced.

During long experiments, there were often a few Calanus lost by death or misadventure. Where possible, allowance was made for these in computing the results.

It was found that there was no measurable reduction in the oxygen content of samples of filtered sea-water at the beginning and end of an experiment so that the effect of any microplankton or bacteria passing through the filter was negligible. Moreover the duration of the experiments was too short to allow of any appreciable growth of bacteria in the bottles containing the Calanus. Thus the reduction in the oxygen content can be attributed to the Calanus alone.

A possible objection to the method used is that the Calanus remained for four hours in the same body of water which was not renewed till the end of this period. There was a progressive diminution of dissolved oxygen and an accumulation of carbon dioxide and waste products in the course of each experiment. Except, however, in those cases dealing specifically with low oxygen content (page 10) and a few other cases mentioned in the text, the oxygen used was on an average not more than a fifth of that present at the beginning. Owing to the difficulty of making sufficiently accurate analyses of the carbon dioxide content of sea-water no estimations of the respiratory quotient were made.

The Calanus were not anæsthetised and the Stage V Calanus usually swam about actively ; males and females were more sluggish and remained on or near the bottom of the bottle. It was thought, however, that it would be desirable to obtain a value for their respiration near the normal value in the sea rather than the basal oxygen consumption obtainable by anæsthetising the animals. Since good duplicates could be obtained it was felt that this was justified.

Oxygen Consumption under Standard Conditions.

In measurements of the oxygen consumption time must be taken into account. It is frequently found that during the first few hours after capture the oxygen consumption is considerably above the values obtained subsequently. This is shown in the experiments in Figure 1 and Table I. In the March experiment (Figure 1, B) male and female Calanus were picked out as soon as possible after capture and subjected to consecutive four-hourly periods of experimental conditions at constant temperature for thirty-six hours. This was followed by later estimations at 48 and 72 hours after capture. The females showed a sharp fall from the first to the second four-hourly period, followed by a slow irregular fall up to thirty-six hours after capture. The values remained approximately the same at 48 and 72 hours, and duplicate bottles of female Calanus, not used before, gave at 30 and 73 hours after capture values not far removed from those given by the experimental Calanus. The parallel experiment on males was not begun early enough to show the rapid initial fall and subsequent results were irregular, showing after 48 hours a rise to a value higher than the original value found. A similar fall is shown in an earlier experiment in February (Figure 1, A, and Table I) in both males and females, but on this occasion there was a longer time between capture and the beginning of the experiment and the initial rapid fall is not shown. This rapid fall in respiration is most marked in females and less marked in males while Stage V, which were used in only a few experiments of this type, showed no fall. The fall is apparently more definite in winter than in summer.

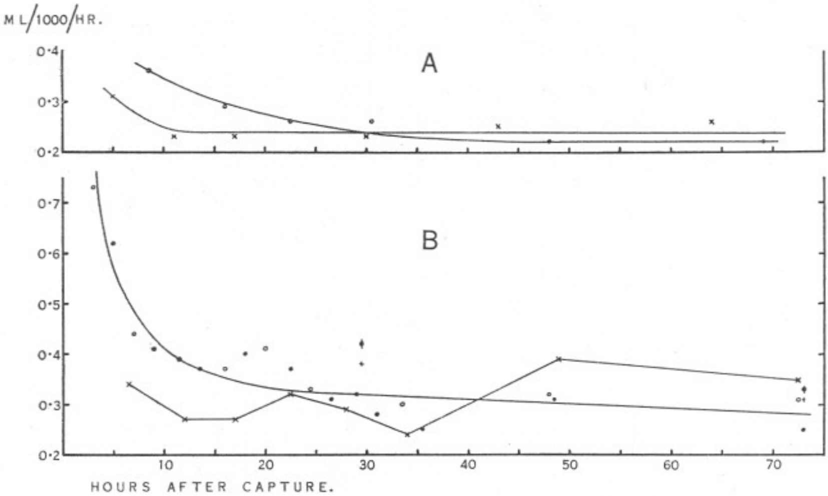

FIG. 1.—The fall in oxygen consumption with time.
A. 25–28.2.31 B. 1–4.3.32
× Males. ° Females. ● + Control Females.

Two explanations for this rapid initial fall have been put forward in similar work on respiration. In the first place the animals may have been so much disturbed by the processes of capture and picking out that their metabolism was greatly increased and only gradually fell again to normal values. It is known from work on fish (Keys, 1930) that values for their respiration are above normal for some time after they have been put under experimental conditions. In the second place, the keeping of Calanus in the laboratory and their exposure to varying conditions in small bottles is injurious to them. Usually in the course of a prolonged experiment several died, and it seems probable that the metabolism of the whole number, and therefore their respiration, decreases gradually. Kreps (1929) working on the respiration of *Balanus crenatus* found a gradual slight fall in the respiration from day to day and attributed this to the unfavourable conditions of a laboratory. Calanus kept in the usual filtered water showed no difference in respiration from others given a supply of phytoplankton. This indicates that the Calanus, under the conditions of these experiments, were not suffering from a lack of food.

TABLE I.

FALL IN RESPIRATION WITH TIME.

120 Calanus in each bottle. Exposed 4 hours at 10° C.

25–28.2.31

Males.		Females.		
Hours after capture.	O_2 used in ml./1000/hr.	Hours after capture.	O_2 used in ml./1000/hr.	Mean.
5·0	0·31	8·5	0·34 0·38	0·36
11·0	0·23	16·0	0·28 0·29	0·29
17·0	0·23	22·5	0·26 0·25	0·26
30·0	0·23	30·5	0·24 0·27	0·26
43·0	0·25	48·0	0·21 0·23	0·22
64·0	0·26	69·0	0·23 0·21	0·22

1–4.3.32

Males.		Females. A.		Females. B.	
Hours after capture.	O_2 used in ml./1000/hr.	Hours after capture.	O_2 used in ml./1000/hr.	Hours after capture.	O_2 used in ml./1000/hr.
6·5	0·34	3·0	0·73	5·0	0·62
12·0	0·27	7·0	0·44	9·0	0·41
17·0	0·27	11·5	0·39	13·5	0·37
22·5	0·32	16·0	0·37	18·0	0·40
28·0	0·29	20·0	0·41	22·5	0·37
34·0	0·24	24·5	0·33	26·5	0·31
49·0	0·39	29·0	0·32	31·0	0·28
72·5	0·35	33·5	0·30	35·5	0·25
		48·0	0·32	48·5	0·31
		72·5	0·31	73·0	0·25
		*29·5	0·38	*29·5	0·42
		*73·0	0·31	*73·0	0·33

A possible explanation of the fall in respiration in Calanus is that shortly after capture they were exposed to daylight for one or more hours. As is shown on page 14 this has the effect of raising their metabolism considerably and it might be that the observed fall is a result of initial abnormally high consumption caused by exposure to light after capture.

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* Females not used before (see text).

There are, however, several facts which contradict this explanation. In the first place Stage V Calanus, although as sensitive to light as adults, show no definite fall. Secondly, Calanus whose respiration has been increased by exposure to light show a rapid fall to normal or sub-normal values in the dark (see page 17). Finally, in an experiment designed to test this point, in which the fall in respiration of male and female Calanus caught in the light and in the dark was compared, there was no significant difference.

THE EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS.

Temperature.

That Calanus can survive over a considerable range of temperature was shown by experiments in which they were subjected to gradually rising temperatures until the lethal point was reached. The first of these experiments was done in July, 1930, and 100 each of females and Stage V were used, distributed in a number of bottles of suitable size. The bottles were immersed in the experimental tank at 17° C. in the usual way and every hour the Calanus were examined and the temperature raised by 1° C.

All the Calanus remained quite healthy up to 22° C. when the females began to get sluggish and did not swim even if they were shaken up. At 24° C. two of the females had folded antennæ, usually a sign of distress, and the Stage V had become inactive. At 26° C. most of the females were apparently dead; half of all the Calanus were taken out and allowed to cool. At 27° C. the Stage V were apparently dead and the experiment was stopped. By the next morning, of those taken out at 26° C., no females and 7 Stage V had recovered and of those taken out at 27° C., 1 female and 3 Stage V had recovered. Calanus raised suddenly to temperatures of 21° C. and 25° C. behaved similarly to those raised gradually. The experiment was repeated in April, 1932, with the same general results except that the lethal temperature was about 2° C. lower. Of 50 taken out at 24° C. and allowed to cool, 3 females and 5 Stage V recovered and of 50 taken out at 25° C. no females and 1 Stage V recovered. Males were also used on this occasion but they looked unhealthy from the beginning of the experiment, and all but one were dead at 24° C.

The experiments show that male and female Calanus are less resistant to high temperatures than Stage V, and that the lethal temperature is higher in summer than in winter. This difference is probably to be ascribed to the difference in the temperature conditions under which the Calanus used in the two experiments developed. Those of the April experiment were Calanus of the first brood and developed when the sea was at its coldest while those of the July experiment, Calanus of a late summer brood, had much warmer conditions throughout their lives.

The lethal temperature for Calanus has also been measured by Huntsman and Sparks (1925). They found that Calanus taken from the sea at temperatures of 6–10° C. died between 26·5° C. and 29·5° C. This is a limit apparently higher than ours, but the temperature was raised rapidly (about 1° C. in 5 minutes) and the animals may have been able to survive such temperatures for a short time. Huntsman and Sparks do not record any difference between summer and winter Calanus. Brown (1929), however, studying Cladocera, has found that northern species and those with spring and autumn maxima have a lower lethal temperature than southern species and those with summer maxima.

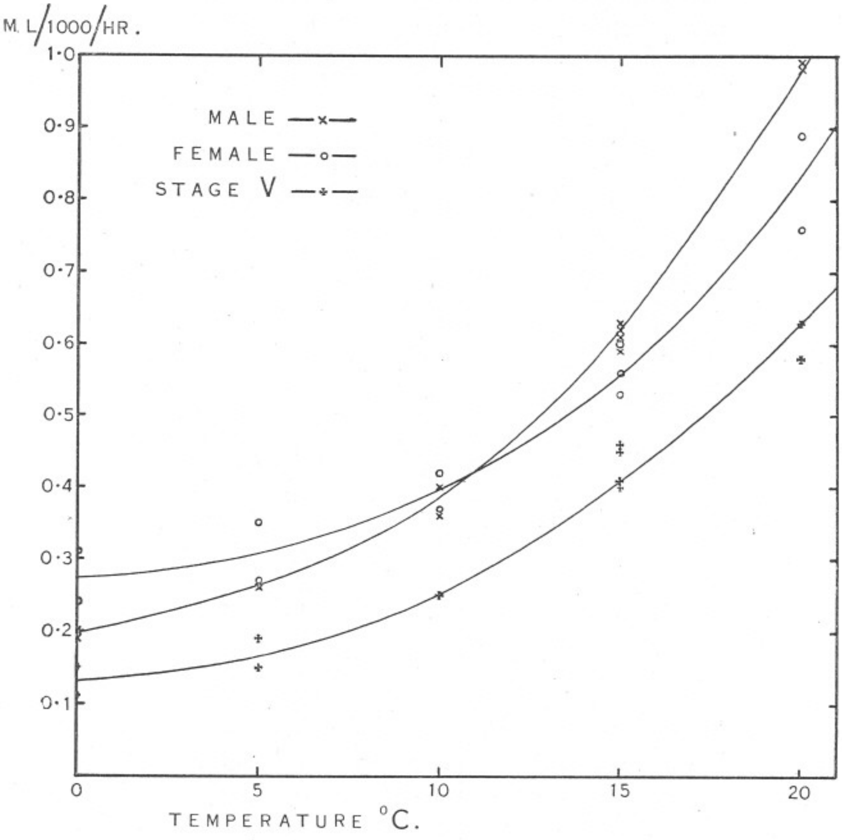

FIG. 2.—The effect of temperature on oxygen consumption. 13–14.8.31.

The effect of temperature on oxygen utilisation by Calanus was measured at intervals of 5° C. from 0° C. to 20° C. The respiration was first measured at 10° C. or 15° C. (according to the time of year), and this was followed by successive experiments from 0° C. to 20° C., the initial temperature being repeated to find if respiration had reached a steady value. The results of a typical experiment (in August, 1931) are shown in Figure 2 and Table II. The Calanus were allowed to stand for over 18 hours before the beginning of the experiment and the initial fall was thus avoided. The oxygen consumption at 0° C. is only about half that at 10° C. but above this the curves rise more rapidly and the oxygen consumption at 10° C. is less than half that at 20° C. Oxygen utilisation is higher in adults than in Stage V. In this and in one other experiment the values for males lay on a steeper curve, beginning lower and ending higher than those for females, but in most experiments there was very little difference between them. Above 20° C. the Calanus were close to their lethal temperature and in one experiment where the respiration was measured at 25° C. there was no further increase in respiration and the Calanus were all moribund at the end of 4 hours. Indications that even at 20° C. there may be a harmful effect was shown only in one experiment, in March. At these high temperatures the oxygen consumed amounted in some cases to about a third of that initially present. The oxygen content of the bottle, however, was never so low as to affect the respiration of the Calanus.

TABLE II.

EFFECT OF TEMPERATURE ON RESPIRATION. 13–14.8.31.

About 120 Calanus in each bottle, exposed for 4 hrs. Two bottles used for each stage.

	Males.		Females.		Stage V.	
T. in °C.	O_2 used in ml./1000/hr.	Mean.	O_2 used in ml./1000/hr.	Mean.	O_2 used in ml./1000/hr.	Mean.
15	0·61	0·62	0·60	0·58	0·41	0·41
	0·63		0·56		0·40	
0	0·20	0·20	0·31	0·28	0·15	0·13
	0·19		0·24		0·11	
5	0·26	0·26	0·35	0·31	0·19	0·17
	0·26		0·27		0·15	
10	0·36	0·38	0·42	0·40	0·25	0·25
	0·40		0·37		0·25	
15	0·59	0·61	0·60	0·57	0·45	0·46
	0·62		0·53		0·46	
20	0·98	0·99	0·89	0·83	0·58	0·61
	0·99		0·76		0·63	

Similar experiments were done at different times of the year and the sizes of the Calanus measured. The value for the oxygen consumption at 10° C. may vary by as much as 0·2 ml. in different experiments. The differences show no relation to size except that Stage V Calanus, which are always smaller than adults, have always a lower oxygen consumption.

It is interesting that in the early part of the year, Stage V have about the same weight as adults and in summer are considerably heavier (Orr, 1934 ; Marshall, Nicholls and Orr, 1934). In spite of this, and the fact that they are always more active, their oxygen consumption remains consistently lower. Moreover there is no consistent difference between the consumption in summer and winter for Stage V Calanus, although they are much heavier in summer than in winter.

As has been found by other workers (Ege and Krogh, 1915–16 ; Bělehrádek, 1930), the increase in oxygen consumption with temperature does not follow van't Hoff's law.

Hydrogen-ion concentration.

It is well known that changes in hydrogen-ion concentration are in many cases of importance to animal life. The changes in sea-water are small compared with those in fresh water, but it has been shown (Powers, 1930) that even the changes in the sea may be of importance. To find if these changes had any effect, Calanus were put in sea-water the pH value of which ranged from 6·7 to 8·5. The pH value was lowered by bubbling carbon dioxide through the sea-water and raised by the addition of dilute sodium hydroxide. After two days' exposure to pH 6·7 the Calanus were apparently unharmed and in no experiment was there evidence of any injurious effect at high values. Similarly it was found that the effect of changes in hydrogen-ion concentration from pH 7·3 to pH 8·5 had little or no effect on the respiration of male, female or Stage V Calanus. In several cases there was a small but continuous fall from the beginning to the end of a series of experiments. When, however, separate lots of Calanus (females) were used for measurement of the respiration (Table III) at normal, low and high pH values, this fall was not shown, so that it may safely be concluded that the effect of pH change on the respiration of Calanus is negligible within the limits studied.

TABLE III.

EFFECT OF PH ON RESPIRATION. 30.4.32.

About 100 female Calanus in each of the six bottles, exposed for 4 hrs. at 12° C.

pH.	O_2 used in ml./1000/hr.	Mean.
8·08	0·39 0·37	0·38
8·47	0·39 0·39	0·39
7·40	0·35 0·37	0·36

Oxygen content.

Variations in the oxygen content of the sea may at times be quite large. While raised oxygen content is not likely to harm Calanus, a reduction below a critical value will be lethal. This was demonstrated by an experiment in August, 1931. At this time of the year the normal oxygen content is about 6 ml. per litre. The oxygen content was reduced by passing through the sea-water hydrogen washed successively with alkaline lead acetate, silver nitrate and distilled water to remove any traces of injurious gases. That the washing was successful was shown by re-oxygenating the water by agitation with air and leaving a number of Calanus in it for 48 hours during which time they were not harmed. An experiment was then done in which 100 each of male, female and Stage V Calanus were used at 5° C. and 15° C. and the oxygen content lowered by stages to about 3·3, 2·5, 1·4 and (for Stage V) 0·7 ml. per litre. The Calanus were left at each concentration for two hours and examined every hour.

Males at 15° *C.* Six were dead after two hours at 3·3 ml. per litre; after an hour at 2·5 ml. per litre the rest were apparently normal, but after two hours they were definitely sluggish and 20 more were dead. When the oxygen was reduced to 1·4 ml. per litre, all died within an hour.

Females at 15° *C.* After two hours, even at 3·3 ml. per litre, they were sluggish but survived one hour at 2·5 ml. per litre. After two hours at this oxygen content nearly half were dead and only 2 survived after one hour at 1·4 ml. per litre. These did not survive the second hour.

Stage V at 15° *C.* These were definitely more resistant to low oxygen tensions. They were sluggish after two hours at 2·5 ml. per litre; most were moribund after two hours at 1·3 ml. per litre and all were dead after one hour at 0·7 ml. per litre.

At 5° C. the resistance to lowered oxygen content was increased.

Males at 5° *C.* Five were dead after two hours at 3·3 ml. per litre; 4 more were dead and the remainder were sluggish after two hours at 2·4 ml. per litre; only one survived two hours at 1·4 ml. per litre, the remainder having died within an hour.

Females at 5° *C.* These became moribund at 1·4 ml. per litre but a few were alive after two hours at this low value.

Stage V at 5° *C.* These were sluggish throughout but the mortality was low until the oxygen was reduced to 0·7 ml. per litre when almost all were dead within one hour.

A low oxygen concentration affects the respiration of Calanus directly as is shown in Figure 3 and Table IV. This experiment was done, using the earlier method (see page 2), on females and Stage V at 15° C. and 5° C. With females, when the oxygen concentration fell below about 3 ml. per litre, the consumption also fell even though at the end of four hours they had not used up more than about 20% of the oxygen initially present. At the end of this experiment over 90% of the Calanus were still fairly active. At a lowered concentration (2·2 ml. per litre) the fall in consumption was still more marked but towards the end of the experiment the Calanus were dying rapidly. The final oxygen content in this experiment was 1·8 ml. per litre. The Calanus were killed off completely at an oxygen concentration of about 1 ml. per litre. The Stage V Calanus were more resistant and their respiration was little affected even at 2·2 ml. per litre; at 1 ml. per litre, however, the majority were dead at the end of four hours.

When the experiment was repeated at 5° C. (Table IV) the resistance of both females and Stage V was greater; a larger number survived at low oxygen concentrations and the drop in consumption was not so marked.

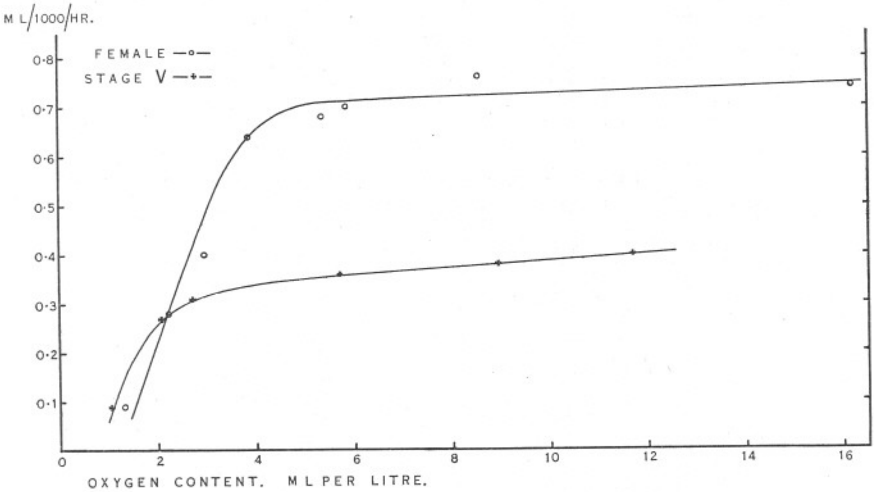

FIG. 3.—The effect of oxygen content on the oxygen consumption of female and Stage V Calanus, at 15° C.

Raising the oxygen content even as high as 19 ml. per litre had no effect on the respiration. The absence of any injurious gases in the commercial oxygen (washed with distilled water) used to raise the oxygen content was demonstrated by estimating the respiration of Calanus in a sample of water of which the oxygen content had first been raised to 18 ml. per litre and then reduced to normal by agitation with air. The oxygen consumption was the same as in normal sea-water.

It is apparent from these experiments that the respiration of Calanus is unaffected by changes in dissolved oxygen concentration except at and below about 3 ml. per litre.

Similar conclusions were reached by Henze (1910) who states that in cold-blooded animals with good circulation and branchial respiration the oxygen consumption is, within wide limits, independent of the oxygen tension. Hyman (1930) found the same to hold in planarians and the oxygen tension below which respiratory activity decreased (3 ml. per litre) was much the same as that found in Calanus.

TABLE IV.

EFFECT OF OXYGEN CONTENT ON RESPIRATION. 31.7.30–7.8.30.

Oxygen used, in ml. per 1000 per hour based on triplicate samples of about 20 Calanus.

15° C.				5° C.		
Females		Stage V				
O_2 content in ml./litre.	O_2 used in ml./1000/hr.	O_2 content in ml./litre.	O_2 used in ml./1000/hr.	O_2 content in ml./litre.	O_2 used in ml./1000/hr. Females.	Stage V.
16·17	0·74	11·69	0·40	2·04	0·28	0·16
8·54	0·76	8·94	0·38	1·21	0·09	0·09
5·85	0·70	5·71	0·36			
5·36	0·68	2·72	0·31			
3·84	0·64	2·06	0·27			
2·94	0·40	1·03	0·09			
2·21	0·28					
1·31	0·09					

Salinity.

Fluctuations in salinity in the sea are not very great except in coastal waters but this is a factor of the environment which may sometimes affect Calanus. If the salinity of the water in which Calanus are living is

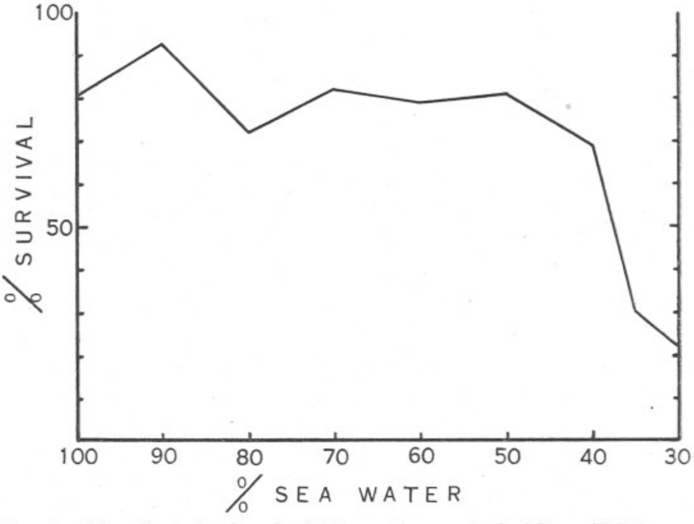

FIG. 4.—The effect of reduced salinity on the survival of Stage V Calanus.

changed in the course of two or three hours from normal (about $34^{\circ}/_{\circ\circ}$ for this area) to about 66% sea-water, they die within a short time. If, however, the change is accomplished more gradually, they can become acclimatised. This is shown by an experiment carried out from May 24th to June 9th, 1932. A thousand Stage V Calanus were picked out and put by fifties into beakers. Two hundred of these Calanus were kept in normal sea-water, the rest were then transferred at once to 95% sea-water, that is, to freshly drawn sea-water diluted in the correct proportions with glass distilled water. The water was changed daily and at the same time the salinity was reduced by 5%. The Calanus in the first four beakers remained in normal sea-water (about $33{\cdot}9^{\circ}/_{\circ\circ}$) throughout ; the Calanus in the next pair of beakers were brought down to 90% sea-water (in two steps) and remained at 90% for the rest of the experiment ; the Calanus in the next pair were reduced to 80% (in four steps) where they remained, and so on. By the twelfth day the Calanus in the last four beakers were in 35% sea-water ($S = 12{\cdot}0^{\circ}/_{\circ\circ}$) and the mortality was high. It was noticed that as the salinity was reduced the Calanus became less active. The Calanus in two of the last four beakers were kept in 35% sea-water and those in the other two reduced to 30% at which the majority of those remaining died. On June 6th some of the Calanus were used for a respiration experiment and on June 9th the experiment was stopped and the survivors counted. The results are shown in Figure 4 and Table V. There was no definite injury and the death rate was irregular and not very high until 40% sea-water was reached. Below this the animals died off

TABLE V.

EFFECT OF LOWERED SALINITY ON THE SURVIVAL OF FEMALE CALANUS.

Salinity.	100% S.= $33{\cdot}91^{\circ}/_{\circ\circ}$	90%	80% S.= $27{\cdot}18^{\circ}/_{\circ\circ}$	70%	60%	50% S.= $16{\cdot}93^{\circ}/_{\circ\circ}$	40%	35% S.= $11{\cdot}98^{\circ}/_{\circ\circ}$	30% S.= $10{\cdot}22^{\circ}/_{\circ\circ}$
% survival	81	93	72	82	79	81	69	30	22

TABLE VI.

EFFECT OF LOWERED SALINITY ON RESPIRATION. 6.6.32.

About 80 female Calanus in each bottle exposed for 4 hrs. at 15° C.

Salinity.	100% $S.=33{\cdot}91^{\circ}/_{\circ\circ}$	80% $S.=27{\cdot}16^{\circ}/_{\circ\circ}$	50% $S.=16{\cdot}97^{\circ}/_{\circ\circ}$
O_2 used in ml./1000/hr.	0·37		
	0·37	0·38	0·25

rapidly. The mortality at the high salinities is to be accounted for by the long duration of the experiment.

The respiration values (Table VI) show that, whereas the Calanus in 80% sea-water ($S=27{\cdot}2\,^{\circ}/_{\circ\circ}$) had an oxygen consumption as high as the Calanus in normal sea-water, those in 50% ($S=17{\cdot}0\,^{\circ}/_{\circ\circ}$) had one which was definitely lower. Thus Calanus can become acclimatised to salinities as low as 12–$17\,^{\circ}/_{\circ\circ}$ although respiration is reduced before these values are reached.

In several marine invertebrates (*Nereis* sp., *Procerodes* (*Gunda*) *ulvæ*) a decrease in salinity causes at least an initial increase in respiratory activity (Beadle, 1931), which is contrary to the effect found for Calanus.

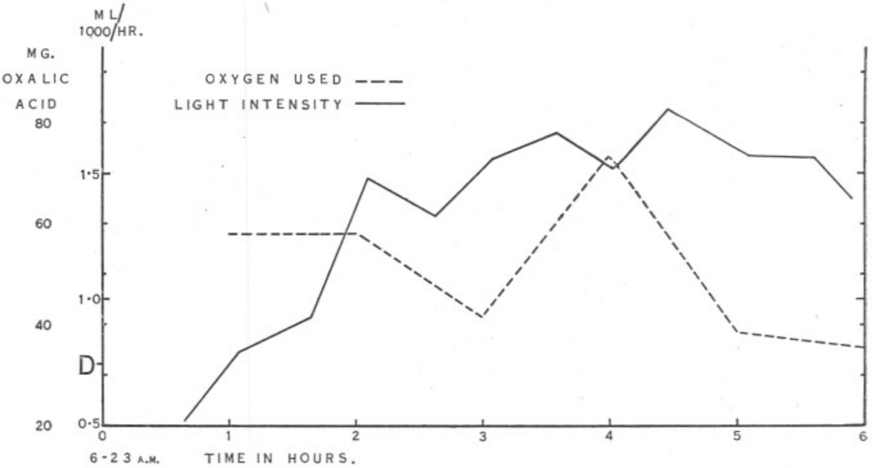

FIG. 5.—The oxygen consumption of female Calanus in bright sunshine compared with the light intensity over the same period. 10.5.32. D = Value for respiration in the dark.

Kreps (1929), however, working on Balanus found that although at salinities from $12\,^{\circ}/_{\circ\circ}$ to $35\,^{\circ}/_{\circ\circ}$ respiration was almost independent of salinity, below $12\,^{\circ}/_{\circ\circ}$ it decreased.

Light.

When exposed outside in ordinary clear glass bottles, there is no apparent increase in the activity of Calanus but there is a very considerable increase in oxygen uptake. In bright diffuse light or in sunshine, the respiration may be even double what it is in the dark (Table IX). The increase is not due to a rise in temperature for care was taken to keep all the experimental bottles at a constant temperature. The light intensity at which this increase in respiration was obtained is comparatively low. Calanus which were exposed out of doors shaded from direct sunlight gave values as high as those which were fully exposed during the same time. Similar high values were obtained also on a cloudy day. Exposure to artificial light* or to the diffuse light indoors in front of a north window had no appreciable effect.

To find the effect of continued exposure to sunlight, 12 bottles containing female Calanus were exposed for periods from one up to six hours on May 10th, 1932, two bottles being removed each hour (Figure 5 and Tables VII and VIII). Only 50 Calanus were put in the bottles which were to be exposed for four hours or more so that they should not suffer from lack of oxygen. The remainder contained 100. There were, besides, four controls in the dark. The temperature during this experiment was 12° C. The light intensity (measured by Anderson and Robinson's method (1925)) rose during the first two hours and remained more or less constant during the rest of the experiment. The oxygen consumption although it fluctuated considerably showed no relation to the variations in

TABLE VII.

THE EFFECT OF SUNLIGHT ON THE RESPIRATION OF FEMALE CALANUS AT ABOUT 12° C.

Experiment began 6.23 a.m. G.M.T. 10.5.32.

Time exposed in hours.	Actual consumption of O_2 in ml./100 Calanus.	O_2 used in ml./1000/hr.	Mean.	†Calculated consumption in successive hours.
1	0·114	1·14	1·26	1·26
	0·137	1·37		
2	0·254	1·27	1·26	1·26
	0·247	1·24		
3	0·343	1·14	1·15	0·93
	0·348	1·16		
4	0·543	1·36	1·31	1·57
	0·501	1·25		
5	0·573	1·15	1·17	0·87
	0·594	1·19		
6	0·667	1·11	1·16	0·81
	0·723	1·21		
3 (dark)	0·220	0·73		
3 ,,	0·218	0·73	0·74	
3 ,,	0·228	0·76		
3 ,,	0·216	0·72		

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* The source of artificial light was a 110 V. 60 W. Phillips " Argenta " gas-filled lamp with a parabolic reflector, at a distance of about 25 cm.

† Obtained by subtracting the sum of the means for the preceding hours from the actual consumption at any given hour.

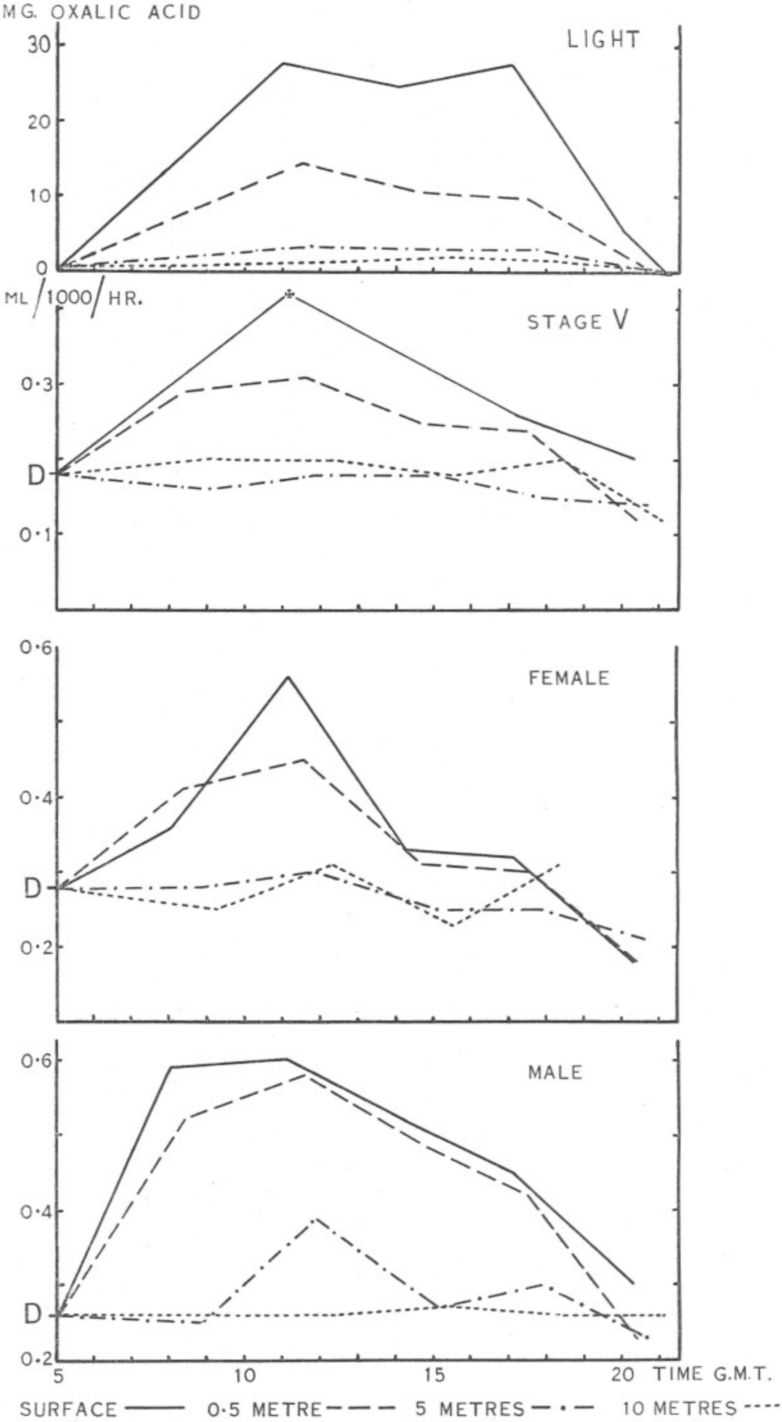

Fig. 6.—The oxygen consumption of Calanus at different depths in the sea and its relation to light intensity. 13.4.32. D=Value for respiration in the dark.

light intensity, even at the beginning. This indicated that, beyond a certain point, light intensity has no further effect on respiration. After the experiment all the bottles which had been exposed to the light were kept in the dark for three hours or (with bottles containing only 50 Calanus) six hours, and the respiration measured. In all cases but one, the value was much below that of the controls in the dark, suggesting that the exposed Calanus had been injured by light.

It will be noticed that the value for respiration in the dark was in this experiment unusually high (0·74 ml. at 12° C.). The Calanus were taken in a surface tow-netting during the day and the metabolism of those living at the surface is probably higher than that of those living in deep water.

TABLE VIII.

LIGHT MEASUREMENTS ON 10.5.32.

Time in hours.	Mg. oxalic acid used per hour.
0·65	20·8
1·08	34·6
1·65	41·5
2·10	68·8
2·63	61·5
3·08	73·0
3·58	78·1
4·03	70·9
4·57	81·4
5·08	73·5
5·60	73·2
5·90	64·9
6·48	76·7

Since light must therefore have an effect under natural conditions, Calanus were exposed at different depths in the sea to find out to what depth its influence could be detected. The bottles were put in small wire cages and these were attached to a buoyed and anchored rope. The surface bottles were on a small float attached to the buoy.

In a preliminary experiment on males on April 5th, 1932, the dark value was 0·28 ml., that at the surface was 0·64 ml. and that at 5 metres was 0·36 ml., showing that a slight effect may be noticed even at 5 metres. A long experiment was then carried out to measure the variations in the respiration of male, female and Stage V Calanus at different depths throughout a day. Bottles were exposed at the surface, 0·5, 5 and 10 metres for five periods of about three hours from 5 a.m. onwards. Since washing through the bottles with fresh sea-water at the beginning of each period took a considerable time, two sets of bottles had to be used for each depth. Thus one set was exposed from 5 a.m. to 8 a.m., 11 a.m. to 2 p.m. and 5 p.m. to 8 p.m. while the second set was used from 8 a.m.

to 11 a.m., and from 2 p.m. to 5 p.m. The different sets of bottles are indicated in Table IX by letters. The results are shown in Figure 6 and Tables IX and X. Although the Calanus were caught on April 12th and were left overnight after picking out, it will be seen that there is a fall in respiration in most bottles from one period of exposure to the next. This may be caused in part by the fall with time and in part by the injurious effect of exposure to light. Both these factors complicate the results. The light intensity, measured over the same time, showed that there was a rapid increase up to a maximum at 11 a.m., a slight decrease at 2 p.m. and a rise to a maximum value again at 5 p.m. after which it fell off rapidly. It will be seen that the respiration values from 5 p.m. to 8 p.m. in all cases, and those at 5 and 10 metres throughout the day, with one exception, can be taken as equivalent to dark values. The exception is in males at 5 metres from 8–11 a.m. when there was a slight increase. In all cases the maximum values at the surface and 0·5 metre were from 5 to 8 a.m. and from 8 to 11 a.m. and the two were sometimes much the same in spite of the difference between the light

TABLE IX.

THE RESPIRATION OF CALANUS AT DIFFERENT DEPTHS IN THE SEA DURING THE DAY. 13.4.32.

About 100 Calanus in each bottle at about 7·5° C. Secchi disc reading 5·5 m.

O_2 used in ml./1000/hr.

Time of exposure.	Males.	Females.	Stage V.
	Surface.		
5·05— 8·05	A 0·59	A 0·36	Lost
7·50—11·10	E 0·60	E 0·56	E 0·42
10·55—14·15	A 0·52	A 0·33	Lost
14·05—17·10	E 0·45	E 0·32	E 0·26
17·00—20·20	A 0·30	A 0·18	D 0·20
	½ metre.		
5·25— 8·25	B 0·52	B 0·41	B 0·29
8·15—11·35	F 0·58	F 0·45	F 0·31
11·20—14·40	B 0·49	B 0·31	B 0·25
14·30—17·30	F 0·42	F 0·30	F 0·24
7·20—20·25	B 0·23	B 0·18	B 0·12
	5 metres.		
5·35— 8·50	C 0·25	C 0·28	C 0·16
8·35—11·55	G 0·39	G 0·30	G 0·18
11·45—15·05	C 0·27	C 0·25	C 0·18
14·50—17·55	G 0·30	G 0·25	G 0·15
17·40—20·40	C 0·23	C 0·21	C 0·14
	10 metres.		
5·55— 9·15	D 0·26	D 0·25	D 0·20
9·00—12·20	H 0·26	H 0·31	H 0·20
12·00—15·30	D 0·27	D 0·23	D 0·18
15·05—18·20	H 0·26	H 0·31	H 0·20
18·05—21·05	D 0·26	Lost	E 0·12

intensities at these depths. Figure 6 shows that the light was equally bright from 11 a.m. to 5 p.m. but in spite of that the respiration fell off. It must be remembered, however, that all the Calanus were then being used for the second time. The males showed the effect of light most clearly and the females and Stage V showed it to a lesser degree. The light during the brightest hours was therefore sufficient to affect Calanus at the surface and at 0·5 metre, but at 5 metres and 10 metres there was little or no change.

TABLE X.

LIGHT MEASUREMENTS ON 13.4.32.

Time of exposure.	Mg. oxalic acid used.	Mg. per 3 hours.
	Surface.	
5·45— 7·56	9·7	13·4
8·00—11·00	27·7	27·7
11·00—14·05	25·2	24·6
14·07—17·03	27·0	27·6
17·05—20·05	5·2	5·2
	½ metre.	
6·10— 8·27	5·9	7·8
9·25—11·30	10·0	14·4
12·30—14·40	7·7	10·6
15·40—17·30	5·9	9·7
18·30—20·30	0·7	1·0
	5 metres.	
6·10— 8·45	1·7	2·0
8·45—11·45	3·3	3·3
11·45—15·05	3·3	3·0
15·05—17·45	2·7	3·0
17·45—20·45	0·2	0·2
	10 metres.	
5·54— 9·00	0·9	0·9
9·00—12·15	1·4	1·3
12·15—15·25	2·1	2·0
15·25—18·15	1·4	1·5
18·15—21·06	0·2	0·2

Light may have an effect greater than that indicated in the above experiments since the glass of the bottles absorbs a certain amount, particularly the ultra-violet which according to Klugh (1929) is lethal to Calanus (see p. 21).

All the foregoing light experiments, with the possible exception of that on May 10th, were done on the first brood which is occasionally found near the surface (Marshall, Nicholls and Orr, 1934). Stage V Calanus from a summer brood were used for a similar respiration experiment in the sea in August at the surface, 0·5, 2·3, 5 and 10 metres (Figure 7 and Table XI). There was a marked increase in respiration at the surface and one slightly smaller at 0·5 and 2·3 metres while at 5 metres there was an increase but so small that it was not much beyond experimental error.

It may thus be stated that light has no effect on respiration, or at least none that can be measured, below 5 metres.

One might expect the influence of light on the behaviour of Calanus in the sea to be reflected in the response of Calanus to light under experimental conditions, but it is difficult to find any quantitative relation between them. Huntsman (1925) has shown that light is injurious by keeping numbers of Calanus on the laboratory roof in unshaded, shaded and completely darkened jars. Those in full light all died within two or three days and only those in the dark survived for any length of time. He found that light had an equally injurious effect on many other marine animals. Our experiments on the effect of light on the respiration of Calanus lasted at most eight hours and although they were not apparently injured yet the low value of their subsequent respiration in the dark indicates that they were not unharmed. In October, in the course of

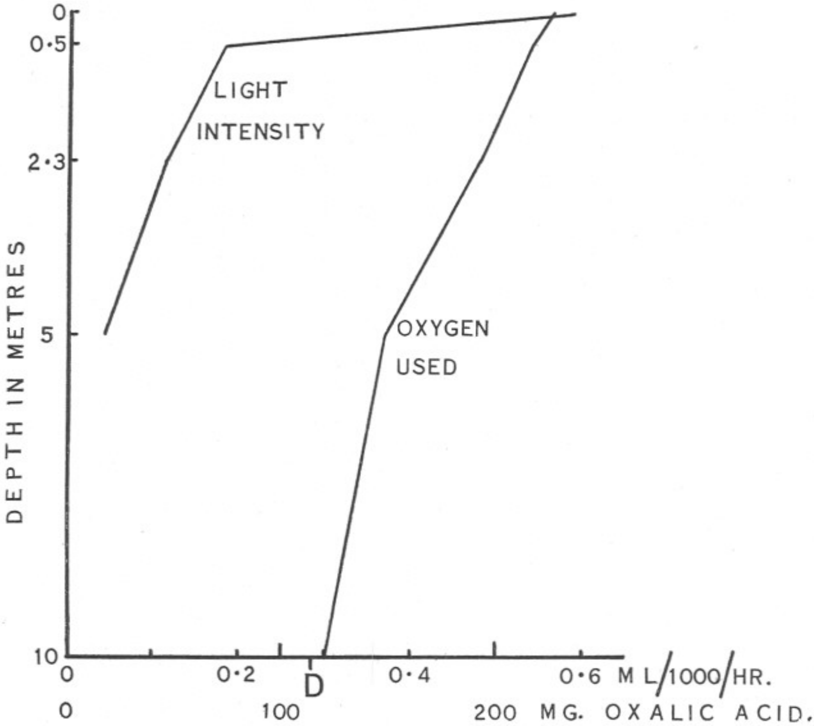

Fig. 7.—The oxygen consumption of Stage V Calanus at different depths in the sea from 10·40—14·40 G.M.T., in relation to light intensity. 22.8.32. D=Value for respiration in the dark.

another experiment, a hundred Calanus were exposed to diffuse daylight out of doors for the greater part of the day, but after four days they all died. Even when they were given only two hours exposure daily, the death rate was much higher than when kept indoors. Klugh (1929, 1930) has shown that the lethal effect is due chiefly to the ultra-violet component of sunlight and that shallow water organisms are resistant to this. Harvey's work (1930), besides confirming Huntsman's results, showed that the rate of heart beat was decreased on exposure to sunlight and that blue light was most injurious. He dealt only with the visible rays. Klugh's results were confirmed by an experiment in which the respiration of female Calanus in plain glass bottles was compared with the respiration in bottles of Uviol glass (transparent to light of short wave length).

TABLE XI.

THE RESPIRATION OF STAGE V CALANUS AT DIFFERENT DEPTHS IN THE SEA FROM 10.40–14.40 G.M.T. IN BRIGHT SUNSHINE. 22.8.32.

Secchi disc reading >10 m.

Depth in metres.	O_2 used in ml./1000/hr. during exposure.	Light. Mg. oxalic acid used during exposure.
0	0·56 0·57	234·6
0·5	0·54	72·5
2·3	0·48	46·0
5·0	0·37	17·2
10·0	0·30	
Dark	0·30	
,,	0·26	

Although there was no significant difference in the figures for respiration, the mortality was much higher in the Uviol glass bottles.

Calanus does not normally live near the surface during daylight, but an interesting point is raised by their presence at certain times of the year close to the surface or actually in the surface film. When exposed thus to a high light intensity, their oxygen consumption must be greatly increased. Such a distribution would not be expected from Huntsman's and Klugh's results. Lepeschkin (1931) states that while excess of ultra-violet light may be harmful, small quantities protect copepods (*Paracalanus parvus*, *Oncæa venusta* and *Euterpe acutifrons*) from the lethal effect of the visible rays.

CALANUS IN RELATION TO ITS ENVIRONMENT.

Planktonic organisms as a rule have a wide range of distribution and *Calanus finmarchicus* is no exception. Its centre of greatest abundance is in the North Atlantic, north of 55° N. (Farran, 1911) although it has been recorded from the Azores. It extends eastwards into the Norwegian and North Seas and it is found in the western part of the Baltic but it is less abundant in the southern North Sea and English Channel. In the western North Atlantic it is common as far south as the Gulf of Maine. It is also fairly common in the coastal waters off the west coast of North America as far south as California. Records are lacking for the Pacific as a whole but it has been found to the south of New Zealand and in the Great Australian Bight. It is recorded from the waters of South Africa, the Red Sea, the Mediterranean and the Black Sea.

Calanus normally inhabits water below 15° C. and we have seen from the experimental results that it is killed at temperatures from 25° C. to 30° C. That it can, however, become acclimatised is indicated by the records of its occurrence in the Red Sea although it is apparently not abundant there. The specimens found in warm seas are smaller than those from the North Atlantic.

The changes in pH value in the sea are relatively small and, as has been mentioned, are not likely to affect Calanus. In the Baltic where a large range in pH value is found, Calanus is not present but this is perhaps because of the low salinity.

Calanus in the laboratory is able to tolerate only a moderately low oxygen content but the lower limit is dependent on temperature. The fluctuations in oxygen content in the open sea are small, but in certain areas very low values are met with in deep water. Loch Striven and the Black Sea are examples of such areas. In late summer when Calanus is abundant in the deep water of Loch Striven the oxygen content at the bottom occasionally falls to 2 ml. per litre (Marshall and Orr, 1927). This is near the lethal limit for Calanus as found experimentally, but probably such low values do not persist for long. It is more surprising to find Calanus recorded in the Black Sea at the limiting depth for plankton in February where the oxygen content is below 1 ml. per litre, and the temperature is about 7° C. (Nikitin, 1931).

According to Farran (1911) Calanus is abundant only in water where the salinity of the sea is $35{\cdot}3\,°/_{oo}$ or less. Very low salinities are encountered in the Baltic which suggests that this factor determines its absence from that sea. The lower limit which Calanus can tolerate in the laboratory is about $17\,°/_{oo}$, a value lower than is found in the open ocean.

Since Calanus can always escape from the injurious effect of light by going deeper into the water, this is not a factor which will affect its geographical distribution, but it is possibly one of the causes of its vertical distribution and diurnal migration. Much work has already been done on these lines (Esterley, 1919 ; Russell, 1928) and it need here only be noted that there is a striking contradiction between the fact that Calanus is injured by strong light and that at times it swarms at the surface in bright sunlight.

Food Requirements of Calanus.

Respiration gives a measure of food requirements since there is a quantitative relation between the amount of oxygen used and the amount of material combusted to produce energy. The lack of figures for the carbon dioxide exchange makes it impossible to state the nature of the material utilised.

Ostenfeld (1913) estimated the amount of oxygen used by adult *Calanus hyperboreus* and found it amounted to 0·68 ml. per 1000 per hour which, since *C. hyperboreus* is larger than *C. finmarchicus*, agrees very well with our figures. Pütter made a long series of experiments on the respiration of copepods (1922, 1923, 1924–25), but apparently only a few were done on Calanus. The majority were on mixed catches of small copepods (Pseudocalanus, Centropages, Paracalanus, Acartia and Oithona) and the results varied a good deal from one experiment to another. In general he found that respiration is considerably higher in summer than in winter (at the same temperature), and that copepods require as food from 39% (for Calanus) to 156% (for Oithona) of their body weight daily in summer (1924–25). This involves a very large intake of food. His calculations of the enormous number of diatoms or other organisms necessary are well known. His experimental methods and the assumptions on which his calculations are based have been criticised by Krogh (1931) and from our results it certainly appears that his values for respiration and maintenance are excessive.

For Calanus, at 17·7° C., he records a utilisation of 1·83 ml. per 1000 per hour (1922) or roughly three times our value in the dark. From January to March the Stage V weigh about 15 mg. per 100 individuals (Marshall, Nicholls and Orr, 1934) and the oxygen consumption at 5° C. is about 0·17 ml. per 1000 per hour (Figure 2 and Table II). From April to July they weigh on an average about 30 mg. per 100 individuals and the oxygen consumption is about 0·42 ml. per 1000 per hour at 15° C. From these figures it follows that the food requirements of Stage V Calanus in winter lie between 0·002 and 0·006 mg. per individual per day, and in summer between 0·005 and 0·013 mg. The lower value given is that for fat and the higher that for carbohydrate in each case. The real value lies between these and depends on the composition of the food utilised. These figures indicate that for Stage V (the most abundant form) the amount of food required daily, expressed as a percentage of the body weight (dry) of one Calanus, lies between 1·3 and 3·6 in winter, and 1·7 and 4·5 in summer. For adults the percentage lies between 2·2 and 2·8 in winter, and 6·2 and 7·6 in summer. These values are very much lower than that calculated by Pütter for Calanus (38·7%).

A comparison of the food requirements with the food available as microplankton could be made from our knowledge of the seasonal fluctuations of the latter and the analyses of Brandt (1898) and Brandt and Raben (1919–22). In view, however, of our very incomplete knowledge of the composition of the different organisms used by Calanus and our ignorance of the digestibility of these by Calanus, any attempt at a direct relation should be deferred till further work has been done.

SUMMARY.

1. Experiments have been done to determine the oxygen utilisation by male, female and Stage V Calanus under different environmental conditions.

2. An initial fall in the respiration of adult Calanus was observed during the first few hours after capture. Stage V do not show this clearly. It is found more often in winter than in summer.

3. The lethal temperature varies from 24° C. in winter to 26° C. in summer. Stage V Calanus are more resistant to high temperatures than adults.

4. Respiration rises with increase of temperature from 0° C. to 20° C. The increase does not follow van't Hoff's law. The oxygen consumption of males and females is about the same, while that of Stage V is lower. Above 20° C. there is a harmful effect.

5. Within the limits studied (pH 7·4–pH 8·5) change in hydrogen-ion concentration has no effect on respiration.

6. Calanus are unaffected by an increase in the oxygen content of the water, but are sensitive to low oxygen tensions. Below a concentration of about 3 ml. per litre the respiration decreases. At concentrations between 1 and 2 ml. per litre they are killed. They are more resistant at 5° C. than at 15° C. and Stage V are more resistant than adults at both these temperatures.

7. Calanus can become acclimatised to salinities as low as 35–40% seawater ($\mathrm{S}=12^{\circ}/_{\circ\circ}$–$13{\cdot}6^{\circ}/_{\circ\circ}$), but their respiration is lowered at a salinity of 50%.

8. Light has a striking effect on Calanus. It may increase the respiration by 100% or more. This effect can be detected also in the sea, but not below 5 metres. Continuous exposure to light is harmful.

9. The bearing of these results on the distribution of Calanus is discussed.

10. From the amount of oxygen used in respiration, calculations of the food required are made and these are compared with the results given by Pütter for Calanus and other copepods.

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