

ON THE NUTRITION AND METABOLISM OF ZOOPLANKTON

I. PRELIMINARY OBSERVATIONS ON THE FEEDING OF THE MARINE COPEPOD, *CALANUS* *HELGOLANDICUS* (CLAUS)

By E. D. S. CORNER

The Plymouth Laboratory

Although much is already known about the diets of certain marine animals and the culturing of various unicellular algae, there is still a good deal to be learned about the quantitative aspects of energy transfer in oceanic food chains. One aspect of this problem which has attracted considerable attention is the grazing of the phytoplankton by certain species of zooplankton, and particular interest has been shown in the feeding habits of the copepod *Calanus finmarchicus* (Gunnerus). Studies made with this animal have been concerned with determining various species of diatom or flagellate on which it will feed in the laboratory; also with the proportion of ingested food it actually assimilates; and, especially, with attempts to estimate the rate at which it filters food from the surrounding medium (Clarke & Gellis, 1935; Fuller & Clarke, 1936; Fuller, 1937; Harvey, 1937; Raymont & Gross, 1942; Gauld, 1951; Marshall & Orr, 1955*b*, 1956; Conover, Marshall & Orr, 1959).

Results, in general, show that *C. finmarchicus* grazes at markedly different rates, depending on the diet used; in addition, there is even considerable variation between different estimates of the grazing rate found with one particular species of diatom (e.g. *Ditylum brightwellii*) or flagellate (e.g. *Chlamydomonas*); and more recent work by Conover *et al.* (1959) has shown that these values can vary greatly depending on the method of estimation. For example, a grazing rate determined by the technique of counting cells can be as high as 224.7 ml./animal/day, whereas that found using ³²P-labelled diatoms can be as low as 11.5.

In order to estimate the amount of food assimilated by *Calanus* it is necessary to take into account several factors other than the filtering rate. For instance, it is equally important to know the quantity of the diet; and the concentration of the food in the surrounding medium. Moreover, in order to assess the adequacy of any filtering rate for sustaining the animal in its natural habitat it is necessary to know the concentration and chemical composition of particulate matter in the sea. Unfortunately, this kind of information has not been obtained by previous workers and it is therefore difficult, without making several lengthy extrapolations, to elucidate the full meaning of their results.

In addition, laboratory experiments with *Calanus* have frequently been carried out in a closed system, the animals being confined in a fairly small volume of sea water enriched with a culture of a single species of phytoplanktonic organism, and always in contact with their excretory products.

These various considerations have been borne in mind during the present study, and the results obtained, unlike those of previous workers, are in complete accord with the view that *Calanus* satisfies its food requirements by filtering particulate material from the surrounding sea water. There is no evidence that dissolved organic substances make a substantial contribution to its diet.

METHODS

Animals. *Calanus helgolandicus* (Claus) (see Rees, 1949; Russell, 1951) was collected with a coarse tow-net (25 m.p.i.), of 1 m diameter, from the surface of the sea in the area between Penlee Point and Renny Rocks, at the entrance to Plymouth Sound. As female *Calanus* eat more than males (Raymont & Gross, 1942) only females were used in the present work; and their separation from the tow-nettings was made easier by the fact that they greatly preponderated, possibly because the hauls were made near the surface (see Russell, 1928). Quite a few of the animals bore spermatophores and others were at the so-called ripe stage, although there was little evidence of egg-laying during the experiments. Once the animals had been sorted they were immersed overnight in sea water containing a mixture of penicillin and streptomycin (25 mg. of each/l.), then washed with fresh sea water and transferred to the apparatus.

Continuous flow assembly. This consisted of two series of six glass vessels, each of 200 ml. capacity, arranged in cascade. A sample of sea water (100 l.), collected from the same sea area as the *Calanus*, was passed through a medium bolting silk (48 m.p.i.) to remove macroplankton, then pumped into two large polythene reservoirs, each holding 40 l. The remaining 20 l. of sea water was used for an estimation of the quantity of particulate matter in the sample. To start the experiment, sea water was run from the reservoirs into the glass vessels and an appropriate number of animals was then added to each. In most experiments, one series of vessels contained 96 animals (16/vessel) and the other series 48 (8/vessel). Sea water was then passed continuously through both series of vessels at a steady rate for 4 days. The inlet at the base of each vessel was covered with a glass bead to prevent the animals from moving down into the feed tube: the outlet at the top was similarly protected so that the animals were not swept out of the vessel in the stream of sea water. As the sea water left the last vessel on each side of the assembly it was filtered through a fine bolting silk (180 m.p.i.) to trap any faeces that had inadvertently been removed from the apparatus. At intervals of 24 h the two batches of sea water, one of which had flowed over 96 *Calanus* and the

other over 48, were separately examined for their contents of particulate material, the difference between the two values being a measure of the quantity filtered off by 48 *Calanus*. This procedure obviated the need to take into account any suspended material lost on the sides of the glass vessels or on the walls of the rubber tubing during the passage of the sea water through the apparatus. Nevertheless, had there been a substantial growth of phytoplankton during the 4-day period, it might have seriously affected the results, and so to avoid this the experiments were carried out at 10° C in total darkness. It is doubtful that either of these factors were adverse to *Calanus*; indeed, Fuller (1937) has found that they feed better at 8° C than at a higher (13° C) or lower (3° C) temperature, and both he, and Marshall (1924), have reported evidence consistent with the view that *Calanus* feeds actively in the dark.

Rate of flow of sea water. As the total volume of each series of vessels was 1.26 l., each of the 96 *Calanus* placed in one side of the apparatus occupied only a little over 12 ml. of sea water. However, because 10 l. of fresh sea water was passed through each series of vessels each day the actual volume of sea water available to each animal was more than 100 ml./*Calanus*/day. Experiments showed that if this volume was markedly reduced many animals did not survive the experiment. On the other hand, if the volume was greatly increased the separation of suspended material to be used in analysis was impracticably long.

The time for which the experiment was continued (4 days) was also dictated by expedience. Thus, if a much longer experimental period was used, the system became contaminated with bacteria; and if the time was greatly shortened, the quantity of food removed was too small for accurate measure.

Usually, more than 90% of the test animals survived the experiment and it might be pointed out that previous workers have recorded that *Calanus* remain healthy for several days (Clarke & Gellis, 1935), or even several weeks (Marshall & Orr, 1955*a*) in fresh sea water to which no additions have been made. However, on one or two occasions in the present work, when the concentration of suspended material in the sea water was low (< 2 mg. dry wt./l.), 20% or more of the animals either died, or turned opaque and moved sluggishly at the bottom of each vessel, a sure sign of distress. When this happened, the experiment was abandoned.

Collection of faeces. It has been found (Marshall & Orr, 1955*b*) that the size of the faecal pellets shed by *Calanus* varies with the diet. Thus, those from females feeding on *Dunaniella* are about 500 μ in length, whereas the corresponding value for animals feeding on *Skeletonema* is 950 μ . In the present work, at the end of each experiment, the animals were pipetted out of the glass vessels, from which the sea water was then strained through a suitably fine bolting silk (180 m.p.i.). The faeces so collected were then added to those retained by the traps below the final vessels in each series of the assembly (see earlier), and the combined sample was then dried and weighed.

Uptake of food. The sea water that flowed each day through each series of glass vessels was filtered under pressure through Oxoid membranes (pore size, $0.5-1.0\ \mu$; membrane diameter, 6 cm), from which, after rapid washing with distilled water, the retained material was removed by scraping with a rubber 'policeman'. After the 4-day experimental period, all the material so obtained was dried, first at 95°C for 30 min, and then at 65°C *in vacuo*, to constant weight (see Collyer & Fogg, 1955). The 20 l. sample of sea water which had not contained any test animals was similarly treated. Thus, three separate amounts of material were eventually obtained, the first being that left in sea water occupied for 4 days by 96 animals (*a*), the second being that remaining in sea water occupied for the same time by 48 animals (*b*), and the third being that present in 20 l. control sea water (*c*). From these values the amount of suspended material removed by the animals was calculated as $(b-a)/(4 \times 48)$ mg/*Calanus*/day.

Analysis of food. The amount of inorganic material in the available food was estimated by ashing; organic nitrogen—and hence protein—by the Kjeldahl method (see Barnes, 1959); lipid by the extraction procedure described by Milner (1948); and carbohydrate by difference.

Selective feeding. Harvey (1937) has shown that *Calanus* have a preference for certain kinds of diet. Therefore, in order to assess the nutritional value of any food, not only was it necessary to analyse its content of carbohydrate, lipid and protein; it was also important to know whether the animals used their feeding mechanism to select certain fractions (e.g. diatoms, flagellates) and reject others (e.g. particulate inorganic material). To examine this latter possibility, comparison was made of the proportion of inorganic material in the particulate matter before and after the animals had fed on it, the assumption being that any change in this quantity would reflect the degree of selectivity practised by the animals during feeding. Unfortunately, it was not possible to measure this accurately after each individual experiment because the animals removed only a small amount of the food available; the estimation had therefore to be based on the combined results of several experiments, and the following example illustrates a typical calculation using data from the last four. Thus, during these experiments the total quantity of suspended material available to the animals was 505 mg (dry wt.) of which 224 mg was in the inorganic fraction and 281 mg in the organic. However, after the animals had fed on this material the total amount left was 461 mg, of which 215 mg was in the inorganic fraction and 246 mg in the organic. Thus, of the 44 mg removed by the animals, 35 mg was organic material and only 9 mg inorganic. Obviously, therefore, the animals must have preferentially selected the organic fraction of their food, for whereas this fraction only accounted for 55.8% of the dry weight of suspended material available, it accounted for nearly 80% of the material actually removed.

Filtering rate. This was estimated as $20,000/4 \times 48 [(b-a)/c](x/y)$ ml./animal/day, where a , b and c are as defined earlier (see 'Uptake of food'), x is the percentage of organic material in the food actually removed by the animals, and y is the percentage of organic material in the food available.

Assimilation of food. The amount of food assimilated by the animals and subsequently used in their metabolism was estimated by subtracting the quantity excreted as faecal pellets from that which had originally been ingested. The quantities of faeces obtained were much too small to be used for analysis, and all calculations are therefore based on the assumption that the faeces had the same chemical composition as the diet. This is probably not true, for the faeces may have contained a considerably higher proportion of unwanted inorganic material, which means that the amount of organic material assimilated may be even higher than that reported, and the respiration rate, as calculated from this, even greater than that directly measured by previous workers. No account was taken of metabolically active substances that may have been lost in the urine or across the body surfaces.

Calculation of oxygen consumption. This was based on the volume of oxygen required to metabolize lipid (1 g \equiv 2.03 l. O₂) and carbohydrate (1 g \equiv 0.75 l. O₂) present in the total quantity of food assimilated. Protein was not considered in the calculation because in normal circumstances it is not used primarily as a fuel.

Accuracy of the continuous flow method. All the estimations of food uptake depended on comparisons of the amounts of suspended material left in the sea water emerging from the two sides of the assembly. Moreover, separation of this material from the sea water and its subsequent estimation involved several procedures which might have introduced experimental errors. To check this, 40 l. of the same sample of sea water was passed through each series of vessels, to which no animals had been added, at a rate of 10 l./day. At the end of 4 days, each of the two emergent samples of sea water was analysed for its content of suspended material, using the methods described earlier. It was found that the total quantity of suspended material collected from one batch of sea water was 124.6 and that from the other 126.2 mg dry wt., a duplicate result of encouraging accuracy, in that the difference between these two quantities during experiments with animals was normally 10-15 mg dry wt.

RESULTS

The results of all experiments made between May and September 1960 are shown in Table 1. Throughout this period of the year the sea water was found to contain 0.95-2.50 mg insoluble organic material/l., values in fair agreement with those of 1.15-1.77 mg/l. reported by Armstrong & Atkins (1950), but less than that of 3.2 mg/l. reported by Jones & Wills (1956). Analyses of this organic fraction showed that carbohydrate accounted for

TABLE 1. THE FEEDING OF *CALANUS HELGOLANDICUS* IN A CONTINUOUS FLOW OF 'OUTSIDE' PLYMOUTH SEA WATER

All experiments conducted in the dark at 10° C. Calculations as described in text.

Expt.	Time of year	Particulate material in sea water (mg dry wt./l.)		Quantity removed daily by <i>Calanus</i> (μg dry wt./animal)	Quantity assimilated daily by <i>Calanus</i> (μg dry wt./animal)	Daily assimilation of organic material (μg dry wt./animal)			Dry wt. of <i>Calanus</i> (μg /animal)	% Dry wt. digested daily	Filtering rate (ml./day/animal)	Oxygen consumption ($\mu\text{l.}/\text{day}/\text{animal}$).		Measured for <i>C. finmarchicus</i>
		Inorganic	Organic			Carbo-hydrate	Lipid	Protein				Calculated from amount needed to metabolize digested food	Carbo-hydrate	
1	May	3.45	2.35	34.0	25.8	13.5	5.6	1.7	103	20.2	11.4	10.2	10.1	17.8*
2	May	1.79	0.98	44.5	41.2	20.8	8.8	2.7	—	31.4	36.0	17.9	15.6	—
3	June	2.56	1.77	36.5	31.2	15.6	6.4	1.9	117	20.4	16.3	12.6	11.7	6.7*
4	June	3.64	2.50	31.7	24.2	13.7	3.9	2.2	—	16.9	10.0	7.9	10.3	—
5	July	1.75	0.95	43.0	40.5	22.7	5.6	3.6	102	31.2	35.5	10.2	17.1	12.9*
6	July	1.38	1.72	58.3	43.5	24.4	6.1	3.9	—	33.5	25.7	12.4	18.3	—
7	Aug.	1.48	1.88	26.0	21.0	10.6	4.2	1.7	104	15.9	10.9	8.5	7.9	8.6*
8	Aug.	1.29	1.84	66.5	47.1	23.7	9.9	3.8	—	35.6	28.4	20.2	17.7	—
9	Sept.	1.34	1.68	42.6	33.6	16.3	6.5	2.6	98	25.9	20.1	13.2	12.2	14.4†
10	Sept.	2.76	1.92	51.1	39.1	19.8	7.9	3.2	—	31.5	21.1	16.1	14.8	—

* Data from Marshall, Nicholls & Orr (1935); † Data from Raymont & Gauld (1951). All values temperature-corrected to 10° C.

64.1–70.8%, lipid 17.7–27.1% and protein 8.3–11.5% of the dry weight, average values being 66.5, 23.4 and 10.1 respectively, as compared with corresponding values of 63, 8 and 29 reported by Brandt (1898) for the marine diatom *Chaetoceros* and those of 85, 1.4 and 13.6 reported by Ketchum & Redfield (1949) for the marine dinoflagellate *Ceratium*.

Usually, about 80% of the food taken up by the animals was actually digested and it should be noted that high values for this have also been obtained by Marshall & Orr (1955*b*) when feeding *Calanus* with certain diatoms and dinoflagellates. Moreover, because of their selective feeding, the animals assimilated a diet containing a high percentage of organic material, and the average daily quantities of carbohydrate, lipid and protein which they digested were 18.1, 6.5 and 2.7 μg , respectively. The total amount of organic material assimilated daily, expressed as a fraction of the dry weight of the animals, is also shown in Table 1 and the average value, based on the results of ten experiments, is 25.3%.

It should be noted that the animals used in the present work were much smaller than those studied earlier at Plymouth by Bogorov (1934). Thus, in the present investigation, the dry weight varied between 98 and 117 μg per animal with an average value of 106 μg , whereas Bogorov reported May–September values between 254 and 193 μg with an average of 210 μg . A possible explanation of this discrepancy is that dry weights in the present work were determined after the animals had been dried to constant weight at 100° C, whereas in Bogorov's experiments the animals were dried in a desiccator at room temperature, and then not necessarily to constant weight. It should be pointed out that Marshall, Nicholls & Orr (1934) describe the dry weight of female *Calanus* caught during the period May–September in the Clyde area as varying between 250 and 132 μg ; but Marshall & Orr (1955*a*) also report a sample caught in July 1932 with an average dry weight of 113 μg , which closely accords with the values found in the present work. It seems, therefore, that the size and weight of *Calanus* can vary considerably with time and place, doubtless reflecting changes in the temperature or nutrient content of the sea water (see Russell, 1935, 1936, 1939).

DISCUSSION

Pütter (1909) claimed that many marine animals, including copepods, could not obtain sufficient food in the open sea by filtering off suspended material, but must make substantial use of dissolved organic substances which are absorbed through their body surfaces. In support of this hypothesis, Pütter quoted values for the amounts of dissolved organic matter in sea water which later workers (Moore, Edie, Whitley & Dakin, 1912; Krogh, 1931) have shown to be much too high. He is also thought to have overestimated the oxygen requirements of *Calanus* (Pütter, 1922), claiming that at 17.7° C it uses 33.5 $\mu\text{l.}/\text{day}$, whereas Marshall, Nicholls & Orr (1935) report a corresponding

value of only 17.8 μ l./day. Again, Pütter calculated that *Calanus* requires 38.7% of its dry weight/day as food, whereas according to Marshall *et al.* adult *Calanus*, during the summer, need only between 6.2 and 7.6%.

Nevertheless, although Pütter's hypothesis has long been regarded as suspect, the results of many workers who have studied the feeding of *Calanus* have lent indirect support to his views. Thus, the respiration rate of *Calanus*, as determined by Marshall *et al.* (1935), has often been used in order to calculate the quantity of food that the animals need daily; and the rate of filtering required to accumulate this quantity of food in the open sea has been compared with the corresponding values determined in the laboratory. Usually, the filtering rate, as measured in the laboratory, is much too low. Thus, Fuller & Clarke (1936) estimated that their animals would have to filter sea water at a rate of 72 ml./day in order to accumulate their requirements of food, whereas the maximal rate measured in the laboratory was only 5.61. Likewise, Fuller (1937) found that the rate of filtering under laboratory conditions was only about one-tenth of what was needed to sustain the animals in the open sea. A similar conclusion was reached by Raymont & Gross (1942) and by Marshall & Orr (1955*b*). However, Harvey (1937) and Gauld (1951) reported much higher filtering rates, and Gauld concluded that the copepods used in his experiments could probably have obtained sufficient particulate food in the open sea by filtering a daily volume of sea water at a rate corresponding to that which he measured in the laboratory. Unfortunately, however, he did not provide any data concerning the amount of food in the phytoplankton available to his animals in the sea, and his results did not therefore exclude the possibility, re-stated by Marshall & Orr (1955*a*), that an apparent gap exists between the amount of particulate food present in the sea and the quantity required daily by *Calanus*.

Cushing (1959) draws attention to some results which he thinks may possibly resolve this difficulty. In experiments with the copepod *Temora longicornis* he found that the filtering rate of the animal greatly increased with the volume of sea water in which it was confined. For example, raising this volume from 25 to 100 ml. increased the daily filtering rate from 26 to 150 ml. However, Marshall & Orr (1955*b*) found that although *Calanus* had a very low filtering rate when confined in a small volume of sea water (1 animal/ml.), once this volume was increased beyond a certain level (1 animal/3.3 ml.), there was no further rise in filtering rate. Furthermore, the results of the present work are in agreement with this in that the volume of sea water available to the animals was much larger (1 animal/100 ml.) and yet their filtering rates, which varied between 10.0 and 36.0 ml./animal/day (average 21.5), are all within the range (1-84 ml.) reported by Marshall & Orr.*

* Marshall & Orr (private communication) now consider their values for filtering rates to be underestimated by 10-50%. They also report further work with *Calanus* and *Temora* in which they have been unable to confirm Cushing's findings.

Interpretation of the results obtained in the present work does not require the assistance of Pütter's hypothesis. Indeed, the quantities of lipid and carbohydrate assimilated by the animals were considerably in excess of those needed to account for an oxygen consumption similar to that reported by Marshall *et al.* (1935) or by Raymont & Gauld (1951). Thus, the average amount of lipid digested was equivalent to 12.9 and the average quantity of carbohydrate equivalent to 13.6 $\mu\text{l. O}_2/\text{animal}/\text{day}$; whereas the average respiration rate, as determined by previous workers, was only 12.1 $\mu\text{l. O}_2/\text{animal}/\text{day}$. It appears, therefore, that the animals digested roughly twice the quantity of food needed to account for their respiration rate, and in order to explain this difference it is necessary to consider the following possibilities.

The first of these is that all the food taken in by the animals may not have been metabolized; some may have been stored in the tissues. To account for the difference between the amount of food digested/day/animal (25.3% of the dry body weight), and the quantity equivalent to the oxygen consumption (which varies from 5.6 to 15.2%, depending on whether all fat or all carbohydrate is metabolized), the animals would have had to increase their dry body weight daily by a value between 10.1 and 19.7. As the number of animals used never exceeded 150, it was not possible to make an accurate estimate of the extent of their growth during any one experiment. However, a single determination, carried out using animals pooled from all ten experiments, indicated that the daily increase in dry body weight was only 2-3% which, far from accounting for the difference between food digested and food metabolized, is barely equivalent to the daily assimilation of protein.

A second possibility is that the animals do not metabolize carbohydrate by a purely oxidative mechanism but rely to a considerable extent on anaerobic glycolysis and excrete the lactic acid so formed. This, however, seems unlikely because Marshall *et al.* (1935) have shown that *Calanus* quickly die in sea water of markedly lowered oxygen content.

A third, and more likely, possibility is that the animals, under the conditions of the present experiments, respired at a rate faster than those described by previous workers; and, concerning this point, it seems relevant to draw attention to the considerable variation in the values so far reported. Thus, for stage V *Calanus* at 17° C, Clarke & Bonnet (1939) describe a value of 22.8 $\mu\text{l. O}_2/\text{animal}/\text{day}$, whereas Raymont & Gauld (1951) report a corresponding figure of 12.5, and Marshall *et al.* (1935), for a slightly lower temperature (15° C), quote a value of 10.1. In addition, of course, these results have all been obtained using *C. finmarchicus*, comparatively few observations having been made with *C. helgolandicus*, the species used in the present work. Marshall & Orr (1958), however, have recently made some measurements of the respiration rate of this latter species and, from their results with both ripe and unripe females caught during winter months (no summer values are

quoted), the average value determined for animals at 10° C may be calculated as 8.4 μ l. O₂/Calanus/day, as compared with a corresponding figure of 7.4 for *C. finmarchicus*. There is therefore evidence that *C. helgolandicus* respire at a slightly faster rate. In addition, it might be expected that animals which are continuously feeding, as in the present study, have a metabolic rate higher than that of animals kept in filtered sea water, as in studies on respiration rates by previous workers: and evidence consistent with this view has been reported by Conover (1956) who showed that the respiration rates of certain copepods are markedly lowered when the animals are starved. Thus, that of *Acartia clausi*, averaged over a 4-day period, falls from 0.92 to 0.71 μ l. O₂/animal/day; and that of *A. tonsa* from 1.57 to 1.10. These various considerations stress an obvious need to measure the respiration rate of *Calanus helgolandicus* under conditions similar to those used in the feeding experiments; and it seems premature to discuss the wider implications of the present findings until these measurements are made.

Finally, it should be emphasized that although the results of this work indicate that *Calanus* obtains more than enough food to account for its respiration rate by filtering particulate organic material from the surrounding sea water, this is not to say that Pütter's hypothesis is completely wrong. For even though the animal assimilates the bulk of its diet in this way, it might still have to rely to some extent on traces of dissolved organic substances, absorbed through the body surface, in order to sustain normal, healthy development. Furthermore, all the present experiments were carried out during summer months, and so the question of whether *Calanus* makes considerable use of dissolved organic substances in winter remains undecided.

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SUMMARY

Measurements have been made of the quantity and type of food assimilated by adult female *Calanus helgolandicus* (Claus) during the summer months.

Animals kept in the dark at 10° C under a continuous flow of 'outside' Plymouth sea water (0.95–2.50 mg insoluble organic material/l.) removed 26.0–66.5 μ g/day/Calanus, of which 74–91% was actually digested.

Volumes of sea water filtered daily varied between 10.0 and 36.0 ml./animal, with an average of 21.5.

The animals preferentially selected a diet of high organic content from the particulate food available, digesting average daily quantities of 18.1 μ g carbohydrate, 6.5 μ g lipid and 2.7 μ g protein.

The average amount of food digested daily accounted for 25.3% of the dry weight and was equivalent to an average respiration rate of 26.5 $\mu\text{l. O}_2$ /animal/day, which adequately accounts for the highest values reported by others for *C. finmarchicus* (Gunnerus) kept under similar conditions of light and temperature.

These results, which provide direct evidence that *Calanus* obtains the bulk of its food from particulate material present in the sea, have been discussed with especial reference to Pütter's hypothesis.

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