J. mar. biol. Ass. U.K. (1963) 43, 495–511
Printed in Great Britain

ON THE NUTRITION AND METABOLISM OF ZOOPLANKTON

II. THE RELATIONSHIP BETWEEN THE MARINE COPEPOD *CALANUS HELGOLANDICUS* AND PARTICULATE MATERIAL IN PLYMOUTH SEA WATER, IN TERMS OF AMINO ACID COMPOSITION

By C. B. Cowey

Unit for Biochemical Research bearing on Fisheries Problems,*
National Institute for Research in Dairying, Shinfield, Reading

and E. D. S. Corner

The Plymouth Laboratory

(Text-fig. 1)

The feeding and respiration of different species of the marine copepod *Calanus* have been studied a good deal, and particular attention has been paid by previous workers—notably Marshall & Orr (1955) and Conover (1962)—to the extent to which the metabolic needs of the animal are met by the ingestion and assimilation of food in the form of certain species of phytoplankton. In addition, Corner (1961) has shown with *C. helgolandicus* that the quantities of fat and carbohydrate in particulate material in Plymouth sea water are sufficient to account for the daily needs of the adult animal in summer, as assessed from its respiration rate: and other recent experiments, with *C. hyperboreus* (Conover, 1962), *C. helgolandicus* and *C. finmarchicus* (Marshall & Orr, 1958), have shown that the respiration rate of *Calanus* falls considerably during the winter months, so that the dietary needs of the animal are likely to be smaller at a time when less food may be available.

Although attempts to balance dietary intakes of fat and carbohydrate against metabolic needs are useful, they suffer from the disadvantage of presenting an oversimplified picture. Thus, a good deal of fat and carbohydrate is likely to be stored as reserve material and it is therefore unwise to draw conclusions about *Calanus* nutrition without considering the more important question of protein metabolism. Protein is the most significant protoplasmic constituent. Thus, in certain circumstances, energy requirements can be met by catabolism of protein, the protein broken down being either of exogenous or

* Grant aided by the Development Fund.

endogenous origin, and, as this endogenous fraction includes enzymes, its catabolism (which may occur in *Calanus* during winter) will have far-reaching effects on metabolism as a whole. It would not, however, be so with fat and carbohydrate: reserves of these materials could be reduced without vitally interfering with the metabolism of the remaining protoplasm.

Little is known about protein metabolism in *Calanus*, apart from the seasonal levels of protein in *C. finmarchicus* as determined by Orr (1934) from nitrogen analyses. Therefore, as a beginning to the study of nitrogen metabolism in *Calanus*, we have investigated the amino acid composition of the diet, as well as of the animal itself. Fluctuations in the amino acid composition of the animal would indicate changes in nitrogen metabolism, and fluctuations in the amino acid composition of the diet—probably caused by seasonal changes in available species of phytoplankton—would reflect variations in the biological value of the phytoplanktonic protein for the zooplankton, which could well be a limiting factor in marine productivity.

The techniques of paper and column chromatography are now well established as methods for determining the chemical composition of living organisms. Consequently it is surprising that they are not more widely used in examining important aspects of biochemical ecology. Thus, virtually nothing is known chemically of the marine eco-system comprising: 1, dissolved organic material; 2, particulate organic material; 3, zooplankton. Indeed, there appear to be few previous contributions relevant to the present study. One, by Lasker & Lane (1953), describes qualitative tests for four amino acids present in nanoplankton collected in Biscayne Bay; another, by Tatsumoto, Williams, Prescott & Hood (1961) reports semi-quantitative determinations of 18 amino acids dissolved in surface water from the Gulf of Maine and the Carribean Sea; a third, by Parsons, Stephens & Strickland (1961) reports data on the chemical composition of various species of phytoplankton; and Krey (1958) has estimated protein in plankton by a modification of the biuret reaction.

The present work, which is exclusively concerned with Stages 2 and 3 of the above eco-system, deals mainly with an investigation by column chromatography of the relative amounts of amino acids present in adult female *Calanus* caught off Plymouth, and in particulate material present in water samples from the same sea area (L4), at monthly intervals during the period March–December 1961. Some preliminary results, for the year 1960, have been reported earlier (Cowey & Corner, 1962).

MATERIAL AND METHODS

Animals. As much of the previous work on the biology of *Calanus* has been carried out with adult females, we used these animals in the present investigation. Samples of zooplankton were taken with a coarse tow-net (25 m.p.i.) of 1 m diameter from the surface of the sea at station L4. Adult female *C. helgolandicus* (Claus) were separated

from the other animals present, counted, rinsed quickly with distilled water, then dried to constant weight at 100° C. After the weight had been determined the dried sample was divided, a known quantity being used for an estimation of total nitrogen by a micro-Kjeldahl procedure (Barnes, 1959), and the remainder for an analysis of amino acids. This latter sample was treated as follows. The dried *Calanus* was suspended in a volume of 6N-HCl equal to 5000 times the estimated weight of protein in the sample, and the mixture was continuously boiled under reflux for 24 h. The hydrolysed sample was then evaporated to dryness at 50° C under reduced pressure, the residue was resuspended in distilled water and again taken to dryness, the latter process being repeated until all trace of acid had been removed. The acid-free hydrolysate was then dissolved with gentle warming in 10% isopropanol and stored at 0° C for future analysis.

Particulate material. Samples of sea water from station L4 were collected at various times during each month. Immediately after they had been brought to the laboratory they were freed from small zooplankton by filtration through a fine bolting silk (200 m.p.i.), and particulate material, including phytoplankton, nanoplankton and detritus, was then removed from the filtrate by further filtration, under pressure, through Oxoid membranes (diameter 6 cm, pore size 0·5–1·0 μ), from which, after rapid washing with distilled water, the retained material was easily removed by rubbing with a 'policeman'.

Filtration of sea water through membrane filters is slow, and it is essential to keep changing the membrane, otherwise clogging may cause some of the organisms to be crushed and their contents lost: in addition, removal of the residues from the surface of the membrane becomes more difficult. In the present work, even with sea-water samples containing only small quantities of particulate material, 2 l. was the maximum volume of water filtered through any one membrane. A further difficulty is the adequate separation of all zooplankton organisms from the sea-water sample before membrane filtration is carried out, for contamination of the particulate material with zooplankton would obviously lead to spurious results. If too fine a bolting silk is used, quantities of phytoplankton are also retained: on the other hand, a coarse silk may also let through the smaller species of zooplankton. No hard and fast rule can be laid down. The compromise solution adopted naturally depends on the kind of phytoplankton and zooplankton prevalent in the particular sea water under study. In the present investigation, with bolting silk of about 200 m.p.i., it was found that, although some retention of phytoplankton occurred, irrigation of the silk with a jet of sea water easily washed the phytoplankton through the net. Moreover, as far as zooplankton was concerned, chemical evidence showed that the silk adequately removed these organisms, for, whereas considerable amounts of taurine (a substance known to be present in many species of Crustacea) were always found in the zooplankton fraction, no trace of this compound was ever detected in any of the particulate material examined.

Immediately after the particulate material had been separated from the sea water it was dried at 100° C, weighed, and divided into three portions, one being used in an estimation of total nitrogen by the micro-Kjeldahl method, another for a determination of inorganic content by ashing at 450° C, and the third hydrolysed with a large excess of 6N-HCl and prepared for amino acid analysis as described for *Calanus*.

Quantities of material analysed. Although it varied with the amount of particulate material present, the volume of sea water which had to be treated in order to obtain sufficient material for the various analyses (100 mg dried sample used in each) was usually in the range 100–150 l. This quantity of sea water was collected in 40 l. amounts at roughly 10-day intervals throughout each month, and the acid hydrolysates, dissolved in 10% isopropanol and stored at 0° C, were then pooled at the end of the month and analysed for amino acids as one sample.

More frequent collections of *Calanus* were made, the animals also being used as test material in other studies. Usually, about 100–150 adult females were separated from weekly tow-nettings, dried, and treated as described earlier, the final samples (30–40 mg dry wt) being combined at the end of the month for analysis of amino acids.

Amino acid estimation. The amino acids in the hydrolysates of *Calanus* and particulate material were resolved and estimated by the column chromatographic method of Moore, Spackman & Stein (1958).

The amino acids cystine, cysteine and tryptophan are partially or wholly destroyed by acid hydrolysis and therefore special methods had to be used for their estimation. The cystine and cysteine in the samples were converted by oxidation with performic acid into cysteic acid (Schram, Moore & Bigwood, 1954), which is stable under the conditions used for the acid hydrolysis of the samples of particulate material and *Calanus*; and tryptophan was estimated directly on the sample by the method of Spies & Chambers (1949). Estimations of these three amino acids—which were present in only small amounts—required additional material, and were therefore only carried out when sufficient quantities of *Calanus* and particulate material were available.

Estimation of respiration rate. Estimations of the respiratory rate of *C. helgolandicus* were made by the Winkler procedure as follows. Four adult females were placed in each of eight replicate bottles of 70 ml. capacity. The bottles contained 'outside' Plymouth sea water which had been pasteurised. The oxygen content of the sea water used in the experiments was always between 5·5 and 6·0 μl. oxygen/ml., and the oxygen requirement of the animals was assessed from the amount of oxygen removed from the sea water over a period of 24 h, all experiments being carried out in complete darkness at a constant temperature of 8° C. A correction had to be applied for the loss of oxygen by the sea water alone under these experimental conditions. This was always found to be significant (*ca.* 4 % of the oxygen present in the sea water at the start of the experiment). Oxygen uptake by the *Calanus* was expressed in terms of dry body weight, this latter value being determined on a large sample of animals taken from the same tow-netting.

Starvation experiments. Two experiments were carried out, one in winter, December/January 1961–62, and the other in summer, June/July 1962. Adult female *Calanus* were placed in batches of four, in separate beakers containing 100 ml. of micro-pore filtered sea water containing antibiotics (50 mg penicillin and streptomycin/l.). Because the excretory products, if they had accumulated, might have been toxic, the sea water was changed daily and any dead animals were removed. Both experiments were carried out in total darkness, the winter experiment at a temperature of 7° C and the summer at a temperature of 12° C. At the termination of each experiment (14 days, winter; 10 days, summer) the surviving animals were collected, dried to constant weight, analysed as described above, and compared with controls from the same tow-netting, which had been maintained in unfiltered sea water containing antibiotics.

Analysis of Skeletonema costatum. The culture of *S. costatum* was kindly supplied by Dr M. W. Parke. It was grown in a mixture of 3 parts sea water and 1 part Erd-Schreiber medium at 16° C under continuous illumination from a Mazda 'Daylight' fluorescent strip, at a light energy of 330 ergs/sec/mm^2 in the wave band 380–720 mμ. The crop was harvested after the exponential phase of growth, the cells being collected on a glass filter pad capable of retaining all material down to 2 μ in diameter. The cells were washed rapidly with distilled water, dried to constant weight at 105° C, and analysed for total nitrogen and amino acids by the methods described above.

RESULTS

Amino acid nitrogen and total nitrogen in Calanus *and particulate material*

The relationship between the total amino acid content of *Calanus* and that of its diet is shown in Table 1. During the winter of 1961–62 there was a fall in the level of amino acids in the coastal sea water at L4, the dry weights of individual *C. helgolandicus* collected from the same sea area fell correspondingly; and—on a dry weight basis—there was a fall in the total amino acids in the animals.

TABLE 1. TOTAL AMINO ACIDS IN ADULT FEMALE *CALANUS* AND PARTICULATE MATERIAL FROM PLYMOUTH SOUND, 1961

	March–September	October–December
Dry body wt. of *Calanus* (μg)	158 (129–175)	133 (111–143)
Amino acid content of *Calanus* (% body wt.)	50·3 (45·7–58·2)	45·7 (41·0–50·0)
Amino acid content of sea water (μg/l.)	97 (80·2–113)	81·0 (60·1–100)

It was expected that the values for total nitrogen in the animals would be greater than those for amino acid nitrogen because of the presence in the animal of nitrogenous substances other than proteins and amino acids (e.g. chitin, trimethylamine oxide and betaine). This proved to be so. Amino acid nitrogen varied throughout the year from 6·6 to 9·4 μg/100 μg dry body weight (average value 7·7): whereas the corresponding values for total nitrogen were in the range 7·4–10·4 (average 9·3). Therefore amino acids accounted for approximately 83% of the total nitrogen in the animal. This value is about twice that reported earlier for *C. helgolandicus* collected off Plymouth during the summer of 1960, a figure which led to the view that nitrogenous compounds other than proteins and amino acids were present in exceptionally high concentrations in these animals (Cowey & Corner, 1962). However, none of the animals we have analysed since 1960 has contained such small amounts of amino acids, and in *C. finmarchicus* we found that non-amino acid nitrogen accounted for only about 10% of the total nitrogen (Cowey & Corner, 1963). The amino acid nitrogen in the particulate material varied from 7·7 (December) to 14·6 (August) μg/l. (average value, 11·9 μg/l.), whereas the corresponding figures for total nitrogen were 13·8 to 36·1 μg/l. (average value, 21·9 μg/l.). Therefore, on average, the amino acid nitrogen accounted for only 54% of the total nitrogen present in the samples.

It is noteworthy that the total quantity of particulate material present in the sea-water samples varied throughout the year in the range 2·70–5·77 mg/l. (average value, 3·82); and the organic fraction in the range 0·95–2·41 mg/l. (average value 1·80). The values for particulate organic material agree well with those observed by previous workers: 1·15–1·77 mg/l. (Armstrong & Atkins, 1950); 0·95–2·50 mg/l. (Corner, 1961). In addition, on the basis of

Kjeldahl analyses, protein (i.e. N × 6·25) averaged 9·5% of the organic fraction, which is within the range (8·3-11·3%) reported earlier (Corner, 1961).

Relative quantities of amino acids in Calanus *and in particulate material*

The amino acid composition of *Calanus* throughout the year is shown in Table 2. No values for January and February have been included, because the amounts of material available were too small for reliable analysis. The overall picture was remarkably constant throughout all seasons. This is not surprising as far as tissue proteins are concerned, but free amino acids—especially those in muscle—might be expected to vary in concentration (and thereby change the overall picture) in response to changes in salinity, temperature and diet. However, the free amino acids found in highest concentration in crustacean muscle, i.e. arginine, glycine and proline (Camien, Sarlet, Duchâteau & Florkin, 1951), remained at about the same level throughout the year.

TABLE 2. AMINO ACID COMPOSITION OF ADULT FEMALE *CALANUS* DURING 1961

g amino-acid N/100 g total amino acid N

Amino acid	March	April	May	June	July	Aug.	Sept.	Nov.	Dec.
Arginine	18·39	16·95	16·04	19·08	19·86	17·24	15·30	19·18	15·95
Lysine	11·90	11·89	9·52	10·51	10·08	9·71	12·67	10·40	13·76
Glycine	10·79	9·07	9·98	10·97	10·88	10·28	10·97	11·30	10·60
Glutamic acid	7·66	9·47	6·81	9·03	8·33	8·98	6·99	6·31	8·65
Alanine	8·09	8·86	8·67	8·08	7·29	8·28	10·82	8·23	9·85
Aspartic acid	6·23	6·65	8·02	7·29	7·23	7·44	7·02	6·69	6·65
Leucine	5·43	6·00	6·44	6·05	5·44	5·95	6·60	5·37	6·19
Valine	5·15	5·55	8·21	5·05	4·45	5·22	5·29	4·91	6·50
Serine	3·73	4·10	3·39	3·89	3·69	4·32	3·41	3·66	3·52
Histidine	4·28	4·02	3·57	2·77	2·91	2·99	4·39	4·78	3·02
Threonine	3·69	3·71	3·78	4·21	3·62	4·32	3·50	3·87	3·81
Iso-leucine	3·73	3·57	3·99	3·38	3·47	3·76	3·88	3·45	4·33
Proline	3·89	3·30	2·85	3·38	4·15	3·32	3·71	4·16	5·03
Tyrosine	1·94	2·26	2·60	2·33	2·58	2·64	1·55	2·60	2·68
Phenylalanine	2·29	2·21	2·85	2·38	2·47	1·99	1·79	2·49	2·23
Taurine	1·19	1·18	1·41	1·50	1·79	1·66	2·12	1·25	2·09
Methionine	0·76	1·15	1·78	—	1·33	1·43	—	1·10	0·83
Cystine/2	0·85	—	—	—	0·45	0·99	—	0·45	—

Further evidence of the stability of the amino acid composition of *Calanus* compared with that of its diet is shown in Table 3, which provides values for the relative quantities of amino acids in *Calanus* and particulate material during the summers of two different years. The two sets for the animal are extremely close, but the corresponding data for particulate material show some differences.

These differences are amplified in Table 4, which shows the amino acid composition of suspended matter in sea water from the same area as the *Calanus*. The over-all picture is more variable than that observed with the

animals. Thus, there were relatively small amounts of proline and aspartic acid in June; the amount of glutamic acid increased considerably during winter months; there was a considerable fall in the proportion of lysine in April and of histidine in May and August.

TABLE 3. COMPARISON OF THE AMINO ACID COMPOSITION OF ADULT FEMALE *CALANUS* AND PARTICULATE MATERIAL COLLECTED FROM PLYMOUTH SOUND DURING THE SUMMER OF 1960 AND 1961

Molar ratios relative to leucine (= 1·00).

	Calanus		Particulate material	
	1960	1961	1960	1961
Glycine	1·62	1·71	1·78	2·03
Alanine	1·51	1·42	1·38	1·11
Glutamic acid	1·39	1·37	1·32	0·97
Aspartic acid	1·20	1·20	1·22	1·08
Leucine	1·00	1·00	1·00	1·00
Valine	0·82	0·92	0·90	0·87
Lysine	0·83	0·87	0·99	0·70
Arginine	0·61	0·72	0·55	0·61
Threonine	0·69	0·62	0·79	0·67
Serine	0·82	0·61	1·08	1·14
Iso-leucine	0·53	0·61	0·61	0·65
Proline	0·53	0·57	0·66	0·48
Phenylalanine	0·38	0·38	0·52	0·44
Tyrosine	0·37	0·37	0·29	0·27
Taurine	0·14	0·26	—	—
Histidine	0·17	0·18	0·15	0·13
Cystine/2	—	0·07	—	0·14
Methionine	—	0·18	—	0·10

TABLE 4. AMINO ACID COMPOSITION OF PARTICULATE MATERIAL IN PLYMOUTH SOUND, 1961

g amino-acid N/100 g total amino acid N

Amino acid	Mar.	Apr.	May	June	July	Aug.	Sept.	Nov.-Dec.
Arginine	11·50	13·86	15·75	22·66	14·25	15·08	15·97	11·09
Lysine	9·31	6·60	10·34	10·14	10·82	8·98	9·14	9·19
Glycine	14·90	12·99	12·43	11·37	13·39	13·54	17·65	16·19
Glutamic acid	11·42	7·59	7·55	4·61	6·84	6·84	5·78	10·27
Alanine	7·50	7·47	7·59	6·05	7·46	9·32	7·14	7·47
Aspartic acid	7·72	8·79	8·00	4·61	6·71	6·92	8·35	8·95
Leucine	6·31	6·72	6·72	6·57	6·54	7·25	6·36	5·91
Valine	5·30	6·02	6·52	6·33	5·12	5·81	5·25	5·29
Serine	9·13	9·12	7·71	7·69	7·96	7·25	6·14	8·95
Histidine	2·31	3·34	1·10	3·35	2·31	0·86	2·68	1·87
Threonine	4·41	5·28	4·55	4·65	4·36	4·63	3·20	4·82
Iso-leucine	3·80	3·67	4·34	4·93	3·71	4·44	4·78	5·32
Proline	3·43	4·58	2·05	1·92	3·85	3·75	3·15	3·35
Tyrosine	0·91	1·73	2·13	2·09	1·49	1·49	1·63	1·32
Phenylalanine	1·63	2·23	3·15	2·97	2·82	3·82	2·78	1·47
Taurine	0	0	0	0	0	0	0	0
Methionine	—	—	—	—	0·12	—	—	—
Cystine/2	—	—	—	—	0·12	—	—	—

Respiration and starvation experiments

Conover (1962) and Marshall & Orr (1958) have reported that the respiration rates of certain species of *Calanus* are low in winter. The results of our respiration experiments are shown in Fig. 1. It will be seen from it that the respiration of the animals was much higher in spring and summer months (April–August) than it was in winter (December–February), the average

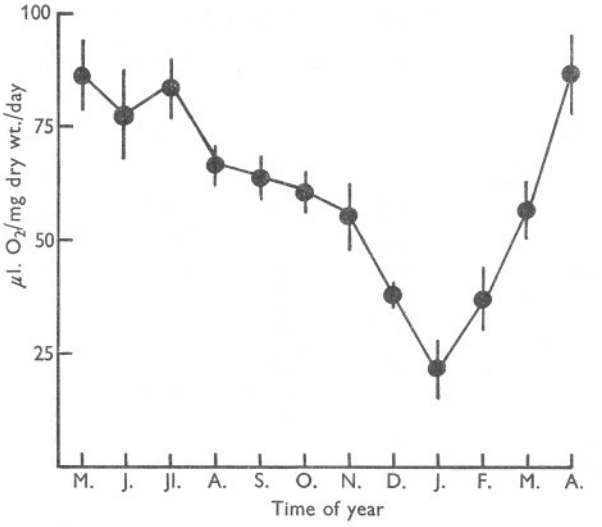

Fig. 1. Respiration of adult female *Calanus helgolandicus* during 1961–62. Vertical lines represent the standard deviation observed in eight determinations, each with four animals.

values for these two periods of the year being 79 and 31 μl. O_2/mg dry body wt./day, respectively. These values can also be expressed as μl. O_2/copepod/day, in which form they are comparable with data by Marshall & Orr (1958), who found that the respiration of *C. helgolandicus* during the period October–April was 4·6–11·5 μl. O_2/copepod/day at 10° C. The corresponding range obtained in the present work was 3·0–13·0 μl. O_2/copepod/day at 8° C, and is therefore in very good agreement. As the respiration experiments were all carried out at 8° C, it is clear that the difference in oxygen consumption by *Calanus* in summer and winter months is a seasonal and not a temperature effect.

Because of the reduced winter respiratory rate it follows that *Calanus*, in winter, is in a condition where its metabolism is greatly reduced. Thus Conover (1962) suggests 'that the life cycle of *C. hyperboreus* seems regulated to take advantage of a single period of food abundance occupying perhaps 10–20% of total life'. The suggestion that *Calanus* may not feed at all during the winter months is implicit in other recent papers (cf. Beyer, 1962).

On the other hand Marshall & Orr (1958) express the view, with respect to *C. finmarchicus* in winter, that 'no "hibernation" seems to take place but the population is living in an economical way'.

Our starvation experiments were carried out to determine whether the extent of losses of various amino acids by *Calanus* in these circumstances were compatible with the view that the animal goes without food throughout the winter. The experiments might also give a first approximation to the daily amino acid requirements of *Calanus* and can lead to an estimation of the filtering rate the animals would need to maintain to replenish amino acids lost.

TABLE 5. LOSSES OF AMINO ACIDS BY ADULT FEMALE *CALANUS* DURING STARVATION

Average dry wt. of individual animals in winter was 109 μg at start and 84 μg after 14 days' starvation: in summer, 200 μg at start and 123 μg after 10 days' starvation. Amino acids shown in lower half of the table are essential for mammals.

	Amount lost as μg/*Calanus*/day	
Amino acid	Winter	Summer
Aspartic acid	0·075	0·521
Serine	0·033	0·193
Glutamic acid	0·110	0·503
Proline	0·130	0·064
Glycine	0·108	0·276
Alanine	0·149	0·305
Tyrosine	0·091	0·264
Taurine	0·018	0·011
Arginine	0·128	0·056
Valine	0·141	0·304
Histidine	0·013	0·071
Iso-leucine	0·096	0·243
Leucine	0·113	0·361
Lysine	0·206	0·428
Methionine	—	0·073
Phenylalanine	0·024	0·224
Threonine	0·064	0·197
Total amino acids lost	1·50	4·10
Total solids lost	1·79	5·20

For comparative purposes a starvation experiment was carried out in summer (June/July) when respiratory activity was particularly high, as well as in winter. There was a high mortality rate in both experiments. 70% of the starved winter animals and 65% of the starved summer animals failed to survive. Mortality in the control animals was less than 10%.

Table 5 shows the quantity of each amino acid lost per copepod per day. The total quantity of amino acids lost during the winter experiment was 21 μg/100 μg dry body weight and accounted for 90% of the total solids lost during the 14 days of starvation. Roughly 1·8% of the dry body weight of *Calanus* was lost as amino acids each day. These figures, together with the high mortality rate observed, are taken as evidence that those *C. helgolandicus* which successfully survive through the winter must feed on whatever is

available, because they could not sustain losses of amino acids of this order for several months on end and survive.

It is remarkable that protein (or amino acids) constitutes so great a proportion of the total solids lost during starvation. It seems probable that protein is mobilized and catabolized long before reserves of fat and carbohydrate are anything like exhausted.

Larger animals were used in the summer experiment and, as was to be expected from the respiration experiments, they lost amino acids at a faster rate, i.e. 2·1% of the dry body weight/day. Again a very high proportion of the total solids lost, some 80%, was protein.

Estimation of filtration rate

For several amino acids the quantity lost during starvation is not necessarily that which must be obtained from the diet, as the animal is probably capable of interconverting amino acids. Mammals can exist without dietary glycine, aspartic and glutamic acids, alanine, serine and proline; these amino acids are therefore not essential amino acids; the animal is capable of making them from other dietary amino acids. Whether *Calanus* has a similar capacity is not known and in the absence of this knowledge it is unwise to attach too much significance to changes observed in those amino acids that are not likely to be essential for the animal. The subsequent calculations are therefore exclusively concerned with essential amino acids, it being assumed from experiments with mammals that these are more likely to represent compounds which *Calanus* is unable to synthesize and therefore needs in its diet. The essential amino acids are shown in the lower part of Table 5.

From a knowledge of the total concentration of each amino acid in the particulate material in the sea (Tables 1 and 4) it is a simple matter to calculate that volume of sea water which contains the quantities of various amino acids lost each day by *Calanus*. To compute the volume of sea water which the animal must sweep clear each day in order to replenish its losses of various amino acids from those present in particulate material, it is essential to know the quantity of food assimilated by the animal and the efficiency with which phytoplanktonic protein is utilized. In the present calculation it has been assumed that 80% of the ingested food is absorbed, this figure being based on the observations of several previous workers (Marshall & Orr, 1955; Conover, 1956; Corner, 1961). For maximum efficiency of utilization of phytoplanktonic protein the essential amino acids present in it must all be biologically available to *Calanus* at the same time. Now the biological value of some protein foods for mammals is less than would be predicted from their essential amino acid content, because not all the essential amino acids in the protein are available to the animal. For example, in some mammalian protein foods the ϵ-amino group of some of the lysine residues may be combined with carbohydrate, thereby rendering these lysine residues biologically unavailable. It is

conceivable that a proportion of the essential amino acids of the phytoplanktonic protein are similarly unavailable to the zooplankton, but in the absence of any evidence for this effect no allowance has been made for 'availability' of essential amino acids in calculating filtration rates. Our estimates of filtration rate—calculated in terms of essential amino acids—are shown in Table 6. They indicate that for most essential amino acids a filtering rate of approximately 30 ml./copepod/day would be sufficient to enable *Calanus* to maintain its complement of essential amino acids. This figure (30 ml.) is low compared with previous values but then these filtering rates relate to winter *Calanus*, which the respiration experiments had shown to be in a fairly inactive state.

TABLE 6. VOLUMES OF SEA WATER SWEPT CLEAR BY WINTER AND SUMMER *CALANUS* IN ORDER TO REPLENISH LOSSES OF 'ESSENTIAL' AMINO ACIDS

Assimilation assumed to be 80%. Winter values for amino acid content of particulate material estimated during November–December. Summer values during April–September.

	Concentration in particulate matter (μg/l. sea water)		Volume swept clear (ml./*Calanus*/day)	
Amino acid	Winter	Summer	Winter	Summer
Arginine	3·8	6·6	42	10
Valine	4·8	6·7	37	54
Histidine	0·7	0·9	23	68
Iso-leucine	3·8	5·5	32	41
Leucine	6·0	6·4	24	55
Lysine	5·2	6·9	50	75
Phenylalanine	1·9	8·1	16	54
Threonine	4·4	5·2	18	45

It is recognized that, in long-term starvation experiments of the type we have performed, protein catabolism may be accelerated because the animal may be compelled to use the carbon skeletons of amino acids as energy sources. In the normal course of events this energy might be supplied by dietary fat and carbohydrate, smaller amounts of protein therefore being catabolized. However, an extremely high proportion of the total solids lost in these starvation experiments was protein: moreover Harris (1959) observed even higher rates of nitrogen excretion in short term (4 h) experiments by a mixed population of zooplankton. It is therefore likely that there is a rapid turnover of protein in *Calanus* under normal circumstances, and our findings on amino acid requirements are probably a fair approximation to the situation which normally obtains in winter *Calanus*.

Filtration rates calculated from losses of amino acids by starving animals in summer are likely to be too low, because we have no means of computing the additional quantities of amino acids required for growth and egg-production by these summer animals.

Selective feeding

Calanus is said to have a preference for certain kinds of diatom in its diet (Harvey, 1937). We have therefore analysed *Skeletonema costatum*, a marine diatom known to be readily utilized by *Calanus*, in order to see how closely its amino acid composition approximates to that of particulate material as a whole, and whether the animal would gain nutritionally by feeding selectively on this diatom in preference to the organic fraction of particulate material.

TABLE 7. AMINO ACID COMPOSITION OF ADULT FEMALE *CALANUS* AND PARTICULATE MATERIAL FROM PLYMOUTH SOUND, 1961, COMPARED WITH THAT OF *SKELETONEMA COSTATUM*

Figures for *Calanus* and particulate material are the means of 9 determinations made throughout the year. Figures in parentheses show the range of values encountered.

	g amino acid N/100 g total amino-acid N		
	Calanus	Particulate material	*Skeletonema*
Arginine	17·9 (15·3–20·9)	15·9 (11·1–22·7)	12·2
Glycine	11·9 (9·1–13·7)	14·3 (11·4–17·7)	10·2
Lysine	11·16 (9·5–13·8)	9·3 (6·6–10·8)	9·1
Alanine	9·1 (7·3–12·0)	7·3 (6·1–9·3)	8·1
Glutamic acid	8·1 (6·8–9·5)	7·7 (4·6–10·5)	8·2
Aspartic acid	7·1 (6·2–8·4)	7·7 (4·6–9·5)	9·1
Leucine	6·0 (5·4–6·6)	6·3 (5·9–7·3)	6·8
Valine	5·7 (4·5–8·2)	5·7 (5·0–6·5)	6·0
Serine	3·9 (3·4–4·7)	8·2 (6·1–9·9)	6·4
Threonine	3·9 (3·6–4·3)	4·6 (3·2–5·3)	4·8
Proline	3·9 (2·9–5·0)	3·6 (1·9–5·2)	4·6
Iso-leucine	3·7 (3·5–4·3)	4·3 (3·4–5·3)	4·7
Histidine	3·6 (2·8–4·8)	2·2 (0·9–3·4)	3·5
Tyrosine	2·5 (1·6–3·4)	1·9 (1·3–2·1)	1·7
Phenylalanine	2·4 (1·8–2·9)	2·7 (1·5–3·8)	3·7
Taurine	1·7 (1·2–2·8)	—	—
Methionine	1·2 (0·8–1·8)	0·8 (0·1–1·5)	0·7
Cystine/2	0·6 (0·3–1·0)	0·11 (0·10–0·12)	0·4

The results, shown in Table 7, emphasize the close similarity in amino acid composition between particulate material and the diatom *Skeletonema*. Obviously, in terms of amino acid composition alone, the animal would gain little nutritional advantage by selecting *Skeletonema* in preference to the organic fraction of the particulate material as a whole.

It will be recalled that in the analyses of particulate material there was a considerable discrepancy between total nitrogen (as determined by Kjeldahl analysis) and amino acid nitrogen (as determined by the method of Moore & Stein), a result which indicates that nitrogenous substances other than amino acids must be present to a large extent in the samples analysed. In the experiments with *Skeletonema*, however, there was no such discrepancy: amino acid nitrogen accounted for more than 95% of the total Kjeldahl nitrogen. *Skeletonema* must therefore be free of these unidentified nitrogenous substances.

Our figures for the amino acid composition of *Skeletonema* are very different from those reported by Parsons & Strickland (1961). Thus their aspartic

acid value is some three times that reported in Table 7. It is possible that this discrepancy is due to differences in the culture medium used and to the harvesting of cells at different phases of growth. Obviously more information is needed on the amino acid composition of important marine algae at different stages of growth and in different conditions of culture.

DISCUSSION

Because the over-all amino acid composition of *Calanus* remains much more constant than that of the particulate material in the sea, *Calanus*, like higher animals, must be capable of 'editing' its diet, possibly by metabolic interconversion and selective excretion. However, it is not yet certain that the amino acids found in the particulate material are all biologically available to the animal: some of them may be confined to fractions which *Calanus* cannot capture or digest. The problem of what *Calanus* can capture as food remains open. Thus, Petipa (1960) has described how *Calanus* can actively catch and feed on *Noctiluca miliaris* (0·5–0·65 mm), Marshall & Orr (1955, 1956) report that organisms less than 10 μ in diameter are not filtered off efficiently either by adult *Calanus* or by the young stages: but Raymont & Gross (1942), in a study of the breeding of *Calanus* under laboratory conditions, found that minute species of nanoplankton ($1{\cdot}7 \times 1{\cdot}2$ μ) were successfully utilized by *Calanus* of all stages. Detritus also cannot be excluded as a possible food for *Calanus*: thus, Jørgensen (1962) concludes that, although it may be of only small importance as a bulk food in the photosynthetic zone, it could have a more important function as a food for animals at a greater depth in the ocean, and possibly be of use as a supplementary food generally; Riley (1959) notes that the quantity of detritus in Long Island Sound is very large, and reports that the zooplankton can assimilate more nitrogen than is found in phytoplankton; and Parsons & Strickland (1961) suggest that because of its quantity and composition detritus in the north-eastern Pacific Ocean must be considered as a diet for zooplankton.

In our work, both nanoplankton and detritus were regarded as possible foods for *Calanus* and were included in all analyses of the particulate material. There is ample justification for this, partly for the reasons just mentioned, but also because results of experiments with *Skeletonema costatum* showed that, in terms of the relative quantities of amino acids required in its diet, *Calanus* would gain no obvious advantages in selecting this diatom in preference to the amino acid containing fraction of the particulate material as a whole.

In addition, there was a distinct advantage to be gained in obtaining accurate quantitative information on all amino acids, both free and combined as peptides and proteins. Thus, although the form of the amino acids (whether peptide-bound or free) might have some bearing on their biological value as food, the relative proportions of all the amino acids is the important factor; it

is well known that the various protein fractions of a food have less biological value individually than that of the entire product, because of mutual compensation.

A further object of the present work was to see whether the amino acid composition of particulate material in the sea was adequate quantitatively, as well as qualitatively, for supplying *Calanus* with its dietary needs throughout the year. Work on this aspect of the problem is still in progress: however, some information has already been provided by starvation experiments which showed that the total loss of amino acids by *Calanus* during a long period of starvation accounted for 0·25% of the fresh weight/day in the winter experiments and 0·34% in the summer. This would seem to be a high rate of loss when one considers that a 70 kg man excretes approximately 1 g amino acids/day, only about 1% of the proportionate quantity lost by *Calanus*. However, it must be emphasized that what was being measured in these starvation experiments was not the amino acids excreted in urine, or passed out across certain body surfaces, but the total change in level of each amino acid in the animal. Therefore, total protein catabolized would be a much better standard for comparison, and for man this figure is 70 g/day (*vide* Hawk, Oser & Summerson, 1949), or 0·1% of the body weight, which is much closer to the corresponding value for *Calanus*. Results for other species of crustacean would be more relevant, but these are rarely expressed in terms of body weight. However, Needham (1957) has shown that the crab *Carcinides maenas* (Pennant), when fasting, excretes urinary nitrogen corresponding to a daily loss of protein amounting to 0·024% of the body weight, which is about one-tenth of the corresponding value for *Calanus*, and possibly reflects the fact that *Carcinides*, in similar circumstances, is a less active animal. On the other hand, Harris (1959) found that nitrogen excretion by mixed zooplankton (mainly *Acartia clausi*) taken from Long Island Sound in summer accounted for a loss of 3·64 μg N/100 μg dry body weight/day (estimated as ammonia), some ten times as great as that found with fasting summer *Calanus*—0·34 μg N/100 μg dry body weight/day (estimated as amino acids). Thus the losses of amino acids from *Calanus* under starvation conditions, corresponding to a loss of protein accounting for 2·04 μg/100 μg dry body weight/day (summer experiments), are much lower than the corresponding value of 22·7 μg/100 μg dry body weight/day as estimated from Harris's figure for excreted nitrogen (average value 2·60 μg at. of nitrogen/mg dry body weight/day) by using the customary conversion factor of 6·25. This large disparity is probably due to the fact that our measurements were made on starving *Calanus*, whereas those of Harris were made on zooplankton which were feeding to excess. As Harris points out, 'the results can only mean that the zooplankton were ingesting more phytoplankton than they could utilize either for growth or for energy... the phosphate and ammonia were being returned to solution more rapidly than the carbon was oxidized'. From the magnitude of zooplanktonic nitrogenous excretion reported by Harris it must be assumed that the animals were

assimilating non-living particulate nitrogen, because the quantity of phytoplanktonic nitrogen available in the water would be insufficient to provide for such a high rate of nitrogen turnover (Riley, 1959).

It would be of considerable interest to know the qualitative and quantitative composition of the particulate material analysed with especial reference to the various species of phytoplanktonic organisms (diatoms, dinoflagellates, etc.) present in it. Perhaps even more important, it would be useful to have some idea of the size and nature of the detritus fraction, for the discrepancy between the total quantity of amino acids on the one hand and of total nitrogen on the other indicates that nitrogenous substances other than amino acids occurred in the particulate material to a considerable extent, and it seems likely that these substances are present in significant amounts in the detritus fraction (Yentsch & Vaccaro, 1958).

We are indebted to Drs F. S. Russell, F.R.S., S. K. Kon, S. M. Marshall, F.R.S. and the late Dr A. P. Orr for their encouragement and advice. We also gratefully acknowledge the untiring assistance of Capt. W. J. Creese and the crew of R.V. 'Sula' in collecting our many samples of sea water and *Calanus*. Finally, we are grateful to Miss Yvonne M. Woodward and Mrs P. Ashton for skilled technical assistance.

SUMMARY

The amino acid composition of adult female *Calanus helgolandicus* Claus and particulate material at station L4 has been analysed from March to December. The average concentration of amino acid nitrogen in *Calanus* was 7·7% of the dry body weight and accounted for 83% of the total nitrogen. The average level of amino acid nitrogen in the particulate material was 11·9 μg/l. and accounted for 54% of the total nitrogen.

The relative quantities of amino acids in *Calanus* remained remarkably constant throughout the year. The relative quantities of amino acids in particulate material were more variable.

The rate of respiration of *Calanus* measured at 8° C varied from 31 μl. O_2/mg. dry body weight/day in winter (December–February) to 79 μl. O_2/mg dry body weight/day in summer (April–August).

Starving winter *Calanus* lost 1·8% and starving summer *Calanus* 2·1% of their dry body weight as amino acids each day. In order to replenish its daily losses of amino acids winter *Calanus* must sustain a filtering rate of about 30 ml./animal/day: the corresponding value in summer is greater than 50 ml./animal/day.

The amino acid composition of *Skeletonema costatum* is so close to that of the particulate material in the sea, that, as far as amino acids are concerned, *Calanus* would gain no nutritional advantage by selecting the diatom in preference to the amino acid containing fraction of particulate material as a whole.

REFERENCES

ARMSTRONG, F. A. J. & ATKINS, W. R. G., 1950. The suspended matter of sea water. *J. mar. biol. Ass. U.K.*, Vol. 29, pp. 139–43.

BARNES, H., 1959. *Apparatus and Methods of Oceanography*. Part I. *Chemical*, 341 pp. London: Allen and Unwin.

BEYER, F., 1962. Absorption of water in crustaceans and the standing crop of zooplankton. *Rapp. Cons. Explor. Mer*, Vol. 153, pp. 79–85.

CAMIEN, M. N., SARLET, H., DUCHÂTEAU, G. & FLORKIN, M., 1951. Non-protein amino acids in muscle and blood of marine and freshwater Crustacea. *J. biol. Chem.*, Vol. 193, pp. 881–5.

CONOVER, R. J., 1956. Oceanography of Long Island Sound 1952–54. VI. Biology of *Acartia clausi* and *A. tonsa*. *Bull. Bingham oceanogr. Coll.*, Vol. 15, pp. 156–233.

CONOVER, R. J., 1962. Metabolism and growth in *Calanus hyperboreus* in relation to its life cycle. *Rapp. Cons. Explor. Mer*, Vol. 153, pp. 190–7.

CORNER, E. D. S., 1961. On the nutrition and metabolism of zooplankton. I. Preliminary observations on the feeding of the marine copepod, *Calanus helgolandicus* (Claus). *J. mar. biol. Ass. U.K.*, Vol. 41, pp. 5–16.

COWEY, C. B. & CORNER, E. D. S., 1962. The amino acid composition of *Calanus helgolandicus* (Claus) in relation to that of its food. *Rapp. Cons. Explor. Mer*, Vol. 153, pp. 124–8.

—— 1963. Amino acids and some other nitrogenous compounds in *Calanus finmarchicus*. *J. mar. biol. Ass. U.K.* Vol. 43, pp. 485–93.

HARRIS, E., 1959. The nitrogen cycle in Long Island Sound. *Bull. Bingham oceanogr. Coll.*, Vol. 17, pp. 31–65.

HARVEY, H. W., 1937. Note on selective feeding by *Calanus*. *J. mar. biol. Ass. U.K.*, Vol. 22, pp. 97–100.

HAWK, P. B., OSER, B. L. & SUMMERSON, W. H., 1949. *Practical Physiological Chemistry*, 1323 pp. Philadelphia: The Blakiston Company.

JØRGENSEN, C. B., 1962. The food of filter feeding organisms. *Rapp. Cons. Explor. Mer*, Vol. 153, pp. 99–107.

KREY, J., 1958. Chemical determinations of net plankton, with special reference to net albumin content. *J. mar. Res.*, Vol. 17, pp. 312–24.

LASKER, R. & LANE, C. E., 1953. The origin and distribution of nitrogen in *Teredo bartschi* Clapp. *Biol. Bull., Woods Hole*, Vol. 105, pp. 316–19.

MARSHALL, S. M. & ORR, A. P., 1955. On the biology of *Calanus finmarchicus*. VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. mar. biol. Ass. U.K.*, Vol. 34, pp. 495–529.

—— 1956. On the biology of *Calanus finmarchicus*. IX. Feeding and digestion in the young stages. *J. mar. biol. Ass. U.K.*, Vol. 35, pp. 587–603.

—— 1958. On the biology of *Calanus finmarchicus*. X. Seasonal changes in oxygen consumption. *J. mar. biol. Ass. U.K.* Vol. 37, pp. 459–72.

MOORE, S., SPACKMAN, D. H. & STEIN, W. H., 1958. Chromatography of amino acids on sulphonated polystyrene resins. *Analyt. Chem.*, Vol. 30, pp. 1185–90.

NEEDHAM, A. E., 1957. Factors affecting nitrogen excretion in *Carcinides maenas* (Pennant). *Physiol. comp. Oecol.*, Vol. 4, pp. 209–39.

ORR, A. P., 1934. On the biology of *Calanus finmarchicus*. IV. Seasonal change in the weight and chemical composition in Loch Fyne. *J. mar. biol. Ass. U.K.*, Vol. 19, pp. 613–32.

PARSONS, T. R., STEPHENS, K. & STRICKLAND, J. D. H., 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. res. Bd Canada*, Vol. 18, pp. 1001–16.

PARSONS, T. R. & STRICKLAND, J. D. H., 1962. Oceanic detritus. *Science*, Vol. 136, pp. 313–14.

PETIPA, T. S., 1960. The role of *Noctiluca miliaris* Sur. in the nutrition of *Calanus helgolandicus* Claus. *Dokl. Akad. Nauk S.S.S.R.*, Vol. 132, pp. 961–3.

RAYMONT, J. E. G. & GROSS, F., 1942. On the breeding and feeding of *Calanus finmarchicus* under laboratory conditions. *Proc. roy. Soc. Edinb.* B, Vol. 61, pp. 267–87.

RILEY, G. A., 1959. Note on particulate matter in Long Island Sound. *Bull. Bingham oceanogr. Coll.*, Vol. 17, pp. 83–6.

SCHRAM, E., MOORE, S. & BIGWOOD, E. J., 1954. Chromatographic determination of cystine as cysteic acid. *Biochem. J.*, Vol. 57, pp. 33–7.

SPIES, J. R. & CHAMBERS, D. C., 1949. Chemical determination of tryptophan in proteins. *Analyt. Chem.*, Vol. 21, pp. 1249–66.

TATSUMOTO, M., WILLIAMS, W. T., PRESCOTT, J. M. & HOOD, D. W., 1961. Amino acids in samples of surface sea water. *J. mar. Res.* Vol. 19, pp. 89–95.

YENTSCH, C. S. & VACCARO, R. F., 1958. Phytoplankton nitrogens in the oceans. *Limnol. Oceanogr.*, Vol. 3, pp. 443–8.