Papers in Marine Biology and Oceanography, Suppl. to vol. 3 of Deep-Sea Research, pp. 110-114.

Experimental feeding of the copepod *Calanus finmarchicus* (Gunner) on phytoplankton cultures labelled with radioactive carbon (^{14}C)

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Summary—A small number of experiments were made on phytoplankton cultures labelled with ^{14}C; they confirmed earlier results obtained when using ^{32}P. With female *Calanus* the volume filtered varied from about 1–40 ml per day, and the digestion of a diatom and two flagellates lay between 50 and 80%.

Since both carbon and phosphorus are important constituents of the algal cell, it is fair to assume that the major part of the organic material is digested. It may be concluded therefore that, during a period of diatom abundance in the sea, most of the food ingested is utilized.

Qualitative work on the feeding of *Calanus* (Dakin, 1908; Esterly, 1916; Lebour, 1922; Marshall, 1924) has shown that it can ingest most of the small organisms present in the marine plankton, but that its diet consists predominantly of diatoms. It is possible that small naked flagellates are also important as food, but, since they have no indigestible skeleton, they can rarely be recognized in the gut, and not at all in the faecal pellets.

It has been possible to measure the quantity of different food organisms consumed by counting the numbers of these in a known volume of culture before and after a known period of feeding (Clarke and Gellis, 1935; Fuller and Clarke, 1936; Fuller, 1937; Clarke and Bonnet, 1939; Gauld, 1951). The earlier workers obtained values for the volume of water filtered of a few ml per day, but Gauld obtained an average of about 70 ml per day, using cultures of *Dunaliella* sp. (*Chlamydomonas*).

Recently some experiments have been carried out by Marshall and Orr (1955) on feeding *Calanus* with cultures of diatoms and flagellates labelled with radioactive phosphorus (^{32}P). These cultures were grown with a limiting quantity of phosphorus present, and were in most cases used after the phosphorus had been almost entirely taken up by the organisms. The initial concentration of ^{32}P in the culture was measured as well as that present at the end of the experiment in the body, faeces and eggs laid (if any). It was then possible to calculate the volume swept clear by the *Calanus* in 24 hours. This varied from less than 1 ml up to about 40 ml with a maximum in a single instance of a little over 80 ml.

The digestion of most of the food organisms was unexpectedly complete, the figures being usually over 60% and often over 90%. With a few organisms only was it apparently very low. It was high even when the *Calanus* were feeding rapidly in rich concentrations of food cells. These experiments show therefore that the phosphorus-containing portion of the algal cell is readily assimilated.

It seemed possible that the high digestion figures might be misleading, since various workers have shown that the phosphorus in algal cells is partly labile, and that this fraction may be either adsorbed on the cell surface or present in inorganic solution within the cell (Gest and Kamen, 1948; Kamen and Spiegelman, 1948; Goldberg,

WALKER and WHISENAND, 1951; RICE, 1953). The error is not likely to be large, for RICE has shown that in cultures a week old with a low phosphorus concentration only about 2% is exchangeable. Most of our cultures were used when more than a week old, and when the phosphorus in solution had fallen to a low value.

It was, however, thought advisable to measure the digestion of some of the same organisms labelled with other radioactive isotopes. One of the most important elements in the plant cell is carbon, and by growing cultures using ^{14}C it was possible to measure the uptake and digestion in a way similar to that used with phosphorus.

There are certain disadvantages in the use of ^{14}C which are not met when using ^{32}P. Carbon is present in sea water as carbonate, bicarbonate and CO_2, and it is never limiting so that the plant cells cannot be expected to remove all the carbon present. The cells have therefore to be removed and re-suspended in non-radioactive water before use. Again carbon is liberated as CO_2 in respiration while the culture is growing, and also during the experiments, and although the value of this second figure may be small it will reduce the amount apparently used.

METHODS

The method adopted was in general similar to that used in the earlier experiments with radioactive phosphorus. The cultures were grown in sterile 250 ml conical flasks, to which were added 100 ml Erdschreiber culture medium and 50 ml of algal culture. The radioactive carbon was added in the form of bicarbonate, and 50 μc was found to be a convenient quantity for this volume of culture medium. The flasks were kept airtight by means of rubber stoppers to avoid possible loss of ^{14}C to the air as CO_2. The cultures were allowed to grow either in diffuse daylight or close to a fluorescent tube. They grew rapidly and well, and took up an unexpectedly high proportion (over 50%) of the added ^{14}C in a few days. For use in an experiment the culture was centrifuged at about 2500 r.p.m. for five minutes, the supernatant liquid decanted, membrane-filtered sea water added, and the organisms dispersed. This process was repeated twice more, and the cells finally suspended in membrane-filtered sea water in a dilution suitable for the experiment. For filtering the sea water a Gradocol membrane was used with an average pore diameter of about 0·9μ.

Since the β-radiation of ^{14}C is relatively weak and readily absorbed, it is necessary either to correct for absorption or to make all the samples tested strictly comparable.

The second procedure was adopted. The methods used are discussed by CALVIN, HEIDELBERGER, REID, TOLBERT and YANKWICH (1949). For sea water samples, uniform flat-bottomed dishes (planchettes) of 25 mm diameter were used. With a 0·2 ml delivery pipette the sample was put in the middle of the planchette and one drop of N/10 NaOH added. A circle of lens tissue of a diameter slightly less than the bottom of the planchette was then laid on the sample to spread the drop evenly so that, on drying, a layer of uniform thickness would be obtained. Drying was carried out on a hot plate. An attempt was made to increase the accuracy by using 0·5 ml instead of 0·2 ml, but it was unsuccessful because the self-absorption was relatively greater than with 0·2 ml, and the samples dried less uniformly.

The object of adding the NaOH was to avoid the exchange of CO_2 between the air and the bicarbonate of the sea water. If this procedure is not adopted, there may be a serious loss, and variable results will be obtained (CALVIN, *et al.*, p. 123). The lens tissue should not be silicone treated, since this type tends to curl up on drying. There was no tendency to curl with the photographic lens tissue used, so that the addition of collodion was unnecessary.

To ensure that no change in absorption of the radiation was caused by variations in salinity, the same sample of sea water was used throughout the experiments.

The activity of each sample was measured by exposing it in a holder at a constant distance from the mica end-window of a G.-M. counter, and the counts were recorded on a scaling unit. The results are expressed as counts per minute, but since we know the concentration of cells in the culture and the activity of both whole culture and filtrate, we can also express them as cell equivalents.

The bodies and faecal pellets of the *Calanus* have an appreciable self-absorption, and even after they had been torn up by needles the losses were considerable. Good duplicates were obtained by

the use of a small disintegrator. This consisted of a narrow tube (diameter 9 mm) about 7 cm long, into which was fitted a perspex piston of almost the same diameter as the tube, and shaped at the foot to fit the bottom of the tube. The *Calanus* or the faecal pellets were put in the tube in about 0·5 ml of sea water, and the piston rotated by a small motor. By raising and lowering the tube the water and the *Calanus* or faecal pellets were forced past the rotating piston and thus disintegrated. After washing down the piston and tube and making the volume up to 3 ml, five aliquot samples were taken for measurement of activity. Microscopical examination showed that disintegration was almost complete. No fragments of a *Calanus* body could be recognized, and there were very few recognizable bits of faecal pellet. The disadvantage of the disintegration method is the dilution of the activity, since only 0·2 ml samples were used from the 3 ml of fluid. This can be countered by taking more sub-samples, or by making much longer counts.

In a feeding experiment a number of bottles of about 70 ml capacity were filled with the diluted culture prepared as described, and sampled for a count of cell number and activity. Into each bottle was introduced a single *Calanus*. Since females feed better than either Stage V or male *Calanus* they were always used. Each bottle was then tied in a black cloth bag (because *Calanus* feeds better in the dark than in the light), and attached to a wheel revolving about once every three minutes in a vertical plane. This keeps the culture cells from sinking to the bottom and so giving the *Calanus* an accumulation to feed on. Control bottles containing filtrate from the culture used were also set up to measure any uptake of ^{14}C from solution. Other control bottles, containing culture but no *Calanus*, were used to measure the ^{14}C returned to solution by the respiration of the plant cells.

After leaving them to feed for a suitable time, usually 15 to 18 hours, each *Calanus* was removed, washed three times to free it from radioactive water, disintegrated and sampled. The contents of the bottle were then poured into a flat-bottomed perspex dish with the inside angles bevelled, and the faecal pellets picked out under a binocular microscope. These too were washed, disintegrated, and sampled and their activity was measured.

The activity of the *Calanus* body added to that of the faecal pellets gives a measure of the total ^{14}C removed from the culture. From these and the culture reading can be calculated the number of cells ingested, the percentage digested and the volume of water swept clear. The results can be expressed either as counts per minute or as cell equivalents. The cell equivalent is a useful figure when considering the total amount taken up, but if we express the activity of the faecal pellets as cell equivalents, it must be remembered that each " cell equivalent " really represents several cells, the number varying according to the percentage digested.

EXPERIMENTAL WORK

Feeding experiments with female *Calanus* were made, using cultures of the diatom *Skeletonema costatum*, one of the more important spring diatoms in the sea, and the flagellates *Cryptomonas* sp. (Plymouth strain 23) and *Syracosphaera carterae*. The cultures when used were only a few days old, and were probably in the exponential growth phase. It was thought that some of the ^{14}C might be present in the inorganic form, either adsorbed on the cells or in solution inside. This was tested with *Skeletonema* by exposing samples on planchettes to the fumes of hydrochloric acid (STEEMANN NIELSEN, 1952), and comparing the activity before and after exposure. About 7% of the total disappeared with this treatment. With *Syracosphaera*, which possesses large numbers of calcareous coccoliths, the loss seemed to be greater but accurate measurements were not made.

The control bottle containing culture but no *Calanus* showed that, as a result of the respiration of the plant cells, the ^{14}C content of the filtrate had risen by 2–5%. This will cause a slight underestimate of the amount taken up by the *Calanus*.

A preliminary experiment was done with a culture of *Skeletonema costatum* in two different concentrations. It was thought that the activity of the faecal pellets and, in the lower concentrations, of the bodies also, would be too weak for the disintegration method, so they were torn up as finely as possible with needles and put in 0·2 ml

of sea water on a planchette. Owing to self-absorption, this was not a satisfactory method, giving results which were too low, and it was therefore impossible to calculate the percentage digested. If we assume that this is the same as in a later experiment (see Table I), a figure can be obtained for the volume filtered which will be approximately correct for the richer concentration but minimal for the weaker. The estimated volume filtered in 24 hours in a concentration of 106,000 *Skeletonema* cells per ml varied from 1·6 to 4·0 ml in 24 hours, and in a concentration of 10,600 cells per ml from 5·7–42·5 ml. It is usual to find that in high concentrations of food cells filtration falls off, and the figures obtained compare well with our earlier experiments using ^{32}P.

Table I
Feeding experiments with Calanus using cultures grown with ^{14}C

Species	*Concentrations*	*Calanus* ♀	*Time of expt. in hours*	*Faecal pellets No.*	*Faecal pellets Counts/min*	*Body less filtrate c/min*	*Total removed c/min*	*% used*	*ml filtered in 24 hr*
Skeletonema costatum	1615 counts/ml/min	1	17½	54	278	817	1095	74·6	0·90
	144000 cells/ml	2	17½	17	192	283	475	59·6	0·38
Culture	0·01 counts/cell	3	17½	47	301	673	974	69·1	0·81
		4	17½	0	—	0	0	—	—
		5	17½	10	59	119	178	66·9	0·14
Filtrate	35 counts/ml/min	A	17½	4	36	38 } 43			
		B	17½	0	—	48			
Cryptomonas	1275 counts/ml/min	1	21½	38	170	585	755	77·5	0·66
	12600 cells/ml	2	21½	51	465	780	1245	62·7	1·10
Culture	0·10 counts/cell	3	21½	80	449	811	1260	64·4	1·13
		4	21½	56	220	454	674	67·4	0·58
		5	21½	57	516	589	1105	53·3	0·98
Filtrate	60 counts/ml/min	A	21½	2	—	0			
		B	21½	Lost	—	0			
Syracosphaera carterae	840 counts/ml/min	1	17	65	289	930	1219	76·3	2·07
	13500 cells/ml	2	17	38	86	180	266	67·7	0·45
Culture	0·06 counts/cell	3	17	45	392	885	1277	69·3	2·16
Filtrate	8 counts/ml/min	A	17	0	—	30			

The results of a second experiment with *Skeletonema* are shown in Table I. In this and subsequent experiments all the bodies and faecal pellets were disintegrated. As is usual in feeding experiments, individual variation was considerable; the number of faecal pellets varied from 10–54, and one *Calanus* did not feed at all. The amount digested varied from 60–75%, a figure very similar to that obtained with cultures labelled with ^{32}P. Unfortunately the *Calanus* were in poor condition and the volume filtered, less than 1 ml in 24 hours, was low. The activity of the culture was not high enough to give very accurate results. In both experiments with *Skeletonema*, the cell concentration was high compared with what is found in the sea.

In an experiment with *Cryptomonas*, which has cells about 20μ long, all the females fed well, although they filtered on an average only about 1 ml in 24 hours (Table I). The digestion ranged from 53–78%, as compared with 51–89% in cultures labelled with ^{32}P.

Finally, the experiment with *Syracosphaera* was carried out using three *Calanus* in a concentration of 13,500 cells/ml. Judging by faecal pellet production, they fed

well, but the maximum volume filtered was just over 2 ml in 24 hours (Table I). The digestion (68–76%), was decidedly lower than that found using *Syracosphaera* labelled with ^{32}P, in which it was usually over 90%. This may be due to the presence on its surface of a layer of coccoliths which would contain ^{14}C in the form of calcium carbonate.

The rate of production of faecal pellets was not high in any of the experiments with ^{14}C, the most rapid being found in a *Calanus* in *Skeletonema* culture, which produced an average of one every twelve minutes. It has been found that even with a much more rapid production, digestion of the phosphorus-containing fraction remains high.

ACKNOWLEDGEMENTS

The experiments with ^{14}C labelled cultures were carried out at The Laboratory, Plymouth, and we are very grateful to the Director and staff for their help. More especially we wish to thank Dr. H. W. Harvey and Dr. B. C. Abbott for their advice and assistance, and Dr. M. W. Parke and Miss D. Ballantine for supplying us with cultures.

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