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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

VIII. FOOD UPTAKE, ASSIMILATION AND EXCRETION IN ADULT AND STAGE V *CALANUS*

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(Text-figs. 1–4)

Introduction

Apart from a few scattered observations (Hensen, 1887; Gran, 1902) the study of the food of *Calanus* began with Dakin (1908). He examined the remains present in the gut, and this was the method used also by Esterly (1916), Lebour (1922) and Marshall (1924). They all found that these remains consisted of a greenish mush containing the skeletons of numerous planktonic organisms, chiefly diatoms and dinoflagellates. Naked flagellates were, however, occasionally seen, and it was realized that the food might in reality consist largely of organisms which had no skeleton and could leave no recognizable remains.

The feeding of *Calanus* can also be studied in the laboratory by keeping the copepods in a suspension of food organisms and seeing if the organism is taken in, and if so whether or not the animal thrives on it. Such experiments have been made by numerous workers (Crawshay, 1915; Clarke & Gellis, 1935; Fuller & Clarke, 1936; Fuller, 1937; Harvey, 1937; and Clarke & Bonnet, 1939). Their criterion for assessing the value of an organism as food was usually either survival time or successful moulting. In female *Calanus* the food value of different organisms can also be tested by their effect on egg production (Marshall & Orr, 1952).

A simple method of finding out whether the food has been taken in or not is by observing whether faecal pellets are produced. This method was first used by Raymont & Gross (1942), and some data were also given by Harvey (1942).

In *Calanus* digestion takes place in the wide anterior part of the gut, and as the food mass passes into the narrow posterior part it is gradually compacted into a cylindrical pellet. When this is ejected, it is surrounded by a delicate pellicle which is sometimes produced into a 'tail' at one end. The pellicle may correspond to the chitinous one described by Forster (1953) on the faecal pellets of decapods.

Descriptions of the variation in size and shape of *Calanus* faecal pellets on several different foods are given by Raymont & Gross (1942) and Marshall & Orr (1955), together with some information on the numbers produced and the differences between male, female and Stage V. The size and shape of faecal pellets show great individual variation; indeed in an experiment in which two *Calanus* had been introduced by mistake into one dish, the two sets of pellets were easily distinguishable. The shape and size also depend on the food from which they are produced. With *Dunaliella* they are short and dark with an average length of about 500 μ for females and Stage V, and about half this for males. In general those produced with flagellates are dark but with *Dicrateria* and *Hemiselmis* the pellets tend to be smaller and paler than those with *Dunaliella*. With *Chromulina* the pellets are very sticky and difficult to deal with. With *Chlorella* they tend to fluff up and disappear. With diatoms the pellets are long and pale in colour; for example, with *Skeletonema* they average in females 950 μ in length. When *Calanus* is fed on thecate dinoflagellates such as *Prorocentrum* and small *Peridinium* spp. the presence of unbroken shells in the pellets gives them a characteristic granular appearance.

The size of the pellets is unaffected by the concentration of the food unless this is low, e.g. with a concentration of *Dunaliella* of 8 cells per mm^3, they measured about 300 μ instead of the usual 500 μ (Fig. 1 and Table V). When food is very scarce indeed a few pellets are still produced but these are 'ghosts' being very small and almost transparent.

In one experiment (Table I) 13 *Calanus* were kept individually in a food culture and examined at frequent intervals. The different females had different rates of faecal pellet production but these remained fairly constant over the 24 h.

Methods

Two methods were adopted to study the food uptake of *Calanus*. The first was to count the number of faecal pellets produced under standard conditions. The individual variations can be very great, and to avoid this source of error experiments were made with duplicate or triplicate samples of 20 *Calanus*, each in a bowl containing about 400 ml. of the culture to be tested. The number of faecal pellets in aliquot samples was counted after a period of about 16–24 h. The volume per *Calanus*, 20 ml., was later found to be too low for optimum results (see p. 502).

Since the amount of food taken varies with the developmental stage and the season of the year, *Dunaliella* was used as the standard food and other cultures compared with it. When different species were compared as food an attempt was made to provide equal volumes of cell substance in the cultures used. This method, however, was superseded by the second, and results obtained by its means are used only to confirm or supplement later observations.

The second method was to use radioactive phosphorus (^{32}P) as a tracer

element. Cultures of many phytoplankton organisms can be grown using ^{32}P as part source of the phosphorus necessary and these can in turn be fed to *Calanus*. The estimation of ^{32}P is rapid and extremely sensitive, and by this means the uptake, assimilation and excretion of *Calanus* can be accurately measured. Carrier-free orthophosphate in dilute hydrochloric acid was used as supplied by the A.E.R.E., Harwell, and from 0·25–2·0 mc was added to each litre of culture solution. When growth was good this gave a reasonably high activity by the time the cultures had reached a stage suitable for use. Many of the cultures were grown using only dilute modified Miquel solution (Ketchum & Redfield, 1938), but to others soil-extract or other growth-promoting substances were added. The cultures were considered suitable for experiment when most of the ^{32}P had been taken up by the cells. Phosphorus is an important limiting element for plant growth in the sea, and is also important in animal nutrition. It is present in all the major food constituents and should therefore give a good idea of the assimilation but some caution is necessary in interpreting the results.

It has been shown by various workers (Kamen & Spiegelman, 1948; Gest & Kamen, 1948; Goldberg, Walker & Whisenand, 1951; Rice, 1953) that when algae are grown in phosphate-rich media, some of the phosphorus in the culture organisms may be labile or very loosely bound. Rice has shown that in cultures with low P concentrations, only about 2% is exchangeable after they are a week old. In our cultures phosphorus was a limiting factor for growth, and as a rule they were not used until the ^{32}P in solution had dropped below 10% and sometimes below 5% of the total. On a few occasions when there was a considerable proportion of the ^{32}P in the filtrate, the organisms were filtered off or centrifuged, washed and re-suspended in membrane-filtered natural sea water.

Since it has been stated that the loosely bound phosphorus can be removed by washing, an experiment was made to find whether the percentage assimilated of the phosphorus in the culture was significantly lower after repeated washing. A culture of *Lauderia* was centrifuged (10 min at 2500 rev/min), the supernatant liquid siphoned off and the cells re-suspended in filtered sea water. This was done four times and the resulting suspension compared as food with the original, uncentrifuged culture, using the method described below. Since two-thirds of the cells had been lost, the original culture was diluted to give approximately the same cell content. The results are shown in Table II. In the centrifuged culture the percentage utilization varied from 87·2 to 93·5, in the untreated from 86·9 to 91·3, an insignificant difference.

After a considerable amount of trial and error, the following method was adopted.

Calanus, usually adult females, were picked out from a tow-netting and kept starved for several days in a cool room in the dark. It was found that they fed better and behaved more uniformly under such conditions than if they were

taken from the sea and used for an experiment the same day. The number of cells in the radioactive culture was estimated, and dilutions made by adding sea water which had been filtered through a 'Gradocol' membrane of average pore diameter 0·9 μ. The usual dilutions were in the ratio 100:10:1. Five bottles of about 70 ml. capacity were used for each dilution and a single *Calanus* introduced into each. In addition, two bottles were filled with filtrate from the strongest by filtering it through a double layer of Whatman no. 42 paper. This was usually sufficiently fine to remove all the organisms. A *Calanus* was also added to each of the bottles with filtrate. When *Calanus* are kept in a solution containing inorganic ^{32}P, some of this is taken up in the body. The results from the two control *Calanus* gave a measure of this, enabling a correction to be made if necessary.

Each bottle was then tied in a dark cloth bag and attached to a wheel which was rotated slowly in a vertical plane (about one revolution in 2–3 min) to avoid settling out of the food organisms. The slow rotation is not likely to have any effect on the behaviour of the *Calanus*. Samples of the culture dilutions and of the filtrate were taken in triplicate or quadruplicate with a 0·2 ml. delivery pipette and dried on a metal disc (planchette) for measurement of the activity.

After a period, usually of about 16–24 h, the experiment was stopped. The *Calanus* was removed from each bottle, washed in three changes of sea water, transferred to a disc in a small drop of water, roughly torn up to avoid self-absorption of the ^{32}P and dried slowly on a warm plate. The radioactivity was then measured. On many occasions a sample of the final wash water was also tested.

The contents of each bottle in turn were then transferred to a rectangular Perspex dish with the inside angles bevelled to facilitate examination of the whole of the bottom. The dish was placed on a Perspex sheet marked off in squares and the faecal pellets and eggs (if present) removed under a binocular microscope. These also were washed three times, transferred to discs and dried for measurement of the activity. A sample of the final wash water was tested in each case.

In some experiments many of the faecal pellets were found broken or crushed at one end. It seems as if the *Calanus* must encounter a considerable number of them when swimming and must damage them. Any appreciable loss of ^{32}P after the pellicle is broken might cause a serious error in the results. To find if this was important nine sets of faecal pellets (20–60 in each) some whole and some broken, were left overnight on coverslips in a drop of water. In the morning the water was withdrawn and tested as well as the faecal pellets. The radioactivity of the water was found to be only just over 1% of that of the pellets and the loss is therefore negligible. When faecal pellets are broken, small fragments may be lost during washing, and although this loss was never serious, it will tend to make digestion results appear higher.

A description of the method of measuring the radioactivity is given in this *Journal* by Spooner (1949). The discs were exposed in a lead castle at a fixed distance from the aluminium end-window of a Geiger-Müller counter and the impulses counted on a scaling unit. Corrections were made for quenching time, distance from the end-window, background and, when the experiment lasted for more than a day, for decay as well. The results can be expressed either as number of pulses per minute, or, since the activity of the culture and the number of cells are known, as cell equivalents.

In Table III are shown the results of a typical experiment with *Skeletonema costatum* culture. It shows a number of characteristic features. In the first place there is a considerable variation in the behaviour of the individual *Calanus*. Some fed well, some not so well and one not at all. Secondly, the volume filtered was much lower at the highest concentration. The number of faecal pellets was approximately the same at the two higher concentrations and considerably lower in the lowest. The cell equivalents per faecal pellet were approximately the same throughout, although this was not often the case in other experiments. The percentage of the phosphorus-containing portion of the diatom utilized showed no significant difference at the different dilutions.

The rate of filtration, F, of the *Calanus* can be calculated (Gauld, 1951) according to the formula

$$F = v \frac{\log_{10} C_0 - \log_{10} C_t}{t \log_{10} e},$$

where v is the volume of the bottle in ml., t the duration of the experiment in days, C_0 the original and C_t the final concentration of the food organisms. The initial concentration C_0 was obtained by measuring the activity in aliquot samples of the culture used and the final concentration C_t was obtained by subtracting from this figure the activity in the body, eggs and faecal pellets of the *Calanus*.

It should theoretically be possible to obtain the same results by measuring the activity in an aliquot sample from the bottle at the end of the experiment or by counting the cells in the bottle at the beginning and end of the experiment. The last method is less accurate because the error of counting cells is relatively high and the count has to be multiplied by a large figure owing to the smallness of the sample. Similarly, by counting the pulses in the culture at the end by the usual method an error within the normal variation of the count may sometimes cause a large difference in the calculated volume filtered. Since the substance of the cells removed from the culture is concentrated in the body, eggs and faecal pellets of the *Calanus*, this figure is not subject to the same error.

The results of an experiment with *Skeletonema costatum*, in which all three methods were used, is shown in Table IV. The cells were pale and the counts were not very satisfactory; the ten initial counts (each of 3·2 mm^3) varied from

24 to 53, although the final counts were more uniform. Using the final cell counts of the three samples in which filtration was highest, the figures are of the same order of size as by the normal method. For counting the activity of the culture at the end a Veall liquid counter (1948) was used with 2 ml. diluted to 10 ml. in each case. The three sets of figures (ml. filtered in 24 h) show a rough agreement but that based on the activity of the *Calanus* body and faecal pellets is likely to be the most accurate.

In other balance experiments, using *Skeletonema costatum*, *Chaetoceros decipiens* and *Syracosphaera elongata*, the agreement was even less satisfactory.

Effect of Different Factors on Food Uptake

Concentration of food

If *Calanus* is purely a filter feeder the number of faecal pellets produced should rise with increasing concentration of food. This has been noted in the sea for copepod pellets in general (Harvey, Cooper, Lebour & Russell, 1935).

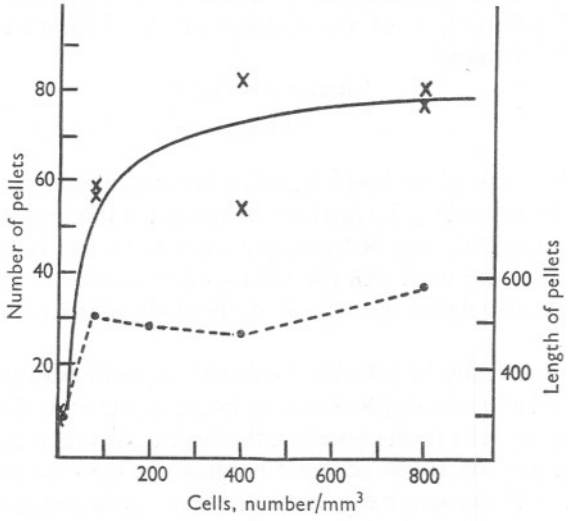

Fig. 1. Effect of concentration of *Dunaliella* on faecal pellet production. ——, number of pellets produced in 24 h; - - -, length of faecal pellet in μ.

Many experiments were made by keeping *Calanus* in bowls containing varying concentrations of the same culture, the number of faecal pellets produced being counted at the end of the experiment. The number usually did increase with increasing concentration but only up to a certain point varying with the organism used. Above this point the number did not increase regularly. The result of one experiment with *Dunaliella* is shown in Fig. 1 and Table V.

It appears that if *Calanus* is a filter feeder, it is not an automatic one but is able to slow down or stop feeding in high concentrations. This confirms observations made by Gauld (1953) that *Calanus* can remain for a long time in a thick suspension of cells, even swimming normally, without feeding.

In most of these experiments there was a tendency for the organisms to settle out or, in the case of some flagellates, to aggregate. The *Calanus* too, especially the females, have a habit of sinking to the bottom of the vessel and rummaging about there, and in dishes where there has been settling out they will get more than the calculated amount. This source of error was avoided in the later experiments with labelled phosphorus by putting the *Calanus* in closed bottles on a rotating wheel.

In a poor concentration of food where few cells are available they will often ingest threads from filter-paper or other such objects and the number of faecal pellets will be higher than one would expect.

When different concentrations were used in an experiment with labelled cultures the highest was much in excess of what is ever found in the sea; the next strength, 10% of this, was equal to or greater than what would be a rich concentration in the sea. Finally it was hoped that the third strength (usually 1%) would represent a moderate concentration in the sea.

In the highest concentration the volume filtered was often lowest. The number of faecal pellets produced in the 10% was always more than a tenth of that in the 100% and was often nearly as high. In the 1% the number produced was usually more in proportion to the concentration of cells, but it was often very low and with a very small number of faecal pellets the loss of one can make a considerable difference to the results. Figures for the weakest concentration may therefore be less accurate; the percentage digested will appear higher and the volume filtered lower than they should be.

To study the feeding of *Calanus* in very low concentrations of cells, such as are found in the sea in winter, a culture of *Chaetoceros decipiens* was grown with a quantity of ^{32}P much larger than usual (1 mc in 200 ml.). With this culture much greater dilutions could be used and reliable counts of pulses still obtained. Even in concentrations as low as 1 cell per ml. and in bottles of 150 ml. capacity the volume filtered in 24 h remained low (under 10 ml.). Similar results were obtained with a much larger diatom, *Ditylum brightwellii* (Table XIV).

Temperature

Most of the experiments were done at room temperature which varied from 10 to 20° C and was always somewhat above sea temperature at the same time. By counting the number of faecal pellets produced by *Calanus* kept at temperatures varying from 5 to 15° C it was found that there was usually a rapid rise in numbers between 5° and 10° C and a lesser rise between 10° and 15° C but the results were variable.

Volume of Container

It was found that the volume of the vessel in which the *Calanus* were kept had an effect on their feeding. In one experiment 15 females were kept individually in dishes of three different volumes (15, 50 and 100 ml.) containing the same culture of *Skeletonema*. These were not stirred during the 16½ h of the experiment. The *Calanus* showed considerable individual variation, but the volumes filtered in the 15 ml. dishes were decidedly lower (average 1·3 ml./24 h) than in the 50 or 100 ml. dishes (average 7·8 and 7·9 ml./24 h). Later experiments were therefore done in bottles of 70 ml. capacity or, in a few cases, 35–40 ml.

Light

It is well known that sunshine or even diffuse light out of doors is lethal to *Calanus* (Huntsman, 1924; Klugh, 1929, 1930); it also affects the respiration (Marshall, Nicholls & Orr, 1935). The harmful effect of bright light reduced the uptake of food in *Calanus* exposed to it (as judged by faecal pellet production), but those of the first generation of the year, which in the Clyde sea area live at the surface, are more resistant to its effects than those of the overwintering generation.

Experiments with radioactive cultures of *Syracosphaera* and of *Lauderia* showed that even in diffuse light indoors the volume of culture filtered was less than with *Calanus* kept completely in the dark. The results with *Syracosphaera* are shown in Table VI. These experiments were done in February in a well-lit room with windows on both sides and overhead, whereas before this all the radioactive feeding experiments had been done well away from the window in a north-facing room. The rather dim diffuse light there is not likely to have affected the *Calanus* appreciably, but subsequently all the experimental bottles were tied in black cloth bags. Since *Calanus* feeds more in the dark than in the light it appears that they do not catch their food organisms by sight.

Age of Culture

It is known that the chemical composition of the cells in a culture may vary with age and with the nutrients present. It is only during the exponential stage of growth that the composition remains approximately constant (Fogg, 1953). Our cultures were almost all grown in sea water enriched with a modified Miquel solution, but it was not possible always to use them at the same age and the amount digested may vary with the growth of the culture.

A number of experiments were made with a single culture of the diatom *Lauderia borealis* at different stages of its growth, and Table VII shows that the percentage utilized decreased with age. The dilutions used in the experiment varied but this did not affect the utilization appreciably. Finally, the old culture was compared with a new young culture of approximately the same

cell concentration and the utilization of the latter was found to be a good deal higher.

In a young diatom culture during the phase of rapid multiplication the cells remain suspended, and it is during this period that the utilization by *Calanus* is high. With the *Lauderia* used this period was about 5 days. The cells then sank to the bottom and became smaller, paler in colour and were united in shorter chains than in a young culture. The change in the amount assimilated indicates that there is a corresponding change in the phosphorus-containing substances built up by the cell.

A later series of experiments with a culture of *Chaetoceros decipiens* up to 18 days old showed only a very slight effect. Similar experiments on a culture of a coccolithophore *Syracosphaera elongata* 16 and 33 days old showed no fall in digestibility, although by the thirty-third day the cells were depositing coccoliths in quantities.

Size of Food Cells

In discussions on the question of the food organisms available and suitable for *Calanus* in the sea much stress has been laid in recent years on the minute flagellates which form an important food of pelagic organisms such as larval molluscs. It was found by Ussing (1938) that the minimum distance between the finest setules on the maxillule in female *Calanus* was 5·7 μ. He suggested that the smallest organisms *Calanus* could filter off must have their greatest diameter more than this. Raymont & Gross (1942), however, kept *Calanus* alive and healthy on a diet of minute flagellates. The possible importance of bacteria also was suggested by Clarke & Gellis (1935), though later Fuller & Clarke (1936) concluded that bacteria were not important as food. The concentrations used were higher than would be expected in the sea.

Various organisms of different sizes were used in feeding experiments on *Calanus* (see Tables XIV–XVI), and the volumes filtered in 24 h are shown in Fig. 2. It is apparent that for organisms below a size of about 10 μ there is a very much lower filtration rate than for organisms of greater size. In the experiments with very small flagellates it was noted that the volume filtered was often greatest in the highest concentration instead of in the lowest as is more usual. This is what might be expected if the cells in a very high concentration gradually clogged up the filtering setae. In calculations of the volume filtered a complete filtration of the food organism is assumed and if it can to any extent pass the filter the calculation is unreliable.

A possible explanation of the failure to take small flagellates might be that they were all unpalatable to *Calanus*, and a search was made for some inert particle of uniform size which could be used instead. Indian ink and carmine are both ingested to a certain extent but the size of the particles is very variable. Dr D. W. Henderson of the Experimental Station, Porton, kindly

supplied us with a suspension of radioactive spores of *Bacillus globigii*, 0·7 μ^3 in volume, and Table VIII shows the results of an experiment made with them. Before use the suspension was washed twice by centrifuging and then resuspending in membrane-filtered sea water. It will be seen that the volume

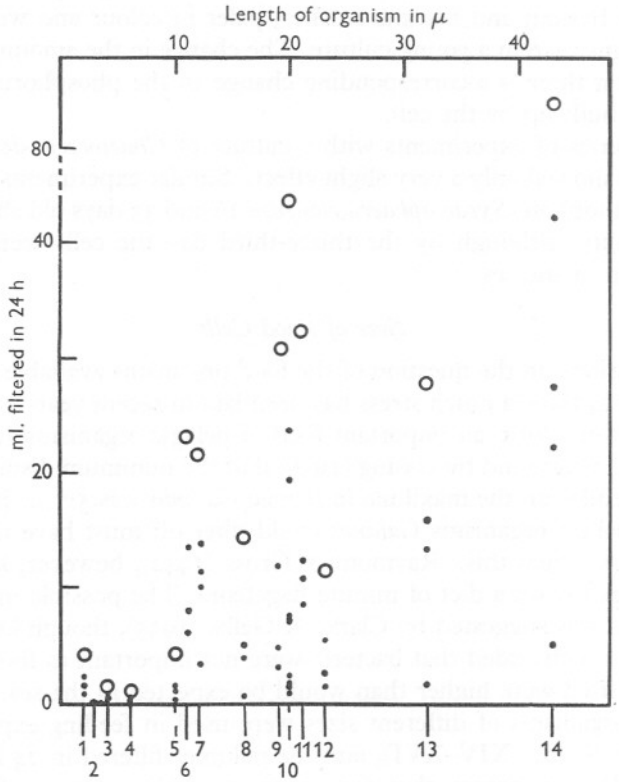

Fig. 2. Relation of size of food cell to volume filtered. •, average with each concentration used in feeding experiments; ○, maximum volume filtered by a single *Calanus*. (1) *Chromulina pusilla*, (2) *Nannochloris oculata*, (3) *Dicrateria inornata*, (4) *Monochrysis lutheri*, (5) *Prymnesium parvum*, (6) *Gymnodinium vitiligo*, (7) *Prorocentrum triestinum*, (8) *Gymnodinium veneficum*, (9) *Chlamydomonas pulsatilla*, (10) *Syracosphaera elongata*, (11) *Cryptomonas* sp., (12) *Platymonas carteriaformis*, (13) *Oxyrrhis marina*, (14) *Prorocentrum micans*.

filtered is extremely low. The small amount apparently digested is probably due to the spores and faecal pellets still in the gut of the *Calanus* at the time it was killed. It is clear that *Calanus* is unable to filter such a small particle efficiently.

Dr M. R. Droop suggested that although the small flagellates might not be taken directly, they might be eaten by larger flagellates which in turn can be eaten by *Calanus*. A culture of the small alga *Nannochloris* (2–4 μ in diameter)

which was itself very little used by *Calanus* was inoculated with a larger flagellate *Oxyrrhis*, which rapidly increased in numbers by feeding on the *Nannochloris* and was then used in a feeding experiment on *Calanus* (Table XVI). The volume filtered and the percentage assimilated showed that the *Nannochloris* could thus be utilized indirectly.

Harvey suggested in 1937 that in a mixture of large and small cells *Calanus* would select the large, and some experiments were made with labelled cultures to test this. The *Calanus* were given a mixture of a very small flagellate (*Dicrateria inornata* 3–5·5 μ) and a larger organism. Previous experiments had shown that in a unialgal culture of *Dicrateria*, *Calanus* produced only a few faecal pellets and filtered only a small volume of water. Feeding was compared in Dicrateria alone, in a mixture of *Dicrateria* of about the same concentration with a large diatom *Lauderia*, and in *Lauderia* alone. Since only the *Dicrateria* was radioactive, feeding in *Lauderia* could be measured only by the number of faecal pellets produced.

The results are shown in Table IX. When *Lauderia* was present nearly five times as many faecal pellets were produced as with *Dicrateria* alone, the amount of *Dicrateria* assimilated was nearly four times as much and over five times the volume of water was filtered. This could be explained by supposing that the *Calanus* in its preference for *Lauderia* was unable to reject the *Dicrateria* which was with it. An alternative explanation is that *Dicrateria* is too small to be efficiently filtered, but that the mucus exuded in cultures of diatoms entangles the cells so that they are ingested with the diatoms. That the second is the more probable explanation was shown by an experiment using *Prorocentrum micans* instead of *Lauderia*. This dinoflagellate exudes much less mucus than *Lauderia* and although the quantities used were greater, the difference between the unialgal and the mixed culture was not marked. With these organisms then it seems that *Calanus* is unable to select the larger from a mixture.

Another experiment was made with *Ditylum brightwellii* and *Chaetoceros decipiens*, two of the genera used by Harvey. Parallel sets were used, one having radioactive *Ditylum* and non-radioactive *Chaetoceros*, the other having radioactive *Chaetoceros* and non-radioactive *Ditylum*. The *Ditylum* used was about fifty times the volume of the *Chaetoceros*. If *Calanus* had shown a preference for the larger of the two diatoms, the volume filtered in the set with radioactive *Ditylum* should have been much the greater, but as will be seen in Table X there is no marked difference between the two.

Choice of Food

To find out whether *Calanus* could acquire a preference for one species rather than another, irrespective of size, a series of experiments was made by keeping *Calanus* in two different kinds of food, and after a few days putting

each set in a mixture of the two foods. Organisms were chosen whose skeletons could be easily recognized in the faecal pellets, and the first experiments were done by examining these faecal pellets.

When a *Calanus* is transferred from one kind of culture into another the change over in the skeletons in the faecal pellets may not be complete until the third pellet has been extruded.

In a qualitative experiment using *Calanus* which had been kept in cultures of *Coscinodiscus centralis* and *Prorocentrum micans* it was found that after some hours in a mixture of the two both lots had taken *Prorocentrum* freely, but that *Coscinodiscus* fragments were present in about two-thirds of the faecal pellets of those fed originally on *Coscinodiscus* and in only one-fifth of those fed on *Prorocentrum*. The diatoms sank to the bottom of the dish while the flagellates swam freely and this may have affected the results. After 3 days, however,

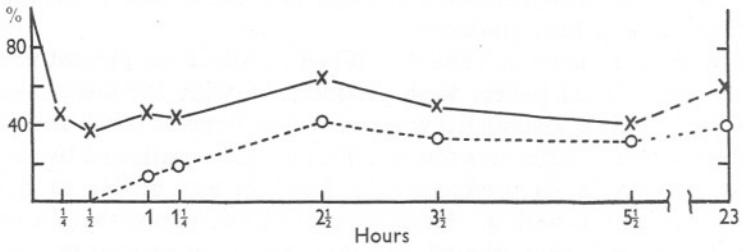

Fig. 3. Choice of food experiment. *Calanus* feeding in a mixture of *Peridinium trochoideum* and *Prorocentrum micans*. ——, % *Prorocentrum* in the faecal pellets of those accustomed to *Prorocentrum*; - - -, % *Prorocentrum* in the faecal pellets of those accustomed to *Peridinium*.

there was no difference between the pellets from the two lots. These experiments suggest that there may be for the first hour or two some power of selection.

Two experiments were then carried out in a similar way comparing Stage V *Calanus* accustomed to *Prorocentrum micans* and *Peridinium trochoideum* respectively. The results were similar but only the second, in which improvements in method were made, will be described (Table XI and Fig. 3). The *Calanus* which had been kept for 10 days in their respective cultures were put singly into dishes containing about 15 ml. of a mixture with about 900 cells of *Prorocentrum micans* and 3900 cells of *Peridinium trochoideum* per ml. This mixture was chosen in an attempt to give approximately equal volumes of each food. The dishes were examined at frequent intervals and the faecal pellets produced by each *Calanus* removed (Table I). These were squashed individually under a coverslip and the skeletons of both organisms counted. The skeletons as a rule remained whole and were easily counted, but occasionally they were so numerous or so many had split into halves that the number was not very accurate. When the number of faecal pellets was small all were

examined but when it was large a sample of three was taken from each *Calanus* and the total number calculated. Individual variation in feeding rate and in the proportions eaten was marked and the average is therefore given. As Fig. 3 shows, there is a considerable difference in the behaviour of the two sets. Those accustomed to *Prorocentrum micans* ate equal numbers of the two species from the first half hour onwards whereas those accustomed to *Peridinium trochoideum* did not begin to eat *Prorocentrum micans* till after half an hour had elapsed and even then the proportion was low. After $2\frac{1}{2}$ h they reached a fairly steady state. The numbers and not volumes are shown in Fig. 3 and Table XI. If we allow for volume there appears to be a preference for *P. micans*, much more marked in those accustomed to *P. micans*.

The number of organisms whose skeletons remain whole and can be counted easily in faecal pellets is very limited, but by growing cultures with ^{32}P one can use foods which cannot be thus recognized. A much more accurate measure of the cells taken up can also be made.

Feeding experiments were done by keeping *Calanus* in two different food cultures and then measuring their uptake in a mixture of the two, only one of which was radioactive. One such experiment compared the utilization of a large diatom (*Lauderia borealis*) with a small (*Skeletonema costatum*). From measurements of the cells in the culture it was calculated that a *Lauderia* had 122 times the volume of a *Skeletonema* cell. Six of each set of *Calanus* were kept in the mixture for $\frac{1}{2}$ h, six for 1 h, six for 2 h, and six for 16 h. A summary of the results is shown in Table XII, and it is clear that there is no significant difference between the two sets.

A similar experiment was made with *Calanus* (fed for a week previously) using *Lauderia* and radioactive *Syracosphaera*, and again there was no significant difference between the two. In a third experiment using radioactive *Lauderia* and *Cryptomonas* (Table XIII) the *Calanus* which had been kept in *Lauderia* did for the first hour eat more *Lauderia* than those kept in *Cryptomonas*, although this difference was not observed in the 2 h samples.

The results of the experiments with ^{32}P do not confirm either Harvey's observations or the results we obtained by counting skeletons in faecal pellets. If selection takes place it does not do so to a marked degree. It is difficult to understand the mechanism of selection since, as has already been mentioned, *Calanus* feeds better in the dark than in the light.

Utilization of Different Species

A variety of different organisms was tested to find their value as food. They were chosen from the main groups found in the phytoplankton and included a variety of size from the 1–3 μ of *Chromulina* to the 100 μ or so of *Coscinodiscus*. Special attention was paid to the very small flagellates since it has often been suggested that they may be of outstanding importance as food for copepods.

Some of these are open sea forms and others were isolated from shore pools.

The following organisms were used:

CHLOROPHYCEAE

Dunaliella sp. (Plymouth strain 81)
Chlamydomonas pulsatilla Wollenweber
Platymonas carteriaformis nom. prov. (M. R. Droop's strain 10)
Chlorella stigmatophora Butcher (Plymouth strain 65)
Nannochloris oculata Droop (M. R. Droop's strain 66)

CHRYSOPHYCEAE

Chromulina pusilla Butcher (Plymouth strain 90)
Monochrysis lutheri Droop (M. R. Droop's strain 60)
Dicrateria inornata Parke (Plymouth strain B)
Pseudopedinella sp. (Plymouth strain 91)
Coccolithus huxleyi (Lohmann) Kamptner (Plymouth strain 92)
Hymenomonas carterae (Braarud & Fagerl.) Braarud (Plymouth strain 17)
Syracosphaera elongata Droop (M. R. Droop's strain 62)
Prymnesium parvum Carter (M. R. Droop's strain 65)

BACILLARIOPHYCEAE

Coscinodiscus centralis Ehrenberg (Plymouth strain 105)
Thalassiosira gravida Cleve (Plymouth strain 112)
Lauderia borealis Gran (Plymouth strain 111)
Skeletonema costatum (Grev.) Cleve (Plymouth strain 106)
Rhizosolenia delicatula Cleve
Chaetoceros decipiens Cleve (Plymouth strain 107)
Ditylum brightwellii (West) Grun. ex Van Heurck (Plymouth strain 110)
Nitzschia seriata Cleve (Plymouth strain 124)

CRYPTOPHYCEAE

Cryptomonas sp. (Plymouth strain 23)
Hemiselmis rufescens Parke (Plymouth strain D)

DINOPHYCEAE

Exuviaella sp. (Plymouth strain 18)
Prorocentrum micans Ehrenberg (Plymouth strain 97)
P. triestinum Schiller (Plymouth strain 98)
Oxyrrhis marina Dujardin (M. R. Droop's strain 18)
Gymnodinium vitiligo nom. prov. Ballantine (Plymouth strain 102)
G. veneficum nom. prov. Ballantine (Plymouth strain 103)
Peridinium trochoideum (Stein) Lemm. (Plymouth strain 104)

Diatoms

Table XIV shows all the experiments made by using cultures grown with ^{32}P. All the diatoms tested were eaten freely and the size of the cell had apparently no effect on the amount taken up. Since diatoms normally occur in chains even those with very small cells will be easily filtered.

With the four species in which assimilation was measured the proportion digested was high, the average being always over 50% and usually over 80%. The percentage did not vary appreciably with the concentration, the apparent slight rise in low concentrations having perhaps been caused by the incomplete recovery of faecal pellets. The percentage is lowest in the spring diatom *Skeletonema* and considerably higher in the three others. Yet *Skeletonema* is undoubtedly the most important diatom food in the Clyde sea area.

As is to be expected the actual quantity of food taken up is greatest when the concentration is highest and this is true also of the number of faecal pellets produced. The quantities are intermediate in the middle concentrations and least in the lowest concentrations, but, as has already been pointed out for faecal pellets, the relationship is not linear. One would therefore expect the volume of water filtered to be lowest in the high concentration and this is usually the case. It must be admitted, however, that in the experiments this volume shows a very great variation, not only among individual *Calanus* but between different experiments on the same species.

Flagellates

Table XV gives the results of the feeding experiments with cultures of flagellates grown with ^{32}P. The relation of volume filtered to size has already been discussed.

The percentage digested is high in most cases, but there are exceptions in *Dicrateria inornata*, *Chromulina pusilla* and possibly *Nannochloris* sp. in which so few faecal pellets were produced and so little taken up in the body that the results are unreliable.

The two very similar forms, *Dunaliella* sp. and *Chlamydomonas pulsatilla* were used. The second is considerably the larger and as can be seen in the table it is a good food. The first is a little smaller and has also been shown by experiments on egg production (Raymont & Gross, 1942; Marshall & Orr, 1952) to be a good food at least when present in high concentrations, but it has the disadvantage that it aggregates readily and is therefore difficult to sample accurately.

Although *Chlorella* can be taken in by *Calanus*, the faecal pellets produced are peculiar in that they seem to consist mainly of a mass of unaltered cells. That some of these at least are viable was proved by Dr M. R. Droop, who grew a culture of *Chlorella* from a faecal pellet. When the pellets are punctured or broken (and they seem to be particularly fragile) a cloud of cells emerges

and the whole pellet soon disappears. *Chlorella* is therefore unsuitable for quantitative work since by the end of an experiment many faecal pellets may have completely disintegrated. In one or two experiments where the *Calanus* were kept in small crystallizing dishes and the faecal pellets removed at frequent intervals, the percentage digested was low, usually below 50% and often only 15 or 20%. The food present in the gut probably accounts for most of the small amount found in the body so that digestion is even lower than it appears to be.

Dinoflagellates

Table XVI shows the results of all experiments with radioactive cultures of dinoflagellates. That with *Peridinium trochoideum* was not done as usual in rotated bottles, but in 50 ml. crystallizing dishes and therefore the figure for volume filtered may not be so reliable.

On the whole dinoflagellates are, with the possible exception of *Prorocentrum triestinum*, digested well. The volume filtered is also high and as one might expect with fairly large cells rises with lowered concentration. Indeed the highest figure we have obtained for volume filtered (84 ml.) was got with one out of the five *Calanus* in a culture of *Prorocentrum micans* containing 5 cells per ml.

The culture of *Oxyrrhis* used (see p. 505) also contained a large number of very small *Nannochloris* cells on which they were feeding. Table XV shows, however, that even in much higher concentrations the amount of *Nannochloris* actually ingested is negligible, so that the results obtained can be considered as due to *Oxyrrhis* alone. This is shown also by the fact that there is an increase in volume filtered with increasing dilution.

Prymnesium parvum is interesting in that, under certain conditions of growth, it is poisonous to fish, both in the sea (Otterstrøm & Steemann Nielsen, 1939) and in fish ponds (Shilo & Aschner, 1953). With *Calanus*, however (Table XV) a culture grown by Dr M. R. Droop showed no evidence of being harmful. *Gymnodinium veneficum*, which has been found to be poisonous to fish by Miss D. Ballantine of the Plymouth laboratory (personal communication), was also tested on *Calanus* (Table XVI). In rich cultures it caused death, but only after one or more days, which is much slower than with fish. That the *Calanus* ate it was shown by the production of faecal pellets. Another culture grown with ^{32}P present was tried in a quantitative feeding experiment, using lower concentrations. Few of the *Calanus* fed freely but in those which did, faecal pellet production was normal and digestion high.

Digestion

When faecal pellets taken in tow-nettings are teased out and examined they sometimes appear to consist largely of unaltered cells of diatoms and other organisms. This, and the rapid production of faecal pellets in cultures in the

laboratory, led observers to suppose that digestion went on only to a limited extent.

For the experimental feeding of *Calanus* it is possible to choose food organisms which give rise to faecal pellets in which the skeletons of the food organisms can be counted and the degree of digestion assessed visually. One such experiment was made by feeding *Calanus* on *Prorocentrum micans*. In the gut the *Prorocentrum* readily breaks up into two halves, and it is possible to see whether these are empty or if they contain an appreciable amount of undigested material. The concentrations ranged from 33 to 1·7 cells/ml. and the dilutions were made with outside sea water which contained at that time a variety of other organisms but no *P. micans*. In addition, the culture contained about 25% of empty shells which were also presumably ingested by the *Calanus*. Five sets each of fifteen *Calanus* were used and the faecal pellets produced were counted. A sample of the faecal pellets from each was crushed, the *Prorocentrum* shells counted and the number fully digested assessed. This varied from 66% in the highest concentration to 100% in the lowest. Estimates of degree of digestion are of course not very reliable. Even when most of the shells were empty, there was still a mass of greenish material sometimes retaining the shape of the original cell body present in the faecal pellet and this was probably only partly digested.

By the use of cultures grown with ^{32}P it was possible to estimate the digestion of a large variety of organisms, and these results are shown in Tables XIV–XVI. Although in most cases digestion appears to be very high, there are exceptions. The percentage digested is usually between 50 and 100% but very low results were obtained with *Dicrateria*, *Chromulina* and *Chlorella*, as well as with the spores of *Bacillus globigii* (p. 504).

From the single experiment on non-radioactive *Prorocentrum micans* it did appear that there was a tendency to digest more in low concentrations. This tendency is not confirmed in the experiments with radioactive cells, for the differences in the percentage digested between the richest and poorest concentration is usually within experimental error.

Faecal pellets are sometimes produced at the rate of one every 5 or 6 min, and it is astonishing that, in these conditions, digestion of ^{32}P is still as high as 80 or 90%. A low content of phosphorus in copepod faecal pellets, as compared with the phytoplankton was also found by Harvey *et al.* (1935), although they thought that the phosphorus was liberated directly into the sea as phosphoproteins and phospholipins.

Nearly all the feeding experiments were made with starved females, and it seemed possible that when first fed after a period of starvation digestion might be more efficient. A number of females was therefore kept for 6 days in a rich culture of *Chaetoceros* before use. They produced a large number of faecal pellets and when used for a feeding experiment the percentage digested was still found to be over 90% and the volume filtered was 24–46 ml. per *Calanus* in 24 h.

Distribution of ^{32}P in the *Calanus* Body

Rough dissections of numerous *Calanus* were made to determine the fate of the ^{32}P taken in. The *Calanus* was fixed in a drop of formalin and then dissected with needles under a binocular microscope on a series of circular coverslips of the same diameter as the planchettes used. These were then dried and their activity measured. The phosphorus begins to leach out immediately after fixation, and up to 12% may be lost overnight so the dissections were done within a few hours of fixation.

The fat was separated off as far as possible on the first coverslip, and then the *Calanus* was transferred to a second and most of the gut taken out and put on a third. The reproductive system was then dissected out. After this the carcass was transferred to a fourth coverslip and the muscles, apart from those inside the appendages, removed to a fifth. As can be well understood the separation of the different tissues was by no means complete. When fat was present (always in Stage V but rarely in females) it was never possible to leave it all on the first coverslip and the whole series contained some. The body fluid came out on the first (fat) or second (gonad) coverslip. It is difficult to disentangle the oviduct from the muscles to which they are attached and some eggs are left with the carcass. Otherwise this consists of the exoskeleton, appendages with their contained muscles, and the nervous system. On several occasions most of the nerve cord was removed and read separately, but the ^{32}P content was very small.

In spite of the crudity of the method, the results were fairly consistent (Table XVII). In females without fat, the carcass and the reproductive system each contained roughly 30–50% of the whole, the muscles 6–16% and the gut usually under 10%. In Stage V and in a newly moulted female the fat contained up to 40% and the gonad was very low. If allowance were made for the small relative volume of the reproductive system the percentage activity would be still higher. In two fat females it was much lower than usual, whereas the fat contained 8 and 24%. In males the gonad was surprisingly low, being not much higher than in Stage V and the muscles were slightly higher, 12–25%. The fat fraction was unexpectedly high. It was never possible to dissect out the thin-walled vas deferens whole and its contents may have been included with the fat.

A number of females were fed for only a short time (5 min up to several hours) to see if it was possible to trace the path of the ^{32}P but in these *Calanus* the gut was usually packed full of the food and its activity was much higher than usual (15–40%). In addition, it was difficult to retain the food within the gut and it contaminated the other coverslips.

The fact that the fat in Stage V and immature females has a high value, whereas the gonad is high in mature females, confirms what had already been suspected, namely that most of the fat as it disappears goes to form the gonad.

Some ripe females were kept in a culture of radioactive *Chaetoceros*; the eggs were removed daily and their activity measured as well as that of each female at the end of the experiment. Unfortunately it was late in the year and egg-laying was poor. Those females which laid 12 to 20 eggs had 17–30% of the ^{32}P present in the eggs besides 30–45% of the ^{32}P in the reproductive system.

In another experiment in April when females were laying actively, ten were kept individually in a culture of radioactive *Dunaliella* for 2 days and on the third were transferred to inactive sea water; the eggs laid (41–158 per female) accounted for 15–50% of the total ^{32}P taken up.

In actively laying females producing perhaps several hundred eggs, the phosphorus contained in the eggs must be many times that retained in the body.

The minimum period of feeding in a radioactive culture before radioactive eggs are laid seems to be about 6–8 h. On several occasions when a female had laid twice during the night the younger eggs were more highly radioactive than the older. Thus a female which was fed for about 18 h in a radioactive culture of *Lauderia* laid two clutches of eggs (69 and 59) during this time, both of which were washed, dried, and measured for activity. The older set which must have been laid after the female had fed for about 6 h had an average of 0·5 counts per egg per min.; the younger which were laid after about 15 h of feeding had about 21 counts per egg per min.

Excretion of ^{32}P in Solution

Apart from the faecal pellets produced *Calanus* must excrete the products of its metabolism. We attempted to measure the rate of this excretion so far as phosphorus was concerned by feeding a number of *Calanus* on a rich radioactive culture for about a week then putting them singly in small dishes of membrane-filtered sea water and measuring the loss of ^{32}P by the *Calanus*. Every 2 or 3 days each *Calanus* was removed, washed in three changes of water and transferred to a planchette. The water was then almost completely removed, the activity measured and the *Calanus* returned to its dish. This handling of the *Calanus* is severe treatment but it lasted only for 1 or 2 min on each occasion and did not apparently lead to any deaths. The reading of activity when the water is removed in this way is only a little below that obtained when the animal is torn up and dried, and in any case the error will remain about the same. The rate of loss is shown in Fig. 4.

It should be possible to measure the increase of ^{32}P in the water in which they are kept, and an attempt was made to do this also. There were, however, in many dishes large and unexplained losses and it is possible that some ^{32}P may have been taken up again by the *Calanus* or adsorbed on the glass or taken up by bacteria adhering to the glass. An experiment to test the last two possibilities gave, however, no definite results.

Two experiments on excretion were made, the first with twenty females fed on *Chaetoceros* when the water in the dishes was changed every time the animal was examined; the second with eight Stage V and seven females fed on *Syracosphaera*, when the water was not changed. All the Stage V which survived had moulted into females by the end of the experiment but the act of moulting had no recognizable effect on the rate of loss of ^{32}P and there was in any case very little difference between Stage V and females.

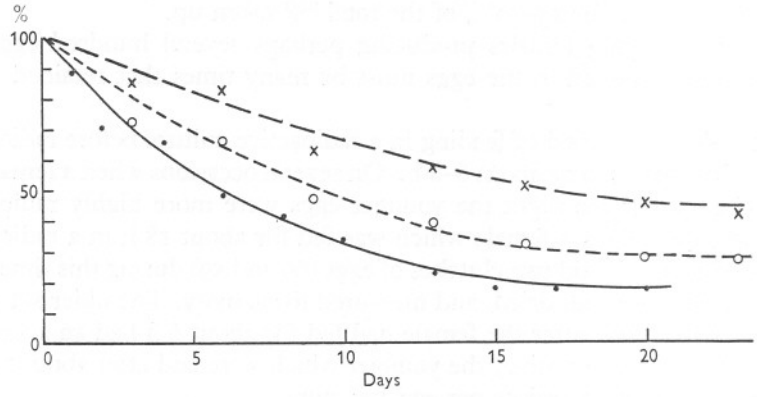

Fig. 4. Excretion of ^{32}P by starved *Calanus*. —●—, *Calanus* ♀ fed on *Chaetoceros*; —○—, *Calanus* ♀ fed on *Syracosphaera*; —×—, Stage V fed on *Syracosphaera*.

There was considerable individual variation, but the curves in Fig. 4 show that excretion is most rapid in the first week of starvation and becomes progressively less in the second and third weeks. The excretion was much higher than was expected. It was observed that unhealthy *Calanus* lost much more than healthy and in the few days before death the ^{32}P content might fall rapidly down to a figure of as little as 2 or 5%. This seems an impossibly low figure for the body of even a moribund *Calanus*, and it is possible that the newly acquired ^{32}P is not in equilibrium throughout the body or is metabolized first. In view of the handling of the *Calanus* involved the results given should be considered as over—rather than under—estimates; these losses will also be accentuated by the starvation of the *Calanus* after a period of feeding on a rich source of phosphorus.

Uptake of ^{32}P from Solution

It has already been mentioned that *Calanus* kept in a cell-free solution of ^{32}P will become radioactive. The means by which they take in the phosphate is unknown. It may be by 'drinking' either by the mouth or by the anus, or it may be taken through the thin parts of the exoskeleton. The amount taken up varies directly with the concentration but there is a great deal of individual

variation. No measurements were made of the non-radioactive P in solution, and it is possible that the uptake of ^{32}P by *Calanus* may depend on the total inorganic P present. In low concentrations (as in the filtrates of the cultures used in feeding experiments) the uptake is very low and is usually negligible when compared with the amount found in feeding *Calanus*. It is interesting, however, that after keeping females some hours in a solution of inorganic ^{32}P the eggs laid are slightly radioactive, indicating that, however the phosphate is taken in, it is rapidly distributed throughout the body.

Comparison of Feeding in Males, Females and Stage V *Calanus*

In our experiments females fed best, especially after being kept in the laboratory some days without food. A number of feeding experiments were also done with male and Stage V *Calanus*, but these were much more erratic in their feeding and very often produced no faecal pellets and took up very little ^{32}P. When they were feeding and could be compared with females it was found that the males produced usually fewer and always smaller faecal pellets and filtered less water. Stage V *Calanus*, on the other hand, on the few occasions when they fed freely, compared well with females in number and size of faecal pellets and volume of water filtered. There was no evidence that the utilization of any food organism differed with these stages of *Calanus*.

Utilization of Iodine

The utilization of phosphorus was so much higher than had been expected that it was thought advisable to test some other element which might occur in limiting concentration in the sea. Other radioactive isotopes besides ^{32}P are available for studies on feeding, and from each new information can be gained. Radioactive iodine (^{131}I) has a long enough half-life (8 days) for phytoplankton cultures to be grown and then used in feeding experiments on *Calanus*. *Lauderia borealis* was grown in Miquel culture solution with ^{131}I added. The culture grew rapidly and, after a week, had passed its period of optimum growth, although only 27% of the ^{131}I had been taken up by the diatom cells. The *Lauderia* was therefore collected on a filter and then re-suspended and used in different dilutions for a feeding experiment. Even after this treatment there was a considerable amount of ^{131}I in solution, and the *Calanus* in the filtrate from the 100% strength took up a substantial amount. Table XVIII shows the results of an experiment with female *Calanus* lasting 18 h. The total removed, faecal pellets produced, and volume filtered was remarkably small in the highest concentration as compared with the next, one-tenth of its strength, but this is not unusual. The percentage digested is very low when compared with that in experiments with ^{32}P and rises with decreasing concentration. As already mentioned, the higher figures in the 1% however may

be partly due to the loss of one or two faecal pellets. From the results we may conclude that iodine in diatoms is retained by *Calanus* only to a slight degree.

A culture of *Syracosphaera* grown in Miquel with added ^{131}P took up so little of the iodine that it could not be used.

Discussion

The two most striking results which emerge from this series of experiments are the low volumes of water filtered daily by *Calanus* and the high percentage of the food digested. Both these results are at variance with generally accepted opinion. The volume of culture filtered varied from less than 1 ml. to about 30–40 ml. per day; one or two individuals exceeded this but they were exceptional. Digestion was usually over 50% and often over 90%.

In the early experiments made to determine the volume of water which a *Calanus* could filter daily (Fuller & Clarke, 1936; Fuller, 1937) volumes of 1 to 5 ml. were found. The *Calanus* were, however, kept in a very small volume of water during the experiment (3 ml. per *Calanus*) and it seemed that Gauld's later work (1951), when he kept individual *Calanus* in 100 ml. beakers, would be more reliable. He found a good deal of individual variation (42–101 ml.), but his average value was about 70 ml. a day. Harvey (1937), in the course of some short experiments on selective feeding, found values much higher even than this, reaching with *Ditylum brightwellii* rates of 168 and 240 ml. per day. It has already been pointed out that a small error in the estimation of the number of food cells may lead to a large error in the final calculation. Thus in the concentrations which Gauld used (mostly 10 to 20 cells per mm^3) an error of 1 or 2 per mm^3 in the final cell count would lead to an error of 10–50 ml. in the calculation of volume filtered. This type of error is avoided in the current experiments by measuring directly the total quantity taken up.

There is another possible source of error in the earlier work. In almost all cultures some settling out of the cells takes place unless the water is stirred (Ryther, 1954), and since *Calanus* has a habit of feeding on the bottom it will tend to get a richer supply of food than the calculated one.

Recently, Ryther (1954) has found that a substance which inhibits the feeding of *Daphnia* is produced in cultures of *Chlorella*, *Scenedesmus* and *Nitzschia*. On increasing the concentration of the food the volume filtered decreased. When a culture was in the exponential growth phase, little of this inhibitory substance was found in the water, and the *Daphnia* was affected mainly by the food ingested. In senescent cultures the inhibitory substance was present in solution also and the effect on feeding was more marked.

In the experiments on feeding *Calanus* with old cultures of *Lauderia* there was little evidence of a decrease in filtration, though the percentage digested decreased markedly. In high concentrations the volume filtered usually fell

off but it seemed likely that the *Calanus* was unable to ingest the large quantity of food involved rather than that an inhibitory substance was present. There was not an inverse correlation in the lower concentrations, such as Ryther found. Although none of our experiments was designed to test for the presence of inhibitory substances, a general view (Tables XIV–XVI) does not indicate their presence in the older cultures.

A striking fact in all the experiments is the great variation between individual *Calanus* even under the same conditions. In any of the experiments some of the *Calanus* might be eating freely and others not at all, even although they were apparently as healthy and active as the rest. In different experiments on the same food species, differences were even more marked and *Calanus* filtered an average of 23·9 ml. of *Skeletonema* culture on one occasion and, on another, in a concentration little richer, only 5 ml. In Table XIX, for example, the results for *Skeletonema* indicate that more water is filtered in the higher concentrations. Yet a reference to Table XIV will show that in the experiments from which these results come, the opposite is usually true. There are, however, many factors which may influence the result which were not taken into account, e.g. age, or rather state of growth, of culture, time of year, and age or state of maturity of females.

Gauld observed that a *Calanus* can live even in a rich food culture without feeding, and this has been confirmed by our own observations. This does indicate that they are not automatic filter feeders in the sense that they must ingest all the time they are moving. Esterly (1916) has described how a food pellet collected on the filtering setae may be scattered again by a flick of the maxillipedes and this may be the usual method of rejection. The very diverse movements which Lowndes (1935) has described for the mouthparts of a *Calanus*, particularly for the second antenna, may also enable them to divert the feeding current.

The low filtration figures found make it difficult to understand how *Calanus* can maintain itself in the sea. If we accept the respiration figures found experimentally for *Calanus* (Marshall *et al.*, 1935; Clarke & Bonnet, 1939) there is apparently still a gap between the food required and the food normally present in the sea. This difficulty cannot be got over by postulating the presence of large numbers of μ-flagellates since *Calanus* seems unable to filter them off.

According to Fuller & Clarke (1936) and Fuller (1937) a *Calanus* needs to filter from 40 to 70 ml. per day to maintain itself when the phytoplankton is moderately rich. Gauld, who found daily filtrations of this order, himself showed that because of vertical migration *Calanus* will spend only part of the day feeding in the phytoplankton-rich waters. Thus the rate of filtration would have to be increased considerably for them to obtain enough.

It has been suggested by Fuller & Clarke (1936) that the fat reserve in *Calanus* might help to tide it over a period of scarcity. In Stage V *Calanus*,

however, in which it is prominent, it often persists till they moult at the end of the winter and then disappears gradually. The dissection results (Table XVII) show that when Stage V moult to females, the fat may be used to build up the ovary. Digby (1954) has made the suggestion that during the winter *Calanus* are cannibals and that this accounts for the large fall in numbers over the winter.

In our experiments females were used in most cases since they fed more regularly than Stage V. Often in winter Stage V fed poorly or not at all even if starved for some days before an experiment. It may be that the metabolism and therefore the food requirement of the over-wintering Stage V *Calanus* is lower than it is during the rest of the year.

When one considers the concentration of food cells usually present in the sea, our low filtration volumes are all the more unexpected. Table XIX shows the maximum number of cells taken up by the best-feeding *Calanus* in all the experiments that were made with concentrations of food cells such as may occur in the sea. The upper limit for this was put at 16,000 per ml. Concentrations of this magnitude certainly occur only for short periods during phytoplankton outbursts, e.g. in the Oslo fjord (Braarud, 1939) and in the Norwegian Sea (Halldal, 1953), but the lowest concentrations used, down to 1 cell per ml. may be found in the sea even in winter. It will be seen that the *Calanus* can ingest up to a maximum of 600 large cells such as *Ditylum brightwellii* per day, up to 12,000 of *Syracosphaera* or *Peridinium trochoideum* and up to 50,000 of small forms such as *Platymonas*, *Cryptomonas* or *Prorocentrum triestinum*. By far the largest number was with the spring diatom *Skeletonema costatum* when nearly 400,000 were ingested. These are also very small cells but they are usually united in chains and so are more easily filtered. With still smaller forms, e.g. *Dicrateria inornata* or *Nannochloris*, the highest number ingested may be quite small (200 to 400), confirming the suggestion that these cells pass through the maxillary filter. *Chromulina* is however an exception to this for, though it is even smaller than *Nannochloris*, up to 18,000 per day were ingested in a concentration of 16,000 per ml. In low concentrations of organisms, such as might be found in the sea in winter, only a few hundred cells at most will be ingested.

While the methods used in the experiments enable us to calculate the volume filtered with some degree of certainty, the estimates of the digestion of the food taken in depend on the phosphorus alone. As is shown in Tables XIV–XVI its digestion in marine phytoplankton forms is unexpectedly high. On the other hand, the experiments with a culture labelled with ^{131}I showed that only a very small fraction of this was retained. The same must be true of other elements such as the silicon in the diatom skeleton.

The possibility that the ^{32}P may have been only loosely bound to the algal cell has already been discussed (p. 497) and Rice (1953) has shown that, under experimental conditions, little of the phosphorus can have been labile.

There were a few species in which digestion was much lower, e.g. *Chromulina*, *Dicrateria*, and possibly *Nannochloris*. In *Chlorella* viable cells were excreted and digestion could not be measured because of the breaking up of the pellets. *Bacillus globigii* spores were presumably completely indigestible, and the apparent digestion gives some measure of the spores present in the gut.

Very high filtrations are not possible in rich food cultures because faecal pellet production beyond a certain rate is not physically possible. The peristaltic movements of the gut are slow and the compacting of the faecal pellets gradual. Two *Calanus* feeding on rich *Chaetoceros* cultures produced 166 and 211 faecal pellets in 15 and 24 h respectively, i.e. a faecal pellet every $5\frac{1}{2}$ or 7 min, yet even at these rates 86% was digested. These are maximum feeding rates, and as a rule faecal pellets were produced much more slowly. In concentrations such as are found in the sea (Table XIX) the highest rates were with *Chaetoceros* and with *Prorocentrum micans*, and amounted to about one every quarter of an hour; with the spring diatom *Skeletonema* the rate was about half this.

The number of cells found in one faecal pellet was counted in the experiment on choice of food already described. The maximum numbers for *Prorocentrum micans* and *Peridinium trochoideum*, which were mixed in the food culture, were 129 and 188, the averages 30 and 40. Many pellets contained 50–70 of each species. This compares well with the average figures calculated from the radioactive cultures, namely 74 and 139.

In a later experiment with *Prorocentrum micans* some of the faecal pellets were taken from three of the *Calanus* and crushed and the skeletons in them counted. The total number of cells in all the faecal pellets was then calculated and compared with the number calculated from the radioactivity of the body and faecal pellets. The number of cells per faecal pellet varied from 5 to 44 but it was not always easy to distinguish the skeletons. The agreement was reasonably good; the number of cells eaten was by radioactive measurement 1304, 1655 and 324, and by visual count 865, 1573 and 252. Although the great majority of the cells in the faecal pellets were completely empty, the percentage digestion was not so high as usual, varying from 42 to 71%.

On the whole then, when a comparison is possible the observations on faecal pellets do confirm the results obtained with radioactive cultures. Reports have often been made of faecal pellets containing many undigested cells, but when we consider the total number which a faecal pellet may represent, e.g. 1000–7000 *Skeletonema*, a few dozen undigested cells would, although apparently important, be only a small fraction of the total. There is, however, always a large amount of unidentifiable debris, and it would be desirable to know the degree of assimilation of other substances besides those containing phosphorus.

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Summary

1. Cultures of diatoms and phytoflagellates were grown with radioactive phosphorus (^{32}P) present and used in feeding experiments on *Calanus*. After feeding the uptake and the volume of culture filtered was estimated by measuring the radioactivity of the body, eggs and faecal pellets.

2. The volume filtered varied from less than 1 ml. to over 40 ml. in 24 h; it is less in high concentrations of food cells but does not increase in very great dilutions.

3. *Calanus* feeds better in the dark than in the light.

4. Digestion of an old diatom culture was in some cases lower than that of a young one.

5. It was found that organisms below a size of about 10 μ could not be readily filtered off, and in such cases the volume filtered decreased with dilution of the culture.

6. Some experiments on the choice of one food rather than another show that if selection does take place it is only to a slight degree.

7. All the diatoms used could be eaten freely and most of the other algae. Digestion was poor in a few of the smallest, e.g. *Dicrateria*, *Chromulina* and *Chlorella*, but in most organisms it was unexpectedly high.

8. A high proportion of the ingested ^{32}P goes to the ovary in females and to the fat in Stage V *Calanus*. In an actively laying female a large proportion of the ^{32}P appears as eggs.

9. The excretion of ^{32}P by females initially well fed and then starved was at first rapid and slowed off after a few days.

10. Radioactive iodine (^{131}I) was used in one feeding experiment, but the utilization was low.

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TABLE I. RATE OF FAECAL PELLET PRODUCTION

Thirteen *Calanus* feeding on a mixture of *Prorocentrum micans* and *Peridinium trochoideum*

Successive time intervals	Rate of faecal pellet production per hour													Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	
15 min	0	0	*	8·0	0	8·0	0	0	0	3·8	7·5	0	4·0	2·6
20–25 min	4·0	3·8	0	0	0	6·7	3·0	3·0	3·0	0	5·7	8·2	2·7	3·1
20–25 min	2·7	2·7	3·0	2·4	2·5	7·2	5·0	2·6	2·9	11·4	5·7	5·0	7·2	4·6
1½ h	2·9	2·9	2·1	1·4	0	5·5	5·3	2·0	3·3	7·1	6·5	6·3	4·2	3·8
1½ h	2·0	4·0	4·1	4·1	0·7	6·1	3·5	5·2	5·8	7·1	7·1	7·2	5·4	4·8
2 h	3·1	4·6	3·6	5·6	0·5	5·8	3·1	6·9	5·8	7·9	7·9	6·4	7·8	5·3
17 hr	3·0	3·7	2·1	4·5	1·8	2·6	0·9	3·5	3·0	7·1	2·6	6·4	5·5	3·6

* Excreted a pellet before the first examination.

TABLE II. COMPARISON OF WASHED AND UNWASHED CELLS

Concentrations	No. *Calanus* used (♀)	Average for 1 *Calanus* in 24 h				
		No. faecal pellets	Counts/min in faecal pellets	Counts/min in body	% used	ml. filtered
460 *Lauderia*/ml. Centrifuged 4 times	4	51	2,450	21,550	89·6	32·9
320 *Lauderia*/ml. Untreated	4	28	2,500	19,090	88·6	29·9

TABLE III. SEPTEMBER 1953. UTILIZATION OF *SKELETONEMA COSTATUM*

0·13 counts/cell. 17 hr

Concentration	*Calanus* (♀)	Eggs		Faecal pellets		Body (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
		No.	(c/min)	No.	(c/min)				
37,100 c/min/ml., 288 cells/mm³	1	11	5,592	125	64,550	74,730	144,872	55·4	4·9
	2	12	4,667	120	45,850	92,510	143,027	67·9	4·8
	3	11	3,311	121	53,830	58,410	115,551	53·4	3·9
	4	0	—	80	43,860	54,620	98,480	55·5	3·3
	5	13	3,108	144	57,770	35,590	96,468	40·1	3·2
9,275 c/min/ml., 72 cells/mm³	1	0	—	*c.* 96	38,900	83,100	122,000	68·1	19·2
	2	0	—	*c.* 110	44,950	81,510	126,460	64·5	20·1
	3	0	—	73	29,060	30,020	59,080	50·8	8·5
	4	0	—	104	78,090	77,840	155,940	49·9	23·1
	5	23	19,070	98	75,360	67,230	161,660	53·4	23·9
1,855 c/min/ml., 14 cells/mm³	1	0	—	20	11,510	12,183	23,693	51·4	17·0
	2	0	—	0	—	28	28	—	—
	3	0	—	28	13,280	24,069	37,349	64·4	28·3
	4	1	215	38	14,150	24,890	39,255	64·0	30·1
	5	0	—	23	10,910	21,531	32,441	66·2	24·1
Filtrate, 1,895 c/min/ml.	A	0	—	0	—	16	—	—	—
	B	0	—	0	—	16	—	—	—

TABLE IV. JANUARY 1955. COMPARISON OF DIFFERENT METHODS OF MEASURING VOLUME FILTERED, USING *SKELETONEMA COSTATUM*

1·0 counts/cell. 23½ h

Initial concentration	*Calanus* (♀)	Bottle (vol. in ml.)	Faecal pellets No.	Faecal pellets (c/min)	Body less filtrate (c/min)	Total removed (c/min)	% assimilated	ml. filtered/ 24 h	Diatom counts: Cells/ml. at end	Diatom counts: ml. filtered/ 24 h (calc. by cells)	Liquid counter: c/min/ml.	Liquid counter: ml. filtered in 24 h
11,410 c/min/ml., 11,200 cells/ml. Liquid counter 4642 counts/min/ml.	1	70	47	40,530	120,246	160,776	74·7	16·1	—	—	3,751	15·24
	2	71	33	44,680	130,646	175,326	74·5	17·7	9,100	15·06	3,575	18·93
	3	71	23	28,460	82,006	110,466	74·2	10·6	—	—	4,225	6·83
	4	71	33	34,410	98,916	133,326	74·2	13·0	—	—	4,163	7·90
	5	72	27	26,323	79,106	105,429	75·1	10·1	—	—	4,710	—
	6	72	46	38,290	103,046	141,336	72·9	13·8	—	—	4,136	8·48
	7	73	80	70,650	176,046	246,696	71·4	26·2	7,200	32·94	3,474	21·61
	8	73	28	42,690	66,376	89,066	74·5	8·4	—	—	4,384	4·26
	9	76	*c.* 40	43,250	175,646	218,896	80·2	22·6	7,000	36·47	3,354	25·23
	10	75	33	40,160	117,946	158,106	74·6	15·6	—	—	4,088	9·72
Filtrate, 435 c/min/ml.	A	38	1	5	65 } 54	—	—	—	—	—	159	—
Liquid counter 185 counts/min/ml.	B	38	0	—	43 }	—	—	—	—	—	135	—

TABLE V. EFFECT OF CONCENTRATION OF *DUNALIELLA* ON FAECAL PELLET PRODUCTION

Cells/mm³	8	80	200	400	800
Faecal pellets/ *C*/24 h	11 } 10 } 8	59 } 58 } 57	69 } 67 } 64	81 } 68 } 55	82 } 80 } 78
Size of faecal pellets in μ	296	510	490	470	576

TABLE VI. FEBRUARY 1954. UTILIZATION OF *SYRACOSPHAERA* IN LIGHT AND DARK

16 counts/cell/min. 7.30 a.m.–5.30 p.m.

Concentration	*Calanus* (♀)	Faecal pellets No.	Faecal pellets (c/min)	Body minus filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
11,335 c/ml./min, 720 cells/ml. Dark	1	13	493	22,731	23,224	97·9	5·0
	2	28	1,423	43,902	45,325	96·9	9·9
	3	15	459	42,112	42,571	98·9	9·3
	4	13	629	31,142	31,771	98·0	6·9
	5	11	199	20,759	20,958	99·1	4·5
11,335 c/ml./min, 720 cells/ml. Light	1	12	506	30,552	31,058	98·4	6·7
	2	4	268	10,401	10,669	97·5	2·3
	3	2	252	10,933	11,185	97·7	2·4
	4	0	—	273	273	—	—
	5	4	138	7,713	7,851	98·2	1·7
Filtrate, 135 c/ml./min	A	1	0	11 } 8	—	—	—
	B	0	—	6 }			

TABLE VII. EFFECT OF AGE OF CULTURE ON ASSIMILATION

Age of culture in days	Number of *Calanus* (♀)	Concentration used, cells/ml.	% used	Volume filtered in 24 h
5	5	155	98·4	18·2
10	8	460 320	88·1	31·4
25	6	174	66·7	14·6
32	6	1,590	58·5	8·5
5	6	1,310	73·0	19·7

TABLE VIII. SEPTEMBER 1954. UTILIZATION OF SPORES OF *BACILLUS GLOBIGII*. 18 h.

Concentration	*Calanus* (♀)	Faecal pellets No.	Faecal pellets (c/min)	Body less filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
1 %, 9520 c/ml./min	1	23	536	55	591	9·3	0·09
	2	32	344	49	393	12·5	0·07
	3	16	401	59	460	12·8	0·07
	4	19	422	51	473	10·8	0·07
	5	19	729	54	783	6·9	0·11
0·5 %	1	12	36	3	39	7·7	0
	2	26	248	24	272	8·8	0·11
	3	21	362	15	377	4·0	0·14
	4	21	188	11	199	5·5	0·05
Filtrate, 60 c/ml./min	A	0	—	2 } 1	—	—	—
	B	1	1	0 }			

TABLE IX. TEST FOR SELECTION BY SIZE, USING RADIOACTIVE *DICRATERIA*

Concentrations in cultures	No. *Calanus* used	Average for 1 ♀ *Calanus* in 24 h: No. faecal pellets	Counts/min in body	Counts/min in faecal pellets	Total counts/min	ml. filtered
54,000 *Dicrateria* and no *Lauderia*/ml.	5	10·7	2,613	2,030	4,657	0·57
43,020 *Dicrateria* and 1,070 *Lauderia*/ml.	5	50·8	9,856	6,046	16,050	2·43
1,070 *Lauderia* and no *Dicrateria*/ml.	5	49·7	—	—	—	—
456,000 *Dicrateria* and no *Prorocentrum micans*/ml.	5	47·4	7,204	12,847	20,051	4·73
456,000 *Dicrateria* and 108 *Prorocentrum micans*/ml.	5	104·1	7,059	18,403	25,462	6·27
108 *P. micans* and no *Dicrateria*/ml.	4	60·0	—	—	—	—

TABLE X. TEST FOR SELECTION BY SIZE, *DITYLUM–CHAETOCEROS*

Concentrations of food cells	*Calanus* (♀)	Eggs No.	Eggs (c/min)	Faecal pellets No.	Faecal pellets (c/min)	Body (less filtrate)	Total removed (c/min)	Total removed Cell vol. $\mu^3 \times 10^3$	% used	ml. filtered/ 24 h
Culture										
122 *Ditylum* (radioactive) cells/ml.	1	12	803	110	7,843	15,120	23,766	23·0	67·0	5·7
	2	0	—	0	—	79	79	0·7	—	—
11,500 *Chaetoceros* cells/ml.	3	24	284	46	1,369	9,367	11,020	10·7	87·6	2·6
	4	18	1,641	85	9,326	25,573	36,540	35·4	74·5	8·8
8,575 c/ml./min	5	11	607	80	6,808	17,128	24,543	23·8	72·3	5·8
	6	25	1,916	89	11,669	28,242	41,827	40·5	72·1	10·1
	7	4	45	15	452	7,686	8,183	7·9	94·5	1·9
Filtrate, 950 c/ml./min	A	0	—	0	—	17 } 18	—	—	—	—
	B	11	4	3	3	19 }				
Culture	1	34	138	1	2	882	1,022	0·7	—	—
343 *Ditylum* cells/ml.	2	25	3,353	105	952	48,965	53,270	38·1	98·2	5·7
	3	15	697	98	2,102	63,345	66,144	48·0	96·8	7·2
10,500 *Chaetoceros* (radioactive) cells/ml.	4	0	—	29	129	13,236	13,365	9·7	99·0	1·5
	5	15	10	68	1,309	58,815	60,134	43·6	97·8	6·5
	6	16	1,256	90	3,994	120,675	125,925	91·3	96·8	13·9
18,945 c/ml./min	7	11	42	70	1,332	38,295	39,669	28·8	96·7	4·2
	8	24	130	77	1,687	67,075	68,892	49·9	97·6	7·4
Filtrate, 840 pulses/ml.	C	0	—	1	0	26 } 25	—	—	—	—
	D	0	—	1	34	24 }				

TABLE XI. CHOICE OF FOOD IN A MIXTURE OF *PERIDINIUM TROCHOIDEUM* AND *PROROCENTRUM MICANS*

Time of examination	7 *Calanus** kept 10 days in *P. trochoideum*						6 *Calanus* kept 10 days in *P. micans*					
	Faecal pellets produced	Faecal pellets examined	Skeletons excreted				Faecal pellets produced	Faecal pellets examined	Skeletons excreted			
			P. trochoideum		*P. micans*				*P. trochoideum*		*P. micans*	
			No.	%	No.	%			No.	%	No.	%
11.46 a.m. to 12.01 p.m. (¼ h)	4	4	205	100	0	0	4	4	162	54·9	133	45·1
12.05–12.16 p.m. (½ h)	3	3	117	100	0	0	5	5	299	63·6	171	36·4
12.17–12.38 p.m. (1 h)	5	5	367	86·1	59	13·9	9	9	431	51·8	401	48·2
12.39–1.03 p.m. (1¼ h)	8	8	471	80·9	111	19·1	13	13	586	53·2	515	46·8
2.02–2.42 p.m. (2½ h)	29	17	790	59·1	548	40·9	50	17	1,774	42·7	2,334	57·3
3.30–4.11 p.m. (3½ h)	37	18	1,493	68·4	835	31·6	54	16	1,879	50·7	1,828	49·3
5.25–6.14 p.m. (5½ h)	52	19	2,861	67·9	1,354	32·1	87	18	3,205	57·7	2,351	42·3
10.35–11.35 a.m. (23 h)	315	21	15,258	61·1	9,725	38·9	484	18	9,671	38·8	15,259	61·2

* 1 ♂ included.

TABLE XII. CHOICE OF FOOD

Radioactive *Lauderia* with non-radioactive *Skeletonema*. 6 *Calanus* used in each set

Time in mixture (h)	*Lauderia*-fed, average/*Calanus*				*Skeletonema*-fed, average/*Calanus*			
	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)
½	3·5	438	4,478	4,916	2·2	313	3,994	4,307
1	8·2	1,270	4,972	6,242	4·5	714	4,580	5,294
2	10·3	1,961	7,580	9,541	12·5	2,236	8,242	10,478
16*	106·7	11,047	32,855	43,790	88·3	11,021	27,594	38,615

* Count of pulses for bodies includes eggs laid.

TABLE XIII. CHOICE OF FOOD

Radioactive *Lauderia* with non-radioactive *Cryptomonas*. 6 *Calanus* used in each set

Time in mixture (h)	*Lauderia*-fed, average/*Calanus*				*Cryptomonas*-fed, average/*Calanus*			
	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)
½	6·8	352	907	1,259	5·0	364	520	884
1	9·7	913	1,540	2,453	7·8	612	916	1,528
2	15·7	1,562	2,456	4,018	14·5	1,507	1,986	3,493

TABLE XIV. UTILIZATION OF DIATOMS

Species	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
Lauderia borealis, 29 μ av. diam., 17–43 μ length	*c.* 14	627	5	77	77–88	80·4	6·6–21·2	11·5
	c. 14	63	5	40	83–90	85·6	12·0–36·4	26·5
	c. 14	6	5	8	77–95	87·8	2·7–26·2	11·3
	c. 15	645	5*	46	68–81	73·1	0·9– 9·4	4·0
	5	155	5	4	—	—	6·4–32·0	18·2
	10	320	4	27	87–91	88·6	24·3–38·0	29·9
	10	460	4	51	87–94	89·1	29·8–38·7	32·9
	25	174	6	10	66–68	66·7	11·2–20·0	14·6
	32	1,590	6	118	49–66	58·5	3–12	8·5
	5	1,310	6	113	67–79	73·0	10–33	19·7
Skeletonema costatum, 4 μ in culture	13	288,000	5	183	40–68	54·5	3– 5	4·0
	13	72,000	5	144	50–68	57·4	9–24	19·0
	13	14,400	4	33	51–66	61·5	17–30	23·9
	c. 6	194,000	5	115	78–84	80·7	3– 5	3·8
	c. 6	19,400	4	48	81–87	83·8	3– 6	4·9
	c. 6	970	5	1	—	—	0·1– 1·6	—
	c. 7	770	5	3	79–96	87·0	1– 2	1·3
	11	41,700	6	75	71–89	77·6	2·4–16·8	9·8
	5	36,700	5	16	46–87	72·2	0·5– 2·3	1·2
		3,670	4	3	52–72	62·3	2·2– 2·2	2·2
	7	11,200	10	40	71–80	74·6	8·4–26·2	15·4
Chaetoceros decipiens, 14–21 μ diam., size range 20–78 μ	28	945	4	51	89–94	90·7	2–12	7·2
	28	170	5	2	—	—	0– 7	1·8
	28	17	5	3	—	—	3	2·9
	4	630	5	95	93–97	95·8	3·6–43·3	22·4
	4	63	5	31	91–98	95·3	3·8–24·7	19·9
	4	3	5	28	83–98	93·9	8·3–14·4	10·4
	8	68	5	8	97–100	98·2	1·9–10·3	6·3
	8	7	5	6	98–99	98·5	1·7– 6·2	4·1
	8	1	5	17	90–97	92·7	3·4– 8·4	5·8
	15	490	4	67	87–89	89·1	4·7– 8·1	6·1
	15	110	6	24	93–97	95·1	4·3– 8·9	6·6
	15	22	6	7	98–99	98·2	3·0– 8·6	5·0
	18	78	5	42	82–91	86·1	5·6– 8·9	7·7
	18	78	5†	12	90–98	94·4	0·2– 3·7	1·1
Ditylum brightwellii, 20–60 μ diam.	*c.* 20	63	4	76	85–89	87·2	1–10	7·0
	c. 20	13	5	28	90–95	91·7	2–16	9·4
	c. 20	1	4	6	87–98	93·4	2–18	11·7

* Males. † Stage V.

TABLE XV. UTILIZATION OF FLAGELLATES

Species	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
Chlamydomonas pulsatilla, 19 × 12 μ	3	62,000	5	85	88–90	89·0	3·0 – 5·6	4·57
	3	6,200	5	11	93–98	96·4	1·1 –31·0	11·83
	3	620	5	5	98–99	98·7	1·5 – 6·1	3·21
Platymonas carteriaformis, 20–26 μ × 14–16 μ	15	41,500	6	95	61–93	77·5	2·1 – 3·1	2·59
	15	4,150	5	20	76–95	81·4	0·5 –11·6	5·15
	15	415	4	11	57–83	75·1	0·1 – 3·2	1·4
Nannochloris oculata, 2–4 μ	5–25	2,368,000	5	5	61–87	74·3	0	0
	5–25	119,000	5	1	27·3	—	0	0
	5–25	12,000	5	1	66·7	—	0	0
Chromulina pusilla, *c.* 1–3 μ	28	1,636,000	5	36	27–59	39·4	1·1 – 4·0	2·24
	28	163,600	5	15	46–68	54·2	1·4 – 1·7	1·56
	28	16,360	5	3	47–63	54·9	0·1 – 1·1	0·44
Monochrysis lutheri, 5–7 × 5–7 × 2 μ	7	188,000	4*	34	50–84	71·1	0·7 – 1·2	0·45
	7	18,800	4*	10	60–96	81·8	0·2 – 0·8	0·42
	7	1,880	4*	9	69–97	82·0	0 – 0·7	0·22
Dicrateria inornata, 3–5·5 μ	18	2,180,000	3	59	15–41	25·9	0·2 – 0·8	0·53
	18	106,000	3	10	38–63	48·7	0·1 – 0·2	0·16
	18	10,600	3	2	62–67	64·5	0 – 0·3	0·18
	25	534,000	5	56	33–38	35·1	0·3 – 1·3	0·84
	25	279,000	4*	50	39–59	48·5	0·2 – 0·9	0·51
Syracosphaera elongata, 18–30 × 12 μ	7	3,100	5	32	96–98	97·0	0·7 – 1·4	1·03
	7	200	5	29	98	98·0	3·6 –29·3	19·3
	7	20	5	8	98–100	98·7	3·1 –43·5	23·7
	11	720	5	38	97–99	98·1	4·5 – 9·9	7·1
	16	4,500	6	31	87–94	91·5	1·14– 1·83	1·43
	16	1,125	6	36	91–96	93·9	3·50–11·45	7·70
	16	2,800	5	15	88–99	92·6	0·5 – 3·5	1·62
	33	280	5	9	92–97	94·9	2·2 –25·6	7·35
	33	28	5	3	—	—	0·4 – 6·7	2·16
	13	575	4*	15	97–99	98·3	0·2 – 4·1	1·80
Prymnesium parvum, 9–12 × 5 μ	14	40,000	5	8	92–99	96·6	0·10– 0·47	0·37
	14	4,000	5	8	78–99	90·6	0·10– 4·22	1·77
	14	400	5	6	—	—	0·86– 1·76	1·32
Cryptomonas sp., 19–27 × 6–10 μ, average 22 × 8 μ	16	16,000	5	112	51–84	62·0	4·9 –16·7	8·7
	16	1,600	4*	24	58–89	70·0	15·7 –32·4	22·6
	16	160	5	14	64–86	77·4	5·0 –24·7	10·8

* One omitted because it did not feed.

TABLE XVI. UTILIZATION OF DINOFLAGELLATES

Species and size	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
Prorocentrum micans 43 × 27 μ	*c.* 27	68	5	69	70–86	79·3	15·3–27·6	22·2
	27	540	5	54	86–96	89·6	0·5– 9·4	5·3
	27	54	5	25	89–96	93·4	12·7–37·1	27·7
	27	5	5	17	92–99	97·1	29·8–84·3	42·5
P. triestinum 10–14 μ	*c.* 30	12,200	5	67	43–54	47·3	2·4– 4·2	3·5
	c. 30	1,220	5	31	49–73	61·7	6·2–17·8	13·7
	c. 31	1,020	5	39	53–65	60·2	6·9–15·6	11·3
	c. 31	102	5	12	49–83	72·6	2·2–21·5	10·1
Oxyrrhis marina, 20–45 × 15–30 μ, with *Nannochloris oculata**	14	20,400	5	52	86–92	88·7	0·6– 2·1	1·7
	14	2,040	5	34	92–95	93·9	8·2–21·7	13·4
	14	204	5	8	97–99	97·9	1·5–28·1	16·1
Gymnodinium vitiligo 6–12 μ, 14 μ long	42	7,840	5	33	86–94	88·8	5·1– 8·5	6·2
	42	784	5	15	94–97	95·8	4·8–19·2	13·6
	42	78	4 †	8	97–99	98·0	1·4–23·2	9·7
Gymnodinium veneficum 15 × 13 μ	28	13,800	5	24	75–92	83·5	0·1– 1·3	0·6
	28	1,380	5	23	90–99	94·0	0·5–11·1	5·1
	28	138	5	3	99	98·6	0·3–14·5	3·9
Peridinium trochoideum 25 × 19 μ	32	1,750	10	81	68–85	76·4	0·7– 7·5	4·8

* 56,000 *Nannochloris*/ml. in highest concentration.
† One which did not eat omitted.

TABLE XVII. PARTITION OF ^{32}P IN THE *CALANUS* BODY

Date	Stage of *Calanus*	Fat (%)	Carcass (%)	Reproductive system (%)	Gut (%)	Muscles (%)
23. xii. 53	M.	25·4	34·6	14·0	0·7	25·2
23. xii. 53	M.	23·3	51·9	5·0	1·1	18·7
28. xii. 53	M.	26·2	54·9	1·7	5·5	11·8
28. xii. 53	M.	37·6	42·1	1·0	1·3	17·9
28. xii. 53	M.	31·0	49·5	0·9	1·6	17·0
28. xii. 53	M.	35·1	45·2	0·6	4·6	14·4
28. xii. 53	M.	33·0	50·9	1·0	2·1	13·0
1. xii. 53	F.*	24·4	43·8	17·8	3·1	11·0
1. xii. 53	F.*	8·3	47·5	28·9	4·0	11·4
10. xii. 53	F.†	12·6	55·8	4·2	2·8	7·6
30. xi. 53	F.	—	49·5	25·2	14·4	10·7
30. xi. 53	F.	—	45·7	38·4	8·3	7·5
30. xi. 53	F.	—	41·1	40·8	7·1	11·0
4. xii. 53	F.	—	45·5	32·3	6·0	16·2
4. xii. 53	F.	—	38·5	47·1	5·2	9·2
4. xii. 53	F.	—	45·3	44·4	1·4	8·9
4. xii. 53	F.	—	48·8	44·0	1·1	6·1
10. xii. 53	V	39·8	36·7	8·2	2·3	13·0
10. xii. 53	V	25·4	48·3	11·3	6·7	8·3
18. xii. 53	V	35·3	43·5	4·9	7·6	8·7
18. xii. 53	V	34·1	48·7	2·5	10·2	4·5
18. xii. 53	V	25·7	56·0	0·4	6·6	11·3

* Fat females. † Newly moulted.

TABLE XVIII. UTILIZATION OF *LAUDERIA BOREALIS* WITH ^{131}I

18 h at 15–17° C

Concentrations	*Calanus* (♀)	Faecal pellets: No.	Faecal pellets: c/min	Faecal pellets: c/faecal pellets/min	Body less filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
4775 c/ml./min, 5215 cells/ml. 100 %	1	82	3,404	42	421	3,825	11·0	1·09
	2	55	1,182	21	314	1,508*	21·6	0·46
	3	80	3,763	47	611	4,374	14·0	1·26
	4	54	1,668	31	248	1,916	13·0	0·57
	5	60	1,639	27	224	1,863	12·0	0·57
10 %	1	66	2,496	38	593	3,093†	19·3	9·04
	2	72	3,001	42	788	3,789	20·8	11·20
	3	72	3,383	47	823	4,206	19·6	12·55
	4	73	3,376	46	816	4,192	19·5	12·48
	5	89	2,540	29	628	3,168	19·8	9·38
1 %	1	3	189	63	320	509	62·9	15·46
	2	7	74	11	109	183	59·6	5·23
	3	7	227	32	104	331	31·4	9·69
	4	5	164	33	217	381	57·0	11·27
	5	3	38	13	88	126	69·8	3·66
Filtrate 3070 c/ml./min	A	0	—	—	103 } 122	—	—	—
	B	6	4	1	142 }			

* Including 12 from eggs laid. † Including 4 from eggs laid.

TABLE XIX. MAXIMUM UPTAKE OF ONE *CALANUS* IN SEA-WATER CONCENTRATIONS

Species	No. cells/ml.	Counts/ cell	Time of expt. (h)	No. faecal pellets/24 h	Cell equiv. per faecal pellet	Total for 24 h: No. cells taken up	Total for 24 h: ml. filtered
Skeletonema costatum	14,000	0·15	17	53·7	6,953	373,000	30·1
	11,200	1·02	23½	81·7	3,023	247,000	26·2
	3,670	0·28	16½	8·7	932	8,134	2·2
	970	0·66	18	1·3	1,443	1,924	1·6
	770	0·94	65	2·2	589	1,305	1·7
Lauderia borealis	627	31·0	17	112·9	11	1,198	21·2
	63	31·0	17	38·1	5	192	36·4
	6	31·0	17	7·1	2	15	26·2
	645	25·8	16	91·5	6	575	9·4*
	460	2·6	15½	52·7	290	15,310	38·7
	320	2·6	15½	37·2	275	10,230	38·0
	174	0·5	48½	11·4	279	3,176	20·0
	155	3·9	10	2·5	1,332	3,197	21·8
Chaetoceros decipiens	945	1·35	15½	54·2	211	11,440	11·7
	170	1·35	15½	7·7	143	1,106	6·8
	17	1·35	15½	4·6	11	53	3·2
	630	120·0	16	141·0	159	22,420	43·3
	63	120·0	16	22·5	78	1,752	31·2
	3	120·0	22¼	36·7	1	43	14·4
	490	179·0	17	81·9	46	3,784	8·1
	110	179·0	18½	22·1	34	756	8·9
	22	179·0	18½	11·7	13	146	8·6
	78	311·0	17	31·1	21	667	8·9
	68	87·0	16	19·5	33	651	10·0
	7	87·0	16	10·5	4	42	6·3
	1	87·0	21½	0	—	6	8·4
Ditylum brightwellii	63	385·0	18½	84·3	7	622	10·4
	13	385·0	18½	44·1	4	189	15·9
	1	385·0	18½	14·3	1	15	17·8
Chlamydomonas pulsatilla	6,200	0·68	17	15·5	10,710	166,300	31·0
	620	0·68	17	5·6	653	3,685	6·1
Platymonas carteriaformis	4,150	0·14	16	33·0	1,427	47,000	11·6
	415	0·14	16	22·5	60	1,350	3·2
Nannochloris oculata	12,000	0·03	18	2·7	97	258	0
Chromulina pusilla	16,000	0·01	16¼	4·4	4,077	18,050	1·1
Dicrateria inornata	10,900	0·15	16	3·0	133	400	0·3
Syracosphaera elongata	4,500	1·22	16½	42·2	195	8,230	1·8
	1,125	1·22	16½	40·7	298	12,140	11·5
	3,100	16·1	16	16·5	262	4,330	1·4
	200	16·1	16	49·5	104	5,125	29·1
	20	16·1	16	10·5	64	675	43·5
	720	16·0	10	67·2	101	6,800	9·9
	575	23·2	17½	24·7	97	2,324	4·1
Prymnesium parvum	4,000	0·17	15¾	22·9	739	16,900	4·2
	400	0·17	15¾	9·1	78	718	1·8
Cryptomonas sp.	1,600	0·85	15¼	20·5	1,936	39,630	32·4
	160	0·85	15¼	14·2	252	3,567	24·7
Prorocentrum micans	540	16·0	17	83·3	58	4,827	9·4
	54	16·0	17	22·6	74	1,675	37·1
	5	16·0	17	12·7	25	316	84·3
	68	111·0	9	120·0	15	1,746	27·6
P. triestinum	12,200	0·84	17½	71·3	702	50,060	4·2
	1,220	0·84	17½	38·4	519	19,920	17·8
	1,020	0·76	21½	55·8	274	15,310	15·6
	102	0·76	21½	22·3	91	2,030	21·5
Oxyrrhis marina	2,040	0·60	18½	46·7	839	39,190	21·7
	204	0·60	18½	19·5	254	4,938	28·1
Gymnodinium vitiligo	7,840	1·4	17	25·4	2,566	65,220	8·5
	784	1·4	17	79·9	1,409	13,930	19·2
	78	1·4	17	14·1	117	1,658	23·2
Gymnodinium veneficum	13,800	1·54	18½	68·8	243	16,700	1·3
	1,380	1·54	18½	66·2	218	14,440	11·1
	138	1·54	18½	11·7	159	1,850	14·5

* Male *Calanus*.