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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

IX. FEEDING AND DIGESTION IN THE YOUNG STAGES

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(Text-figs. 1–3)

Little is known about the food of the nauplii and early copepodite stages of copepods, although it has usually been assumed that the food particles must be smaller and perhaps more concentrated than for adults. A few observations have been made on the feeding of the young stages of *Calanus* in the laboratory, but none are available on feeding under natural conditions. It has been suggested (Marshall, Nicholls & Orr, 1934) that the success or failure of a brood of *Calanus* in the sea might depend on the presence of a rich food supply during its development. Experiments were therefore undertaken to find what organisms could be ingested by the nauplii and early copepodite stages, how much of the different foods could be digested, and how much water they could filter in a day.

Methods

The use of food cultures labelled with radioactive phosphorus (^{32}P) enables an accurate measure to be made of the amount taken in, and the methods used in earlier experiments on feeding in adult and Stage V *Calanus* (Marshall & Orr, 1955*b*) were found to be useful for the younger stages also. When photosynthetic organisms are grown in a medium containing inorganic ^{32}P it is rapidly taken up and the culture can then be used in different concentrations to measure the amount filtered and the amount assimilated in a given time. A variety of food organisms of different sizes and belonging to different systematic groups was used. In each experiment the copepods were put in bottles filled with a culture of known concentration of the food to be tested and the bottles were then tied in dark cloth bags and attached to a wheel revolving slowly so that the culture was kept in suspension. All experiments were carried out in a cool aquarium. At the end of the experiment, which lasted from 16 to 24 h, the bodies, and sometimes the faecal pellets also, were removed and washed. They were then dried and exposed on a disc or planchette under the end window of a Geiger counter to measure the radioactivity. The results are shown as counts per minute. Specimens were also kept in filtrate from the radioactive culture to measure the uptake, if any, of ^{32}P in solution.

If faecal pellets were to be collected, the early stages of *Calanus* were kept in bottles which might be as small as 2 ml. capacity. For experiments in which faecal pellets were not collected, as many as 20 Nauplius III were put in a bottle of about 30 ml. capacity and the bodies were usually read in batches of about 10 at a time. The figures given for volume filtered by these are therefore averages. Occasionally, however, a Nauplius III was read singly and it was clear that this stage showed as much variation as any other. The later stages were usually read singly, although several might be kept in one bottle.

It is not so easy to judge the health of the early stages as of the adults. The amount of food taken up varied much in different experiments with the same stage, and it seems likely that those with the higher feeding rate represent the normal individuals. Experiments were therefore usually made with two species of food organism, one of which was known to be a satisfactory food. If this was eaten freely it was assumed that the copepods were in good condition.

Nauplius Stages I, II, III and sometimes IV were usually obtained from eggs hatched in the laboratory. The later stages were usually picked out from tow-nettings taken on the day of the experiment. They were commonest during the spring and summer in fine and medium tow-nettings, but a small number could be obtained throughout the autumn and even part of the winter. Nauplius I usually moults to Nauplius II in less than 24 h and Nauplius II to Nauplius III about 24 h later. Nauplius III remains as such for several days (Lebour, 1916) and mortality at this stage is usually high. The interval between moults is short in the early stages and at the end of an experiment some had remained in the same stage, some had moulted to the next and some were in process of moulting. Only the results from the first group are included in Table IV.

The food organisms were most often used in concentrations such as are found in the sea. Counts were made either with a haemocytometer or, more usually, in 0·2 ml. samples on a ruled slide. The number of cells present does not affect the calculation for the number of ml. filtered, but it can give an idea of the number of cells which the copepod ingests in a given time (Table VIII).

The following organisms were used:

Nannochloris oculata Droop (M. R. Droop's strain 66)
Monochrysis lutheri Droop (M. R. Droop's strain 60)
Dicrateria inornata Parke (Plymouth strain B)
Syracosphaera elongata Droop (M. R. Droop's strain 62)
Skeletonema costatum (Grev.) Cleve (Plymouth strain 106)
Chaetoceros decipiens Cleve (Plymouth strain 107)
Ditylum brightwellii (West) Grun. ex Van Heurck (Plymouth strain 110)
Prorocentrum micans Ehrenberg (Plymouth strain 97)
P. triestinum Schiller (Plymouth strain 98)
Peridinium trochoideum (Stein) Lemm. (Plymouth strain 104)

The dilution of the cultures was always made with water which had been filtered through a 'membrane' filter. This removed particles down to about 1 μ and so ensured that a negligible quantity of non-radioactive food was added. Filtering through filter-paper makes the water harmful to at least the early nauplius stages of *Calanus*. It was noticed that nauplii in Stage I mostly died or at least failed to moult when kept in paper-filtered water. The fine threads which come from the filter-paper probably get entangled in the nauplius limbs and prevent free movement. Rejecting the first portion of the filtrate and so reducing the number of fine threads led to an improvement in survival and moulting and a complete removal of these threads by filtering again through a 'membrane' filter resulted in normal survival and moult.

The Filtering Appendages

Nauplii

Some observations on the feeding mechanisms of copepod nauplii have been made by Storch (1928). He states that the nauplius feeds like the adult, i.e. if the adult copepod is filter-feeding so is the nauplius, and, if the adult is predatory, the nauplius is predatory too. The limbs used in feeding by *Calanus* nauplii must be different from those used by the adult, for the maxilla, which is the main filtering appendage, does not appear until Nauplius VI and

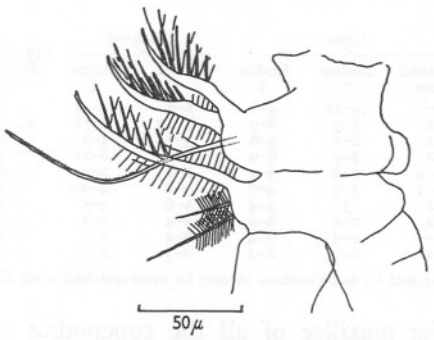

Fig. 1. Coxa and basipod of the antenna of Nauplius V.

is then small and weak. Storch says that in *Diaptomus gracilis* nauplii the filtering is done by the long masticatory processes of the coxa and basipod of the antenna and mandible. In Nauplius I and II of *Calanus* these processes are short and weak and have no setules. As will be shown later, it seems certain that neither of these stages feeds. In Nauplius Stages III to VI the masticatory spines on the coxa and basipod of the antenna have developed further, are longer and stronger and lie close together. Each of the three (Fig. 1) has a double row of strong setules on the distal half about 10–30 μ long and 1·5–4 μ apart. These are the only setules present on the proximal

coxal seta, but on the other two the lower halves have single rows of fine setules on each side. There is a long thin seta on the coxa and two shorter on the basipod, but these may play no part in filtration. It seems possible that the 'masticatory processes' are wrongly named and that their function is filtration rather than mastication. The function of the separate setae is not obvious.

The maxilla, which appears in Nauplius V as a knob, has in Nauplius VI almost the complete number of setae, but these are relatively very short and have no setules.

Copepodites

It may be assumed that in the copepodite filtration is done mainly by the maxilla (Cannon, 1928; Lowndes, 1935). This is a uniramous appendage bearing a series of setose endites. Following Gurney (1931), it may be taken as formed by a coxa bearing two endites and an external seta, a basipod bearing two endites, and a five-segmented endopod, the first two segments of which are produced as endites. The maxilla of *Calanus gracilis* (which differs only slightly from that of *C. finmarchicus*) has been beautifully figured by Giesbrecht (1892, Plate 7, fig. 17).

TABLE I. DISTANCE APART OF SETULES IN COPEPODITE STAGES (IN μ)

		Coxa			Basipod		Endopod			
							Segment 1		Segment 5	
	Stage	External seta	Endite 1	Endite 2	Endite 3	Endite 4	(Endite 5)	Segments 2–4	'Feather'	Others
C. finmarchicus	♂	—	5–12	4–8	6–9	5–11	4–16	5–21	4–5	6–17
C. helgolandicus	♂	2	5–9	8–9	8–10	8–11	12–17	11–19	7–9	13–22
C. finmarchicus	♀	2	2–6	3–6	3–7	3–8	4–7	5–14	3–5	5–14
C. helgolandicus	♀	2	5–9	6–9	4–9	6–12	7–13	11–19	3–9	12–20
C. finmarchicus	V	1–3	2–4	3–4	2–4	2–4	3–6	4–8	2–5	4–10
C. helgolandicus	V	1–3	3–9	4–7	3–8	4–10	3–15	5–17	3	7–20
	IV*	2	3	3–4	3–6	3–5	3–5	4–7	3–4	5–9
	III*	2	2–4	3–4	3–4	2–5	4–7	4–8	3–4	4–11
	II*	—	2–3	3–4	2–4	3–4	3–5	3–7	3–4	3–7
	I*	—	2–4	2–4	2–5	2–5	3–6	4–7	2–4	4–8

* *C. finmarchicus* and *C. helgolandicus* cannot be distinguished until Copepodite V.

The setules on the maxillae of all the copepodite stages and of both *C. finmarchicus* and *C. helgolandicus* were examined and measured to give some idea of the size of organism which could be retained (Fig. 2 and Table 1).

There are altogether twenty-nine setae, of which twenty form a sheet approximately in the same plane, the gaps between them being partly filled by closely set setules. Usually each seta bears two rows of setules set at an angle of about 90° to one another, sloping forward and making an angle of about 60° with the seta. All but one (the external) of the remaining setae are on the inner side of the limb, one to each endite except the fifth and one to each of the three terminal segments; they usually lie at an angle to the main setae. Those on endites 1–4 are shorter and have irregularly spaced setules projecting in all directions. That on the first endite is like the others on the

upper half only; on its lower half there are two regular rows of setules at 180° in the plane of the setae. That on the terminal segment of the limb has two opposite rows of closely set setules (like a feather) but it is naked in the upper half. Finally, there is a short external seta on the coxa with opposite rows of very fine and closely set setules.

Apart from the setules most of the main setae are serrated towards the tip, the serrations sometimes enlarging to become a series of short spines between the setules.

It is not easy to understand the purpose of all this elaboration. Obviously the setules forming a close network between the setae must be the main instruments of filtration, and the fact that the setules and serrations are both directed forward will make it easy for a particle to pass towards the mouth. The function of the special internal setae and of the external seta is unknown. The whole limb is slightly concave viewed from the outer side.

With the exception of the first and sixth endites on which one or two setae are added during development, the main setae are present in all the copepodite stages and indeed almost all are present in Nauplius VI.

The maxillae were dissected off from several specimens of each stage and the distance apart of the setules measured on one or more setae of each endite of the coxa and basipod as well as on most of the segments of the endopod. The smallest divisions on the micrometers used measured 1·86 and 1·67 μ. It was not always possible to make measurements on the same part of the setae or even on the same setae on different individuals, so that the results which are shown in Table I indicate the variations only approximately.

It can be seen that the smallest distance between the setules lies between 2 and 3 μ. In general the setules are closest towards the lower half of the setae and widest towards the tip; one distance may be double the other. There is a gradual but less regular widening of the distance between setules from endite 1 of the coxa to segment 5 of the endopod. On the two terminal setae the spacing is much wider and may reach 20 μ. Although the minimum distance of 2–3 μ is found also in the adult (chiefly at the base of the coxal setae) there is a tendency for the setules to be closer in the younger stages.

There seems also to be a real difference between the adults of the *finmarchicus* and the *helgolandicus* forms; the setules are between 50 and 100% wider apart in the latter. This difference is less marked in Stage V. The distances apart are considerably smaller than Ussing's (1938) estimate. According to him the smallest distance apart of the setules in the adult female is 5·7 μ, in Stage V, 3·8 μ, and in Stage IV, 3·2 μ. His specimens were from the Arctic where the *Calanus* are much larger than ours and the size difference may account for the greater minimum distance apart.

It is not possible to say how efficient the maxilla of *Calanus* is as a filter but we should expect considerably smaller particles to be retained than have been shown experimentally to be important as food. The size of some of the cells

used in the feeding experiments is shown in Fig. 2. It is obvious that the small flagellate *Nannochloris* can easily pass the filter over most of its area.

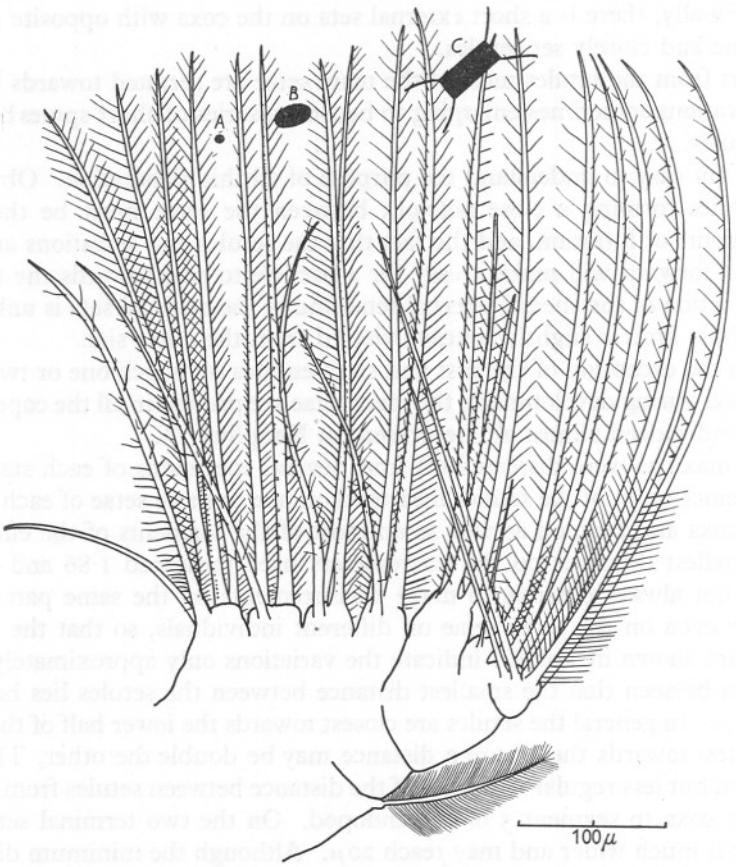

Fig. 2. Left maxilla of *Calanus helgolandicus* ♀ from the right. *A*, *B* and *C* represent the sizes of food organisms used. *A*, *Nannochloris oculata*; *B*, *Syracosphaera elongata*; *C*, *Chaetoceros decipiens* (from culture).

Feeding Experiments

One certain test of whether a nauplius can eat any particular food is to find the skeleton in the faecal pellets. This is sometimes very difficult if the skeleton has no obvious sculpturing. It is probable, for instance, that Nauplius III can eat *Chaetoceros decipiens* but the frustule of this diatom is thin-walled and has no striking features and it has not been identified in faecal pellets. *Skeletonema* frustules are found complete in the faecal pellets of Nauplius III showing that this common spring diatom is a food for even the earliest feeding stages of *Calanus*. Coccoliths are shed in large numbers when a culture of

Syracosphaera is getting old, and it is possible that some may be taken loose. However, the faecal pellets examined from nauplii which had been feeding on a culture of this species were packed full of coccoliths, which had probably come from ingested cells.

These and other observations for the later nauplius stages are shown in Table II and the size of some of the faecal pellets given. It shows a gradual increase with the size of the nauplius, as might be expected. The pellets were usually ovoidal but were very pale compared with those from adults on the same food. The rate of production with a suitable food is somewhat similar to that of the adult *Calanus*.

TABLE II. SIZE OF FAECAL PELLETS IN NAUPLIUS STAGES

(In those marked with an asterisk, the food organism was identified in the faecal pellet.)

Food organism	Size of food organism (μ)	Size of faecal pellets (μ)			
		N III	N IV	N V	N VI
Skeletonema costatum	4–5	48 × 24*	46 × 25*	83 × 33*	87 × 30*
Chaetoceros decipiens	14–21 × 20–78	33 × 20	—	—	—
Syracosphaera elongata	18–30 × 12	31 × 17*	39 × 21*	48 × 28*	*
Prorocentrum micans	43 × 27	—	—	60 × 29*	87 × 32*
P. triestinum	10 × 14	*	—	—	*
Peridinium trochoideum	25 × 19	—	—	—	*
Ditylum brightwellii	20–60 diam.	—	—	—	*

The earlier the stage of the *Calanus* the more difficult are the faecal pellets to recognize. When Janus green is added to the water, they take up the stain and are slightly easier to see, but the risk of losing some is much greater with nauplius stages than with copepodites. In the quantitative experiments in which the faecal pellets were picked out, the percentage of the phosphorus-containing portion digested was always high and it was felt that little was gained by a time-consuming and perhaps unsuccessful attempt to find all the faecal pellets produced. In many of the experiments therefore faecal pellets were ignored and a larger number of individuals could thus be tested. The total variation was so great that in comparing volume filtered with faecal pellets included and excluded, the first does not show a consistently higher figure; the reading of the body alone is therefore justifiable.

Grobben (1881) has stated that the first nauplius stage has no anal opening and this has been confirmed by Miss Judith C. Perryman of King's College, London (personal communication). She finds that the mouth does not open until Nauplius II and the anus not until Nauplius III. From our experiments also it seems certain that neither Nauplius I nor Nauplius II feeds. When Nauplius II were used some of them always moulted during the course of the experiment, and from the results shown in Table III it is clear that only those nauplii which reached Stage III had taken up an appreciable amount of food. The slight radioactivity shown by the Nauplius II may be due to ^{32}P absorbed from solution.

The results of twenty-two out of the thirty-nine experiments done on the late nauplius and early copepodite stages are shown in Table IV. The rest are omitted for one reason or another. In nine the copepods had eaten badly; it was considered that if Nauplius III had filtered less than an average of 0·05 ml. in 24 h they must have been unhealthy. If, however, two food species were tested in the same experiment and the copepods did well in one and badly in the other, both results were accepted. Four experiments on *Nannochloris* and *Monochrysis* are excluded; these are very small flagellates, not likely to be taken freely, but no check against a good food was made. Two experiments on Nauplius II (which does not feed) and two on *Bacillus globigii*, which gave negative results, are also omitted.

TABLE III. UPTAKE OF ^{32}P IN LATE N II AND EARLY N III

(Duration of experiments between 18 and 24 h)

Culture	Age of culture (days)	Concentration cells/ml.	Nauplii put in	Nauplii as read	Counts/ min	Counts/ nauplius/ min
Skeletonema (contaminated)	7	275	20N II	2N II	112	56
				17N III	7096	417
26. viii. 54			20N II	5N II	90	18
				14N III	4405	315
Chaetoceros decipiens	6	1670	20N II	2N II	122	61
				9N III	10146	1127
21. iv. 55				8N III	8134	1017
			23N II	5N II	78	16
				12N III	11133	928
			20N II	4N II	19	5
				8N III	9139	1142
				9N III	5649	628
Ditylum brightwellii	18	57	20N II	5N II	241	48
6. vi. 55				12N III	1176	98
			20N II	7N II	177	25
				10N III	5567	557
Dicrateria inornata	6	71000	20N II	1N II	5	5
21. iv. 55				13N III	5218	401
26. viii. 54	7	43000	20N II	5N II	1	0
				13N III	4180	322
			20N II	9N II	27	3
				9N III	2910	323

In any one experiment also the result was rejected for any individual which had done very much worse than its fellows. Sometimes these were noted as being inactive or unhealthy, but sometimes they were apparently in as good condition as the rest. They may, however, have been near moult (see Tables V and VII). The results given in the table are therefore selected, but as explained above it is thought that they probably show the normal behaviour.

On one or two occasions Nauplii III and IV reared in the laboratory were separated into *Calanus finmarchicus* and *C. helgolandicus* and read separately. The former is always smaller and gave, as might be expected, lower results.

TABLE IV. FEEDING EXPERIMENTS ON NAUPLII AND COPEPODITES

Number of copepods used in each experiment and volume filtered in 24 h

Food species and size	Date	Age of culture (days)	Culture cells/ml.	NIII No. used	NIII Average ml./24 h	NIV No. used	NIV Average ml./24 h	NV No. used	NV Average ml./24 h	NVI No. used	NVI Average ml./24 h	CI No. used	CI Average ml./24 h	CII No. used	CII Average ml./24 h	CIII No. used	CIII Average ml./24h
Skeletonema costatum, 4–5 μ	9. vi. 54	7	10,200	—	—	—	—	—	—	—	—	5	0·53	—	—	—	—
	29. iii. 55	6	9,440	29	0·08	—	—	3	0·26	14	0·30	2	0·40*	4	2·04	2	3·49
	4. iv. 55	12	1,290	61	0·07	—	—	—	—	—	—	—	—	—	—	—	—
	26. viii. 54	7	275†	8	0·24	—	—	2	0·72	3	0·72	5	0·92	—	—	—	—
Chaetoceros decipiens, 14–21 μ diam., 20–78 μ long	17. viii. 54	19	4,090	—	—	—	—	1	0·12*	—	—	—	—	—	—	—	—
	4. x. 54	8	68	49	0·28	15	0·21	—	—	—	—	—	—	—	—	—	—
	19. iv. 55	4	2,260	—	—	—	—	—	—	1	0·54*	2	0·51*	7	1·87	7	1·94
	19. iv. 55	4	2,260	—	—	—	—	—	—	—	—	1	0·77	—	—	—	—
	21. iv. 55	6	1,670	33	0·05	6	0·22	5	0·32	1	0·48	1	0·60	2	0·51*	—	—
	26. iv. 55	11	1,480	—	—	—	—	1	0·31*	1	0·47*	—	—	—	—	—	—
	26. iv. 55	11	1,480	—	—	—	—	1	0·23	1	0·23	—	—	—	—	—	—
	27. vi. 55	4	315	17	0·09	3	0·66	2	0·78	5	1·04	2	1·44	9	3·19	6	5·67
Ditylum brightwellii, 20–60 μ diam.	6. vi. 55	18	57	38	0·05	7	0·14	7	0·15	3	0·35*	9	0·61	3	0·83*	—	—
	6. vi. 55	18	57	—	—	—	—	—	—	9	0·37	—	—	5	1·07	—	—
Nannochloris oculata, 2–4μ	26. viii. 54	17	32,000	15	0·03	5	0·07	5	0·04	6	0·05	3	0·07	7	0·27	—	—
	4. iv. 55	12	19,600	59	0·01	1	0·01	4	0·00	4	0·00	—	—	3	0·11	2	0·07
Dicrateria inornata, 3–5·5 μ	23. viii. 54	4	130,000	51*f*	0·04	7	0·05	—	—	—	—	18	0·14	—	—	—	—
	23. viii. 54	4	130,000	35*h*	0·05	—	—	—	—	—	—	—	—	—	—	—	—
	19. iv. 55	4	112,000	—	—	—	—	—	—	—	—	2	0·56*	3	1·83	4	1·28
	19. iv. 55	4	112,000	—	—	—	—	—	—	—	—	3	0·44	—	—	—	—
	21. iv. 55	6	71,000	38	0·07	1	0·12	4	0·15	1	0·07*	—	—	—	—	—	—
	21. iv. 55	6	71,000	—	—	—	—	—	—	4	0·04	—	—	—	—	—	—
Syracosphaera elongata, 18–30 × 12 μ	9. viii. 54	11	1,250	1	0·04*	1	0·04*	1	0·44*	1	0·67*	—	—	1	0·80*	—	—
	9. viii. 54	11	1,250	9*f*	0·11	3*f*	0·17	—	—	—	—	—	—	—	—	—	—
	9. viii. 54	11	1,250	9*h*	0·25	2*h*	0·25	—	—	—	—	—	—	—	—	—	—
	11. viii. 54	13	570	6	0·08	1	0·41*	3	0·59*	1	0·22*	2	1·08	—	—	—	—
	28. iv. 55	13	64	24	0·08	—	—	—	—	—	—	—	—	—	—	—	—
	9. v. 55	24	370	20	0·05	3	0·06	4	0·10	10	0·12	—	—	—	—	—	—
	27. vi. 55	4	320	26	0·17	3	0·75	11	1·01	11	1·36	7	2·12	13	2·95	14	4·45
Prorocentrum triestinum 10–14 μ	8. vi. 55	22	1,515	21	0·01	5	0·05	10	0·07	6	0·10	9	0·18	8	0·30	—	—

* Faecal pellets included. *h* = *helgolandicus*, *f* = *finmarchicus*. † contaminated with flagellates.

TABLE V. UTILIZATION OF *SYRACOSPHAERA ELONGATA*

(Culture 4 days old. 14·5° C. 27–28 June 1955)

Concentrations	Vol. of bottle ml.	Time (h)	*Calanus* Put in	*Calanus* As read	*Calanus* Counts/min	*Calanus* Total	ml. filtered in 24 h Total	ml. filtered in 24 h /*Calanus*
5120 c/ml./min 320 cells/ml.	34·86	24¼	21N III	8N III	6,613	14,288	2·89	0·17
				8N III	7,384			0·19
				1N IV	291			0·02
	35·28	24	20N III	10N III	7,330	10,854	2·20	0·15
				9N III*	3,524			0·08
	37·50	16	4N IV*	1N IV	3,953	6,233	1·84	1·17
				1N IV–V	195			0·06
				1N IV–V	447			0·13
				1N V	1,638			0·48
	37·50	24	4N IV	1N IV	2,283	5,501	1·09	0·45
				1N IV	3,147			0·62
				1N IV–V	71			0·01
	69·54	22½	10N V	1N V	4,068	27,524	5·96	0·88
				1N V	4,746			1·03
				1N V	4,142			0·90
				1N V	4,706			1·02
				1N V–VI	273			0·06
				1N V–VI	1,704			0·37
				1N V–VI	1,202			0·26
				1N V–VI	404			0·09
				1N VI	2,025			0·44
				1N VI	4,254			0·92
	70·68	19¼	10N V	1N V	6,294	43,835	11·39	1·64
				1N V	3,044			0·79
				1N V	1,607			0·42
				1N V	5,286			1·37
				1N V	4,867			1·27
				1N V	6,587			1·71
				1N V	3,346			0·87
				1N V–VI	3,623			0·94
				1N V–VI	5,929			1·54
				1N V–VI	3,252			0·85
	70·26	21½	10N VI	1N VI	6,803	65,128	15·68	1·64
				1N VI	1,708			0·41
				1N VI	5,063			1·22
				1N VI	4,647			1·12
				1N VI	8,254			1·99
				1N VI–C I	7,558			1·82
				1N VI–C I	6,213			1·50
				1N VI–C I	10,570			2·55
				1C I	6,798			1·64
				1C I	7,514			1·81
	70·93	22¼	10N VI	1N VI	6,894	53,658	12·23	1·57
				1N VI	6,207			1·41
				1N VI	6,938			1·58
				1N VI	4,703			1·07
				1N VI	6,263			1·43
				1N VI*	6,690			1·52
				1N VI–C I	5,529			1·26
				1N VI–C I	4,155			0·95
				1N VI–C I	4,020			0·92
				1N VI–C I	2,259			0·52

* Sluggish.

TABLE V (*continued*)

Concentrations	Vol. of bottle ml.	Time (h)	*Calanus* Put in	*Calanus* As read	*Calanus* Counts/min	*Calanus* Total	ml. filtered in 24 h Total	ml. filtered in 24 h /*Calanus*
5120 c/ml./min 320 cells/ml.	72·79	21¾	4C I	1C I	8,107	39,577	9·02	1·85
				1C I	10,064			2·29
				1C I	12,307			2·81
				1C I	9,105			2·08
	74·70	21¾	5C I	1C I	8,225	35,922	8·14	1·87
				1C I	10,079			2·28
				1C I	7,300			1·66
				1C I*	2,522			0·57
				1C I–C II	7,796			1·77
	66·20	18¼	10C II	1C II	7,818	93,579	28·12	2·35
				1C II	9,662			2·90
				1C II	14,305			4·30
				1C II	2,476			0·74
				1C II	13,967			4·20
				1C II	21,461			6·45
				1C II	12,210			3·67
				1C II–C III	11,680			3·51
	68·72	22½	10C II	1C II	15,751	74,031	17·30	3·68
				1C II	8,570			2·00
				1C II	671			0·16
				1C II	9,292			2·17
				1C II	10,004			2·34
				1C II	14,403			3·37
				1C II–III	282			0·07
				1C II–III	3,940			0·92
				1C III	11,118			2·60
	75·73	15¾	12C III	1C III	4,304	127,284	45·91	1·55
				1C III	13,802			4·98
				1C III	11,032			3·98
				1C III	21,817			7·87
				1C III	11,322			4·09
				1C III	17,568			6·34
				1C III	15,765			5·69
				1C III	25,540			9·21
				1C III*	3,866			1·39
				1C III–IV	2,268			0·82
	76·76	21¾	12C III	1C III	6,990	92,284	22·68	1·72
				1C III	5,744			1·41
				1C III	16,078			3·95
				1C III	5,837			1·43
				1C III	7,912			1·95
				1C III	5			0·00
				1C III	33,109			8·14
				1C III*	4,490			1·10
				1C III*	5,549			1·36
				1C III–IV	6,570			1·61
Filtrate 35 counts/ ml./min	32·38	23	5N III	4N III	0	—	—	—
			1N V	1N V	0	—	—	—
			2N VI	2N VI	0	—	—	—
			2C II	1C II	2	—	—	—
			2C III	1C II	0	—	—	—
				1C III	3	—	—	—
				1C III	2	—	—	—

* Sluggish.

As Table IV shows, the volumes filtered vary much even with the same species of food organisms. On the whole, as is natural, the volume filtered rises from one stage to the next. The very small flagellate *Nannochloris* is clearly used no better by the nauplii and early copepodite stages than it is by the adult. Thus in two experiments the utilization of *Nannochloris* was compared with that of *Skeletonema*. In the first a fairly long range of stages was compared in *Nannochloris* and in a contaminated *Skeletonema* culture. In every one the volume filtered in *Nannochloris* culture was much smaller. In the second a long range of stages was tested in *Nannochloris* and this was checked only by Nauplius III in *Skeletonema*. Both experiments showed that the low results in *Nannochloris* were not caused by unhealthy copepods.

To test whether really small particles could be filtered off, an experiment was made with radioactive bacterial spores (*Bacillus globigii*, 0·7 μ in volume). But, as with adults, none was retained.

It is surprising to find that a large diatom such as *Ditylum* is apparently taken up by even the small nauplii, but possibly debris or broken cells, some of which are always found in old cultures, are being ingested.

The maximum filtration by Nauplius III found in any experiment was 1·0 ml. in 24 h; by Nauplius IV, 1·2 ml.; by Nauplius V, 1·7 ml.; by Nauplius VI, 2·0 ml.; by Copepodite I, 2·8 ml.; by Copepodite II, 6·4 ml.; and by Copepodite III, 9·2 ml. Most of these maxima were reached in one experiment comparing the utilization of *Chaetoceros decipiens* and *Syracosphaera elongata*. Details of the second may be seen in Table V. This experiment, done at the end of June, gave quite exceptionally high figures for all the stages tested. The results also show the great variation which may occur even among copepods of the same stage.

In this experiment, as in others, there were many moults. As a rule animals found in the process of moulting gave a low reading, whereas those which had successfully moulted might be as high as the rest, but this was not invariable. Although several individuals were used in each bottle, the total volume filtered was not a high proportion of its contents except with Copepodite III. In this case the animals may have been too crowded and might have filtered more had they been put singly in similar bottles.

Not all the *Calanus* put in were recovered. Some nauplii may die and disintegrate during an experiment, and occasionally one or two individuals are lost when the stopper of the bottle is put in.

Fig. 3 shows the average results obtained in all the experiments of Table IV for a number of different food species. There appears to be no abrupt change in the volume filtered with the change from nauplius to copepodite, indeed the change from the first to the second copepodite is usually more marked.

The results of three experiments in which faecal pellets were collected are shown in Table VI. That on *Skeletonema costatum* indicates that the percentage of ^{32}P retained is considerably lower than with the other two species.

This comparatively low assimilation of *Skeletonema* was found also with adults. In the experiments on *Ditylum brightwellii* the Nauplius VI have retained a larger proportion than the Copepodite II. This may be partly because not all the faecal pellets were found; even in the unlikely event of a 50% loss, however, the digestion would still be higher than in Copepodite II.

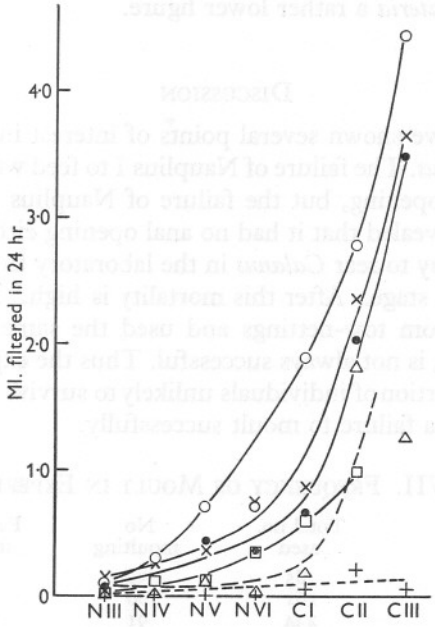

Fig. 3. Average volumes filtered in 24 h by stages Nauplius III to Copepodite III in various food species. ○, *Syracosphaera*; ×, *Chaetoceros*; ●, *Skeletonema*; □, *Ditylum*; △, *Dicrateria*; +, *Nannochloris*.

TABLE VI. UTILIZATION, INCLUDING FAECAL PELLETS, OF THREE FOOD ORGANISMS

Concentration	Age of culture (days)	Date	Time in h	Stage of *Calanus*	Faecal pellets No.	Faecal pellets Counts/min	Body less filtrate	Total removed, c/min	Percentage used	ml. filtered in 24 h
Skeletonema 91,125 c/ml./min 266,000 cells/ml.	7	9. vi. 54	18	CI	11	72	772	844	91·5	0·39
				CI	40	355	569	924	61·6	0·43
				CI	30	293	895	1,188	75·3	0·56
				CI	35	562	538	1,100	48·9	0·51
				CI	57	531	1,092	1,623	67·3	0·78
Ditylum 36,610 c/ml./min, 57 cells/ml.	18	6. vi. 55	20·5	NVI	16	558	10,536	11,094	95·1	0·38
				NVI	18	612	8,529	9,141	93·3	0·31
				NVI	13	425	9,886	10,311	95·9	0·35
				CII	48	5339	24,270	29,609	82·0	1·10
				CII	43	4210	14,733	18,943	77·8	0·68
				CII	33	3396	16,095	19,491	82·6	0·72
Syracosphaera 9725 c/ml./min 1250 cells/ml.	11	9. viii. 54	16	NIII	1	3	228	231	98·7	0·04
				NIV	7	8	2,351	2,359	99·7	0·38
				NV	9	9	2,644	2,653	99·7	0·44
				NVI	26	76	3,873	3,949	98·1	0·67
				CII	15	86	4,442	4,528	98·1	0·80

In the experiment with *Syracosphaera elongata* the faecal pellets of the smaller nauplii were probably not all found. The Nauplius III filtered only a small volume and was probably not in good condition.

In general the proportion assimilated of the phosphorus-containing part of the different food species used varies as it does in adults. *Syracosphaera* and *Chaetoceros* show a high figure (over 90%), *Ditylum*, *Skeletonema* and the small flagellate *Dicrateria* a rather lower figure.

Discussion

The experiments have shown several points of interest in the feeding of the early stages of *Calanus*. The failure of Nauplius I to feed was expected because its gut has no anal opening, but the failure of Nauplius II was unexpected until examination revealed that it had no anal opening either. This, however, explains why it is easy to rear *Calanus* in the laboratory from egg to Nauplius III, the first feeding stage. After this mortality is high. In the experimental *Calanus* taken from tow-nettings and used the same day, moulting, as is shown in Table V, is not always successful. Thus the experimental *Calanus* contain a high proportion of individuals unlikely to survive and this shows in a failure to feed or in a failure to moult successfully.

Table VII. Frequency of Moult in Experiments

Stage	Total no. used	No. moulting	Percentage moulting
NIV	85	30	35·3
NV	161	66	41·0
NVI	234	91	38·9
CI	155	28	18·1
CII	92	21	22·8
CIII	84	12	14·3

Table VII shows the numbers of *Calanus* used in all the experiments made between April and August and the percentage which reached moult. Since the experiments lasted less than 24 h it may be deduced that *Calanus* remains as Nauplii IV, V and VI only 2–3 days each, as Copepodites I and II about 5 days each and as Copepodite III about 7 days. Since Nauplius III was used as a rule immediately after its moult from Nauplius II, nothing can be deduced about the duration of this stage. The times agree fairly well with those given by Nicholls (Marshall & Orr, 1955*a*, p. 78) but are much less than the times suggested for the North Sea by Rees (1949) and Cushing (1955).

One result of these experiments on the young stages of *Calanus* is to throw doubt on the assumption that the earlier the stage the smaller will be the food organism which it requires. Although from a study of the setulation it seems that even an adult *Calanus* should be able to retain particles of 2–3 μ by at least some part of its filtering apparatus, in practice it hardly does so at all. In the

nauplii the distance between setules is not much smaller than in the adult, the filtering screen looks less efficient, and in fact the nauplius seems to feed on much the same as the adult except for really large cells. The filtering apparatus has not a rigid mesh and it would be easy for particles which could be retained by the closest setules to slip through where they are wider, or to escape between the setae.

Cushing (1955) has seen a *Pseudocalanus* take a large *Biddulphia* cell, break it and filter off some of the contents. If *Calanus* nauplii can also break diatoms and remove the contents without ingesting the cell, they may be able to feed on even the largest species.

TABLE VIII. MAXIMUM UPTAKE OF PLANT CELLS BY ONE *CALANUS* IN 24 H IN SEA-WATER CONCENTRATIONS*

			NIII		NIV		NV		NVI	
Species	No. cells/ml.	Counts/ cell	Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered
Skeletonema costatum	10,200	1·5	—	—	—	—	—	—	—	—
	9,440	0·31	2845	0·32	—	—	2539	0·28	4515	0·51
	1,290	5·7	77	$0{\cdot}09_{18}$	—	—	—	—	—	—
Chaetoceros decipiens	68 } 1 }	87·0	{ 64 { 2	0·98 $0{\cdot}34_{11}$	—	—	—	—	—	—
	2,260	22·4	—	—	—	—	—	—	—	—
	1,670	27·8	98	$0{\cdot}06_{7}$	479	0·29	847	0·52	733	0·48
	1,480	29·0	—	—	—	—	448	0·31	652	0·47
	315	125·0	30	$0{\cdot}10_{10}$	211	0·69	251	0·82	385	1·29
Ditylum brightwellii	57	642·0	3	$0{\cdot}05_{10}$	12	$0{\cdot}20_{2}$	15	0·27	31	0·56
Syracosphaera elongata	64	38·0	71	$0{\cdot}11_{10}$	—	—	—	—	—	—
	320	16·0	57	$0{\cdot}19_{8}$	371	1·17	513	1·71	576	1·99

			CI		CII		CIII	
Species	No. cells/ml.	Counts/ cell	Cells taken in	ml. filtered	Cells taken in	ml. filtered	Cells taken in	ml. filtered
Skeletonema costatum	10,200	1·5	6979	0·78	—	—	—	—
	9,440	0·31	5454	0·65	23,800	2·72	35,140	3·90
	1,290	5·7	—	—	—	—	—	—
Chaetoceros decipiens	68 } 1 }	87·0	{ — { —	— —	— —	— —	— —	— —
	2,260	22·4	1602	0·78	5,339	2·77	5,632	3·06
	1,670	27·8	991	0·60	—	—	—	—
	1,480	29·0	—	—	—	—	—	—
	315	125·0	481	1·56	1,272	4·39	2,353	8·24
Ditylum brightwellii	57	642·0	53	0·96	74	1·35	131	2·44
Syracosphaera elongata	320	16·0	849	2·81	1,764	6·45	2,283	8·14

*When more than one copepod was used at one time (N III and N IV) the number is shown as a suffix.

The data in Fig. 3 and Table IV (p. 595) show that the average volume filtered in 24 h by healthy individuals varies from about 0·1 ml. for Nauplius III up to between 2 and 4 ml. for Copepodite III. The rate increases rapidly with development but the variation is great. Compared with adults and Stage V copepodites the volumes filtered are relatively high.

The results can also be expressed as cell equivalents. Table VIII shows the maximum intake of cells in experiments where the culture concentration was such as might be found in the sea, although the numbers in the first two *Skeletonema* cultures would be found only during a rich diatom increase. A single Nauplius III took in *Skeletonema* cells at the rate of about two a

minute and in the same experiment Copepodite III took them up at the rate of twenty-five a minute. Remains of *Ditylum* cells were not recognized in any stage below Nauplius VI so the figures for the earlier nauplii probably mean little.

No data on respiration are available so that the volumes filtered cannot be related to food requirements nor these to the food available in the sea.

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Summary

Nauplii and early copepodites of *Calanus* were fed on radioactive cultures of diatoms and phytoflagellates and the food uptake and volumes filtered were measured.

Nauplius Stages I and II do not feed; Nauplius III can filter up to a maximum of 1 ml. in 24 h. There is an increase of volume filtered with development up to a maximum of about 9 ml. in 24 h for Copepodite III.

Organisms of up to 20 μ (*Skeletonema costatum*, *Prorocentrum triestinum*, *Syracosphaera elongata*) can be eaten by Nauplius III but, as with adults, the very small flagellate *Nannochloris oculata* (2–4 μ) is not taken in to any extent by the young stages.

The filtering appendages are described. The minimum distance between the setules is about 2 μ but for the main filtering surface it varies from 2 to 11 μ, and shows little variation with stage.

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