

THE NUTRITION OF COPEPODS IN RELATION TO THE FOOD-CYCLE OF THE SEA

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The importance of copepods as the chief source of food for several types of commercially valuable fish and whales is well known (Hardy, 1924; Wimpenny, 1929; Hjort and Ruud, 1929; Savage, 1931; and Campbell, 1934). Many other fish and various invertebrates depend upon copepods for food directly or indirectly. Since copepods derive their nourishment from more primitive organisms, their rôle may be regarded as that of "middle-man" in the main food-chain of the sea (Clarke, 1934*a*). The nutrition of copepods is, however, a matter about which very little is definitely known. The experiments described in this paper were therefore undertaken in an attempt to discover the precise organisms upon which copepods feed. This information is desired not only in relation to their growth but also in relation to their distribution (Clarke, 1934*b*). Furthermore, the development of a suitable method of culturing copepods in the laboratory has long been desired in order that the effects of temperature, light, and other important factors upon the growth and behavior of the animals could be investigated under carefully controlled conditions. To accomplish this a knowledge of the food of copepods is obviously a primary prerequisite.

PREVIOUS INVESTIGATIONS

Previous investigations of the food of copepods have been largely inconclusive, as already pointed out (Clarke, 1934*a*; cf. also Yonge, 1931). In general the observations may be divided into those which are claimed to indicate that copepods live on diatoms and those which appear to indicate that other organisms are the chief source of food. Examinations of the alimentary canals of copepods carried out by Dakin (1908), Esterley (1916), Marshall (1924), and Lebour (1922) revealed that a major part of the recognizable material was composed of diatom fragments. Crawshay (1915) kept *Calanus finmarchicus* alive in a pure culture of the diatom, *Nitzschia closterium*, for several weeks. Campbell (1934) believes that diatoms are the chief source of

¹ Contribution No. 66.

food for *Calanus tonsus* in the Strait of Georgia. She states, however, "It does not seem so essential that a large supply of food be available while the copepod is in the adult stage. During the fifth copepodid stage there is probably sufficient food stored as oil to tide the organism over the egg-producing period. . . . The very young stages of the copepod probably do not depend as much as the older stages upon the diatoms for food. Minute Protozoa and bacteria doubtless form the chief constituents of their diet."

Marshall, Nicholls, and Orr (1934) report that in Loch Striven during 1933 the periods of diatom increases coincided with the three main spawning periods of *Calanus finmarchicus*. They assert: "It is, of course, impossible to state what are the actual requirements of a *Calanus*, but the younger stages being less mobile, will require a richer supply than the later stages and adults. The critical time is therefore the period from egg to early copepodite, and the presence or absence of diatoms in the water then, means the success or failure of a brood." In addition they found that "The remaining constituents of the microplankton (chiefly minute flagellates) although at times numerous show no relation to breeding periods or survival." Thus Marshall, Nicholls, and Orr feel that diatoms are essential to the nauplii whereas Campbell believes that diatoms are an important source of food only during the late copepodid stages.

In contrast to these observations are those of Bigelow (1926), Johnstone (1911), and Fish (1925), in which an unmistakable decrease in the amount of zoöplankton was found to take place whenever diatoms were abundant. Fish's explanation is that "the common species [of diatoms] having these swarming periods do not form the food of the zoöplankton so far as I have been able to determine," and that "During the maxima of the large diatoms the smaller members of this group which are eaten by pelagic animals disappear, causing a scarcity in the food supply."

In at least two previous cases copepods have been kept in the laboratory on food other than diatoms. Murphy (1923) reared *Oithona nana* in small Stender dishes in each of which a small piece of fresh kelp was placed. Although a fair growth of *Navicula* and small Protozoa occurred, the copepods were observed to eat the kelp in large amounts. Bond (1933), using a culture of the green flagellate, *Platymonas*, as a source of food, reported that *Calanus finmarchicus* lived for over two months, *Euchaeta* sp. for over five weeks, and *Tigriopus fulvus* for several months including many generations. Bacteria were not excluded from the culture media in either of these cases nor in the experiments of Crawshaw cited above. Since copepods appear to be well

equipped with enzymes (Bond, 1934), their diet should not be limited from lack of powers of digestion.

MATERIAL AND METHODS

The following copepods obtainable within a three hours run of Woods Hole were used in the present investigation: *Centropages typicus* and *hamatus*, *Labidocera aestiva*, *Acartia tonsa*, and *Calanus finmarchicus*. Only about 100 specimens in several liters of water were brought in at one time and the catch was kept cold in the boat's ice box until the laboratory was reached. Immediately upon arrival the copepods were transferred by means of a large-mouthed pipette to the containers to be used for culturing. The containers were placed in one of two constant temperature tanks or in a large refrigerator. The tanks were maintained at different temperatures (kept constant to 0.1° C.) by means of two Kelvinator cooling units operated by Hiergesell thermo-regulators and relays.

The sea water used in the containers was taken from the laboratory tap except where otherwise specified. In certain experiments the water was changed by pouring away all but a little of the original culture medium, leaving the copepods in the bottom corner of the dish and then replenishing with fresh sea water. The effect of stirring and aëration was investigated by using beakers equipped with plungers activated by the Plymouth siphon device (Harvey, 1928) or by employing Erlenmeyer flasks through which compressed air from a tap filter pump was slowly bubbled.

The materials which were tested as possible sources of food for the copepods included, first, the organisms already present in the sea water. In a few experiments the water was centrifuged and the material thrown down added to the culture dishes. Material taken in the harbor with a diatom net was introduced into the containers in other cases. In a large number of the experiments "persistent"² cultures of diatoms and green flagellates grown in the laboratory were used. With the kind assistance of Professor H. H. Gran a culture of *Nitzschia closterium* was started from specimens obtained in the vicinity of Woods Hole. The cells grew chiefly on the bottom and sides of the vessel and the copepods provided with this culture did not survive. Since one supposes that filter-feeders such as copepods (Cannon, 1928) can consume material in suspension only, this culture and others of the encrusting type were abandoned as a food source. Entirely different was the culture of *Nitzschia closterium* kindly sent me by Dr. E. J. Allen from the Laboratory of the Marine Biological Association at Plymouth. In

² By a "persistent" culture is meant one which contains only one species of alga but is not free from bacteria and Protozoa (cf. Allen and Nelson, 1910).

this case the diatoms grew almost entirely in suspension and the cells themselves were quite dissimilar from the Woods Hole form, being smaller and almost straight. The following green flagellates were also found to remain in suspension in pure culture and were tried as sources of food: *Carteria mediterranea*, procured from the Pflanzenphysiologisches Institute der Deutschen Universität at Prague; *Chlamydomonas* sp., obtained from tide pools on Black Rock at the entrance of New Bedford Harbor;³ and a mixed culture of *Dunaliella marina* and *D. salina*, kindly sent me by Dr. R. M. Bond.

TABLE I

The survival of *Calanus* in relation to the presence of diatoms and green flagellates. At 15° C., small crystallizing dishes were used each containing 2 copepods in 35 cc. of filtered harbor water and 5 cc. of the food culture. At 12° C., Erlenmeyer flasks were used each containing 5 copepods in 275 cc. of filtered harbor water and 25 cc. of the food culture.

Food material added	Copepods alive after the following no. of days:					Total number of molts
	0	5	11	19	25	
	Temperature: 15° C.					
<i>Nitzschia</i>	10	9	4	2	2	3
<i>Dunaliella</i>	10	9	7	4	3	3
<i>Carteria</i>	10	10	6	1	0	1
<i>Chlamydomonas</i>	10	10	5	1	1	3
No food added	10	9	3	0	0	0
	Temperature: 12° C.					
<i>Nitzschia</i>	10	10	8	8	7	10
<i>Dunaliella</i>	10	10	5	3	1	1
<i>Carteria</i>	10	8	7	7	6	4
No food added	9	8	3	2	2	2

The diatoms and flagellates were all cultured by the following method recommended to me by Professor H. H. Gran. Sea water from the laboratory tap was filtered, heated to 70° C., and allowed to cool. To each liter was added 10 cc. of the following nutrient solution: 0.1 per cent KNO₃ and 0.01 per cent Na₂HPO₄ in distilled water. Growth was improved by adding also to each liter of sea water 5–10 cc. of a soil extract made as follows: To 1 kg. of rich, dark garden soil add 1 liter of distilled water. Autoclave at a pressure of 15 lbs. for

³ I am indebted to Professor W. R. Taylor for assistance in locating and identifying this organism.

30 minutes. Decant and filter. (Sterilize, if extract is not used immediately.) The diatom cultures were placed in a north window, the flagellate cultures in a south window but shielded from direct sunlight. When heavily inoculated, a good growth was obtained in large Pyrex Erlenmeyer flasks after about 10 days.

EXPERIMENTS USING DIATOMS AND GREEN FLAGELLATES

Preliminary experiments revealed that the copepods would die off rapidly if the temperature was allowed to rise above 20° C. or if the animals were overcrowded. When the culture water was not changed, an allowance of 20 cc. of water per copepod appeared to be adequate provided that the animals did not tend to cluster in one part of the culture dish. When provided with various of the food sources already mentioned (*Dunaliella*, *Chlamydomonas*, and *Carteria* were found to be equally efficacious), survival in the cases of *Centropages*, *Acartia*, and *Labidocera* was improved, but the majority of the animals did not live for more than about two weeks. In most of the cases in which the flagellates were added to the water, green material could be seen in the intestines of the copepods and many excretory casts were found on the bottom of the containers. Molted shells were observed only rarely. Stirring, aëration, and changing the culture water—thus avoiding the accumulation of metabolites—seemed to have no ameliorating effect. However, since we have not yet developed a completely successful method of keeping copepods alive in the laboratory, it is impossible to decide in many cases what the precise cause of the death of the animals was.

In the case of *Calanus finmarchicus* only a slight improvement in survival was found to result from the addition of green flagellates and diatoms to the culture water (Table I). The copepods, the majority of which were in copepodid Stages IV or V when taken, were examined every few days and any dead animals or molted shells were removed from the containers. Two specimens lived for 25 days in water to which nothing had been added. There appears to be no consistent difference in the effects of the several organisms tried as sources of food, but it is clear that more animals survived and more molted at 12° C. than at 15° C.

In a more elaborate experiment with *Calanus* (Table II) it was similarly found that the addition of *Dunaliella* did not enable a significantly larger proportion of the copepods to survive. However, this treatment resulted in a definitely increased number of molted shells. No significant difference appears to exist between the survival in sea water taken from the laboratory tap and in that taken directly from the

harbor. Of the two temperatures, the lower permitted a larger proportion of the copepods to survive but about the same total number of molted shells was observed. At the higher temperature growth would presumably be more rapid and hence a larger number of molted shells would be expected. However, since the period of ecdysis is known to be a critical one (cf. Hagmeier, 1930), it is possible that an increased rate of molting resulted in more deaths. Fewer animals would there-

TABLE II

The survival of *Calanus* in relation to the type of sea water with and without the addition of food material. Erlenmeyer flasks were used each containing 5 animals in 300 cc. water including, in the cases indicated, 25 cc. of the *Dunaliella* culture.

Source and treatment of water	Copepods alive after the following no. of days					Total no. of molts
	0	5	11	17	25	
	Temperature 15° C.					
Lab. supply, untreated, plus <i>Dunaliella</i> . . .	20	18	13	12	11	8
Lab. supply, untreated, no food added. . . .	5	5	3	3	3	5
Lab. supply, autoclaved, plus <i>Dunaliella</i> . . .	20	19	13	10	9	11
Harbor water, untreated, plus <i>Dunaliella</i> . . .	20	18	15	15	15	13
Harbor water, untreated, no food added. . .	5	5	3	3	3	2
Harbor water autoclaved, plus <i>Dunaliella</i> . .	15	12	11	9	5	9
Harbor water autoclaved, no food added. . .	5	5	4	1	0	2
	Temperature 5-6° C.					
Lab. supply, untreated, plus <i>Dunaliella</i> . . .	20	19	19	18	16	15
Lab. supply, untreated, no food added. . . .	5	5	5	5	5	0
Lab. supply, autoclaved, plus <i>Dunaliella</i> . . .	15	13	11	10	10	7
Lab. supply, autoclaved, no food added. . . .	5	5	5	2	2	1
Harbor water, untreated, plus <i>Dunaliella</i> . . .	20	19	19	18	18	19
Harbor water, untreated, no food added. . .	5	5	5	5	4	1
Harbor water, autoclaved, plus <i>Dunaliella</i> . .	15	13	12	10	10	6
Harbor water, autoclaved, no food added. . .	5	5	4	4	4	1

fore be left to produce shells. A high mortality in connection with ecdysis might account in part for the better survival of the copepods at the lower temperature.

In this experiment an even larger proportion of the *Calanus* survived when no food material was added. This aroused the suspicion that the copepods were feeding on microorganisms or other material already present in the culture water. In every case, survival was better

in untreated water than in autoclaved water, and for the most part more shells were molted in the former medium. Although the sterilized culture water became contaminated immediately by bacteria introduced with the living copepods, the untreated water would presumably contain a richer supply of microorganisms. If the copepods could be shown to be able to derive nourishment from these organisms, the better survival in the untreated water would be explained. The possibility existed that in all of the experiments the green flagellates and diatoms were not being assimilated at all, or only to a slight extent, and that the ameliorating effect of adding these materials was due to

TABLE III

The survival of *Calanus* in relation to the presence of microorganisms.

Crystallizing dishes with 2 animals in each were used for the first and third sets of tests, Erlenmeyer flasks with 5 animals in each were used for the second set. Temperature 5–6° C.

Treatment of water (Harbor water)	Original no. of copepods	Copepods alive after 1 month
Berkefeld filtered.....	50	22
“ “ plus <i>Nitzschia</i>	47	8
Untreated.....	50	39
Untreated.....	15	9
Filtered through paper.....	15	9
Berkefeld filtered.....	15	0
Berkefeld filtered plus <i>Nitzschia</i>	15	8
Berkefeld filtered.....	40	0†
“ “ plus bacteria*.....	36	9†

* Culture of common forms taken from Woods Hole harbor and grown on agar slants kindly supplied by Dr. C. L. Carey.

† After three weeks.

the bacteria or other organisms which are present in “persistent” cultures (Waksman et al., 1933) and which might be used by the copepods as a source of food.

EXPERIMENTS USING MICROORGANISMS

To investigate the possibility that the copepods could derive nourishment from bacteria, three sets of tests were run in which the particulate matter was removed by passing the water through a Berkefeld filter (Table III). In the first set of tests less than half the *Calanus* in the Berkefeld-filtered water survived and in the other tests all succumbed. In the untreated water a definitely larger proportion of copepods was

alive at the end of one month. The addition of *Nitzschia* did not improve the survival, and in the first test it was attended by a very high mortality, although the reason for this is not known. Passing the water through a paper filter evidently did not remove material necessary for the copepods, since the survival under these circumstances was no poorer than in the case of the untreated water. Finally, when bacteria were added to the Berkefeld filtered water, the mortality was not as great as it was in the control experiment without the bacteria.

Although the results of these experiments seem to support the suggestion that the copepods were depending more on minute organisms than on the larger diatoms and green flagellates for food, their empirical nature renders them far from conclusive. The complete flora of the culture dishes is not known, nor were its changes followed during the course of the experiment. Furthermore, the length of time that *Calanus* can live without any food is in doubt because even the Berkefeld-filtered water was contaminated as soon as non-sterile copepods were placed in it.

Since no method for sterilizing copepods has yet been devised to our knowledge (cf. Bond, 1933), the interference from extraneous bacteria was minimized by causing fresh culture media to flow continuously through the flasks within which the copepods were confined—a scheme resorted to previously for a similar purpose (Gellis and Clarke, 1935). A continuous flow was provided by running the culture water through glass tubes from carboys placed on a shelf through submerged cooling coils to 500 cc. Erlenmeyer flasks set in the constant temperature tank (15° C.). Each flask was fitted with a two-hole rubber stopper through which inflow and outflow tubes passed. The end of the outflow tube within the flask was covered with netting of bolting silk and its level adjusted so that 400 cc. of liquid remained in the flask. The rate of flow was kept constant by arranging the carboys as “Mariotte’s Bottles” (McCarthy, 1934) and the flow was suitably regulated by stopcocks to allow the entire contents of each carboy (22 liters) to pass through the flask connected with it in 24 hours.

In the first experiment sea water from the laboratory tap was passed through Whatman No. 12 filter paper and placed directly in Carboy *A*. The water for Carboy *B*, after being filtered through paper, was passed through the Berkefeld filter to remove the particulate matter. Although small quantities of water may be rendered absolutely sterile by Berkefeld filtering, complete removal of bacteria is not to be expected when large amounts of water are required. However, the existence of a significant difference in the numbers of bacteria present in the two containers was indicated by a bacterial count (using agar plates with suitable dilutions)

made 12 hours after the carboys had been filled. Carboy *A* was found to contain 680,000 bacteria/cc. and Carboy *B*, 120,000/cc.

Twenty specimens of *Calanus finmarchicus* were placed in each flask. After 20 days a total of 8 dead animals and 5 molted shells had been removed from Flask *A* (paper-filtered water). From Flask *B* (Berkefeld filtered water) 12 dead animals and no molted shells had been removed. In Flask *A* 11 copepods were still alive (one animal missing), whereas in Flask *B* only 2 copepods had survived (6 missing). It is clear, therefore, that in the Berkefeld-filtered water more animals were known to have died, many fewer were found still alive, and none had molted.

These results so strongly supported the idea that the copepods derived an important part of their nourishment from minute organisms that a second experiment using flowing water and designed to provide much greater differences in the numbers of bacteria present was immediately undertaken. To obtain a culture medium more nearly completely devoid of particulate matter, a more effective method of removing the bacteria was required. Resort was therefore made to a Zsigmondy ultra-filtration apparatus, using membrane filters of the "fine" type (average pore size about one micron to fifty millimicrons; said to remove "positively all germs and bacteria" and "the finer colloids"). Harbor water was collected from the Laboratory float and passed through Whatman No. 12 filter paper, then through the Berkefeld filter, and finally through the membrane filter. After this process, which required 6 hours or more, the water was placed in one of the three carboys used in this experiment (Carboy No. 3, Table IV). Into another of these carboys (Carboy No. 1) was put harbor water which had been passed through filter paper only and thus contained its original population of bacteria unaltered. An attempt was made to compare with this "normal" water not only water from which the bacteria had been removed but also water in which the number of bacteria was augmented. Dr. S. A. Waksman had found that if sea water was brought into the laboratory and left standing in the dark at room temperature, the number of bacteria increased enormously, reaching a maximum after 3 days. Accordingly, in the remaining carboy (Carboy No. 2) was placed harbor water which had been passed through filter paper and then allowed to stand for 3 days (or 2 days, see below).

A 24-hour supply of the 3 types of culture media was prepared every day, and just before use, cotton-filtered air was drawn through each for half an hour to insure the presence of sufficient oxygen. In this experiment two flasks were connected to each carboy; the tests were thus run in duplicate. The ends of both the inflow and the outflow of every

flask were covered by netting, thus precluding the possibility of the animals escaping up the tubes. As a control, a fourth pair of flasks was set up which was not provided with flowing water. The paper-filtered harbor water placed in these remained unchanged throughout the experiment. Each of the 8 flasks contained 20 *Calanus* in 300 cc. of water. All were examined every day and any dead animals and molted shells were removed.

At the end of 14 days it was found that the copepods in the flowing, paper-filtered water (Flasks 1*A* and *B*, Table IV) had fared the best, a total of 35 specimens being still alive, and 32 shells having been molted. The next best survival occurred in the controls (Flasks 4*A* and *B*), in which the water remained unchanged, with 33 copepods alive and 20

TABLE IV

The survival of *Calanus* in flowing water in relation to the abundance of bacteria. Twenty copepods per flask. Temperature: 12° C. Results at the end of 14 days. Roman numerals refer to the copepodid stages of *Calanus*.

Flask	Culture water	Total dead	Total molts	Alive
1 <i>A</i> 1 <i>B</i>	Paper filtered	1 Cent.,* 1 Lab. 1 Cent., 2 V	10 V, 5 IV 12 V, 5 IV	2♂, 9♀, 6 V = 17 0♂, 12♀, 6 V = 18
2 <i>A</i> 2 <i>B</i>	Paper filtered, allowed to stand	1♂, 1 V 1 Cent., 3 V, 1 IV	8 V, 1 IV 3 V, 3 IV	0♂, 8♀, 8 V, 1 IV = 17 0♂, 4♀, 6 V, 1 IV = 11
3 <i>A</i> 3 <i>B</i>	Membrane filtered	8 V, 4 IV 1♀, 13 V, 1 IV	0 0	0♂, 2♀, 5 V = 7 0 = 0
4 <i>A</i> 4 <i>B</i>	Paper filtered, water unchanged	1 Cent., 1 V 3 V	5 V, 4 IV 7 V, 4 IV	0♂, 8♀, 9 V = 17 2♂, 9♀, 5 V = 16

* Four *Centropages* and one *Labidocera* were found to have been introduced by mistake. They were all among the first to die.

molted shells. In the paper-filtered water which had been allowed to stand (Flasks 2*A* and *B*) 28 animals were alive and 15 molts had occurred. Survival in the membrane-filtered water (Flasks 3*A* and *B*) was decidedly inferior, only 7 animals being alive and no molts occurring. In five cases the dead and living animals totaled only 19 and in one case they totaled 21. These discrepancies may be due to errors in counting when the copepods were first placed in the flasks. But 4 animals were missing from Flask 2*B* and 5 animals from Flask 3*B*, and in the previous experiment 6 animals from Flask *B* were unaccounted for. Since in the last two cases the culture water was supposedly deficient in food, the possibility suggests itself that the missing animals were devoured by the others (cf. Lebour, 1922). On one occasion a copepod

which had been alive on the previous day was found in a half demolished condition.

Through the kind assistance of Dr. C. L. Carey it was possible to make bacterial counts of the culture media at three times during the course of this experiment. On the first day after the beginning of the experiment the following numbers of bacteria were found:

Flask 1 <i>A</i>	580,000	bacteria/cc.
Flask 2 <i>A</i>	130,000	"
Flask 3 <i>A</i>	6,000	"
Carboy 3	140	"
Flask 4 <i>A</i>	210,000	"

This count showed that a very pronounced difference existed in the sizes of the bacterial populations in Flasks 1*A* and 3*A*, but contrary to expectation there were fewer bacteria in Flask 2*A* than in Flask 1*A*. A more elaborate count was therefore made on the fifth day:

Carboy	Days after collecting	Bacteria per cc.	Flask	Bacteria
1.....	0	6,000	1 <i>A</i>	88,000
2.....	4	9,000	2 <i>A</i>	28,000
2 <i>a</i>	3	1,000		
2 <i>b</i>	2	45,000		
2 <i>c</i>	1	14,000		
3.....	1	8,000	3 <i>A</i>	220,000

The maximum number of bacteria in the standing carboys was evidently reached after 2 days instead of after 3 days. The procedure of the experiment was altered accordingly, and beginning on the seventh day, Carboy No. 2 was allowed to stand only 2 days. Furthermore, the populations in Flasks 1*A* and 2*A* are very much smaller than they were on the first day, and the population in Flask 3*A* has become enormously increased. In all three flasks the numbers of bacteria were much greater than in the corresponding carboys which were supplying them. The explanation for this is not known. Evidently the flora is subject to violent and rapid fluctuations, and in any future attempt to study the precise relationships between bacteria and copepods it will be necessary to follow these changes much more closely than was possible in the present case.

The extremely large number of bacteria found on this occasion in Flask 3*A*, which was supposed to be devoid of particulate matter, aroused the suspicion that the filtering process was faulty. The following counts were therefore made on the seventh day.

Carboy 3 immediately after filtering	9 bacteria/cc.
“ “ “ “ aërating	7 “
“ “ 17 hours after starting flow (1 cc. direct)	88 “
“ “ “ “ starting flow (10-1 dil.)	530 “
Flask 3A “ “ “ starting flow (10-1 dil.)	890 “

These results indicate that the use of membrane filters is as effective in removing the bacteria as could be expected for such large volumes of water. In addition, it seems probable that the large population apparently present in Flask 3A on the fifth day was exceptional, and that taking the whole period of the experiment, the copepods in this flask were provided with a decidedly smaller number of bacteria than those in the other flasks.

There is no question but that the copepods in Flasks 1A and B were thriving throughout the course of the experiment and that those in Flasks 3A and B were suffering adverse conditions. Little can be concluded regarding the copepods in Flasks 2A and B because of the uncertainty as to the numbers of bacteria present. It may be significant, however, that all but two of the molts which occurred in these flasks took place after the seventh day—when the period for which the water was allowed to stand was changed from 3 days to 2 days. Good survival and a relatively large number of molts were found in the control flasks (4A and B) although 40 animals would have been expected to have consumed all the food material present in a very few days. The bacteria originally in the culture water undoubtedly could multiply faster than the copepods would eat them provided that sufficient nutrient substances for the bacteria were present. Although in plain sea water the nutrients would be largely exhausted in three or four days, it is possible that the material excreted by the copepods served as a supplementary source of nourishment for the bacteria and permitted a sizable flora to maintain itself for the fourteen days of the experiment.

If the foregoing interpretation is accepted, the experiment appears definitely to point to the dependence of the *Calanus* on bacteria or other microorganisms. But a possible alternative explanation of the whole experiment should be considered, namely, that none of the copepods were feeding to any significant extent and that the differences in the three tests were due to other factors than the nutritional. Several investigators have suggested that at certain times under natural conditions copepods may live for long periods of time with a greatly reduced diet (cf. Campbell, 1934; Marshall, 1924). In the present experiment the elaborate filtering process used for Flasks 3A and B might conceivably have rendered the culture water harmful in some respect, and the period of standing employed in the case of the water for Flasks 2A and B might have allowed lethal bacteria to multiply unduly. The

slight improvement of Flasks 1*A* and *B* over Flasks 4*A* and *B* could be explained on the basis of a better supply of oxygen and removal of metabolites in the flasks with flowing water. However, the difference between these two sets of flasks is very slight in respect to survival, but in number of molts, the former set is superior by 50 per cent; and in the other cases longer survival is correlated with a greater amount of molting. Although it is possible that stored oil could be entirely depended upon for the production of new shells, the growth entailed in the process would seem to presuppose the taking in of a considerable quantity of nutriment.

Everything considered, the interpretation of the experiment on the nutritional basis seems the more probable one. According to this, the copepods were living and growing on bacteria or other food material small enough to pass through the pores of the paper filter, and when this particulate matter was removed, the animals failed to grow and died off rapidly.

DISCUSSION

Since the time of Lohmann it has been known that the nannoplankton constitutes a large *potential* source of food in the sea, but little has been done to show whether this is actually used by the zoöplankton (cf. Bond, 1933). The foregoing experiments are by no means conclusive, but if, as they indicate, copepods in nature feed to any significant extent upon material as minute as bacteria, a knowledge of the precise organisms and relationships involved will be necessary for an understanding of this part of the food-cycle of the sea. Besides the bacteria, of which there are many types in the sea, other kinds of particulate matter—both living and non-living—should be scrutinized as possible food sources. A fresh-water entomostracan, *Daphnia*, has been shown to be able to derive a certain amount of nourishment from material present in colloidal form (Gellis and Clarke, 1935). Colloidal material undoubtedly exists in sea water, but little or nothing is known of its abundance or availability. Dr. J. B. Lackey, who was conducting a survey of the Protista of Woods Hole Harbor at the time that these experiments on *Calanus* were in progress, has very kindly informed me that of the organisms, other than bacteria, present in the harbor water which would pass in any significant number through Whatman No. 12 filter paper, the following were the most numerous: the spermatozoa of fish and of invertebrates, the gametes or swarm spores of algae, flagellate Protozoa, and unidentified algal cells 2–5 μ in diameter. Of these the flagellate Protozoa would constitute the largest bulk of food material.

The majority of the diatoms—at least the larger ones—was presumably removed by the filter paper; but diatoms and all other larger organisms may be important as a food source when broken into fragments during the course of their disintegration after death. Furthermore, after the bodies of dead plants and animals have passed finally into solution, their substance serves as nutriment for countless bacteria. Larger organisms may thus indirectly furnish nourishment for the copepods. The excretions of living organisms possibly also support a useful population of bacteria. There is some evidence that part of the material synthesized by diatoms dissolves out of the living cells into the surrounding water (Pütter, 1924; Gran, 1927; Marshall and Orr, 1930; and Krogh, Lange, and Smith, 1930). Waksman et al. (1933) found that in the Gulf of Maine many more bacteria existed in association with the zoöplankton and the phytoplankton than in the free water. They state:

“A decided parallelism was observed between the abundance of diatoms in the sea and abundance of bacteria. . . . These results seem to point definitely to the fact that the development of phytoplankton in the sea is accompanied closely by bacterial development. The bacteria feed upon the excretion products of the diatoms, algae, and animal forms and probably upon these plankton forms themselves as soon as they die. . . .” The explanation of those cases in which a correlation is claimed between the abundance of copepods and of diatoms may possibly be that the copepods depend for their nourishment upon the diatoms *indirectly* through the bacteria.

SUMMARY

1. The importance of copepods in the economy of the sea and the inconclusive and contradictory nature of previous observations on their food make it desirable to investigate the nutrition of these animals in a thorough manner. For this purpose and in order to develop a satisfactory method of culturing copepods in the laboratory so that their physiology might be studied under carefully controlled conditions, feeding experiments were undertaken using *Centropages*, *Labidocera*, *Acartia*, and particularly *Calanus*.

2. Small containers without stirrers appeared to be suitable provided that over-crowding was avoided and a low temperature maintained. The materials which were tested as possible sources of food included the organisms present in the harbor water and “persistent” cultures of certain diatoms and green flagellates.

3. Although the survival of the first three copepods mentioned was prolonged by the addition of the diatoms and green flagellates, the

majority died off after about two weeks. In the case of *Calanus* only a slight improvement resulted from this treatment and a large proportion survived when no food organisms were added.

4. Further experiments revealed that the sterilization of the culture water and the removal of particulate matter from it was accompanied by a high mortality of *Calanus* and that the addition of bacteria to the water resulted in improved survival. To test the possibility that the copepods were utilizing the smaller types of microorganisms for food, the growth of bacteria, etc. was minimized by passing membrane-filtered water continuously through flasks in which certain of the copepods were confined. These copepods failed to molt and died off rapidly, whereas those in flasks through which paper-filtered water flowed remained alive and molted in large numbers.

5. These experiments therefore indicate that bacteria and other constituents of the nanoplankton may be an important food for copepods in the sea. Diatoms and other larger organisms may possibly serve as a source of nourishment indirectly through the bacteria, etc., which feed upon them and their excretions.

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