BACTERIA AS FOOD FOR CERTAIN MARINE INVERTEBRATES

By

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Because of their ability to convert dissolved organic matter into cell substances and to synthesize protoplasm below the depths to which radiant energy penetrates sea water, bacteria may play an important role in the nutrition of marine animals. It has been well established experimentally that bacteria serve as an important source of food supply for protozoa (Luck et al, 1931) but it is not definitely known to what extent they may play a role in the nutrition of higher forms of animal life. The solution of this problem requires information on several different points: (1) What animals in the sea may ingest microorganisms? (2) Are the ingested microorganisms assimilated and to what extent may they nourish these animals? (3) Are bacteria sufficiently abundant to constitute a significant part of the food supply of the animals? (4) Are all marine bacteria equally nutritious and may some species indigenous to the marine environment be toxic or poisonous to the animals which ingest the bacteria? Some of these questions are answered by the field observations and laboratory experiments discussed below.

EXPERIMENTS WITH MUSSELS

In the first series of experiments the sea mussel, *Mytilus californianus*, was selected for study as a representative of the lamellibranchs which are believed to derive their nourishment primarily from the finely divided detritus which they swallow (Field, 1922). *M. californianus* is quite abundant in this vicinity, specimens are of convenient size, they grow fairly rapidly, they can be weighed and measured with a fair degree of accuracy and they can be maintained in the laboratory with relative ease.

Healthy mussels measuring 6–9 cm. in length and weighing 20–50 gm. were placed after scraping their shells clean in battery jars containing sea water aged in the dark. The organic matter content of the aged sea water was negligible (less than 2 mg. per liter) after it had been filtered through paper to remove particulate matter and held in the laboratory in the dark for several weeks. After two weeks' starvation the mussels were placed singly in 600 cc. of aged sea water and offered a bacterial meal by adding a suspension of washed bacteria.

* Contribution from the Scripps Institution of Oceanography, New Series No. 40.

For the initial tests two species of bacteria were selected which have distinctive morphological and cultural characteristics by which they can be readily recognized, namely *Rhodococcus agilis* Ali-Cohen and *Bacillus marinus*, n. sp. The former is a motile Gram-positive coccus 0.8 to 1.0 μ in diameter, occurring singly, in pairs and occasionally in fours. On nutrient sea water agar it forms small, circular, rose-red, glistening colonies. *B. marinus* is an aerobic Gram-positive rod 0.8 to 1.8 μ by 7.0 to 9.2 μ, forming central spores which are 1.2 to 1.8 μ by 2.4 to 3.6 μ. The cells occur singly and in chains. It is motile by means of peritrichous flagella. On sea water agar the grayish-white, finely granular, glistening colonies are 5 to 8 mm. in diameter. Large quantities of the bacteria were grown on nutrient sea water agar in Blake bottles incubated at 25° C. Two-day old cultures were harvested and washed by centrifuging in two changes of isotonic salt solution to remove dissolved and adhering nutrients.

Sufficient washed bacteria were given to the mussels to render the water perceptibly turbid (between 500 and 5000 million per cc.). Larger concentrations of bacteria were not well tolerated by the mussels unless the water was oxygenated by bubbling air through it. The mussels removed most of the bacteria from suspension within two hours as indicated by the clearing of the water, whereas in the control jars lacking mussels the water remained turbid for several hours.* Plate counts revealed that the bacterial population was reduced to only a few hundred thousand bacteria per cc. within six hours. However, several thousand or more bacteria per cc. always remained suspended. This is partly attributed to the imperfections in the filtering mechanism of the mussel but mostly to the fact that bacteria multiply at the expense of the metabolic waste products of the mussel. After a day or two virtually all of the two test organisms disappeared or their presence was obliterated by the mixed microflora which developed. It is believed that Moon and Hamilton (1934) underestimate the ability of fresh-water mussels to remove bacteria from water because they overlooked the fact that the excretory products of well-nourished mussels provides for the multiplication of bacteria.

Part of the bacteria from the suspension were merely clumped by the mussels and rejected as pseudo-feces (Dodgson, 1928) but a good many of them were actually ingested. This was proved by the microscopic and cultural demonstration of the bacteria in the digestive tracts of mussels which were dissected within an hour or two after receiving a bacterial meal. The stomachs and especially the head of the style of the mussels which were fed with the red coccus were tinted pink by the ingested bacteria. The intestinal tracts of the control mussels were virtually sterile after two weeks' starvation.

* It has been shown by Fox, Sverdrup and Cunningham (1937) that mussels of this size filter between one and two liters of water per hour.

Most of the bacteria disappeared from the intestinal tracts of the mussels within six hours after being fed. Direct microscopic examinations showed that there were very few more bacteria per unit volume of extruded feces than in the surrounding water, thereby indicating that the bacteria had been digested by the mussels. In fact, bacteria in different stages of lysis were observed in the stomach contents. Incidentally there was a much larger proportion of spores of *B. marinus* in the pseudo-feces of mussels fed on this species than in the original suspension. This may be interpreted as showing a selective exclusion of spores unless spore formation is stimulated in the pseudo-feces by the conditions of the experiment. The spores which were ingested were digested only slowly or not at all because they disappeared very slowly from the intestinal tract and they were many times more concentrated in the feces of the animals than in the surrounding water.

Digestive enzymes which lyse bacteria were recovered from the viscera of mussels. The digestive diverticula, stomachs, styles and intestines of several large well-nourished mussels were dissected free of other tissue and triturated with sand. The resulting extract was filtered through four thicknesses of cheesecloth and preserved in the refrigerator with toluol. A drop of the extract was added to a drop of bacterial suspension in small vials. After two to six hours' incubation at 25° C. stained smears of the mixture were examined microscopically. The cells of all except three of the 31 different species of marine bacteria tested were dissolved by the extract. When the extract was inactivated by heating to 75° C. for ten minutes the bacteria were merely agglutinated or unaffected.

The bacteria which were not attacked by the digestive enzymes of the mussel included two undescribed species of acid-fast *Mycobacteria* and an *Actinomyces*-like rod. The vegetative cells of *Bacillus marinus* were rapidly lysed and its spores seemed to be slowly attacked by the enzyme extract. Incidentally most of the other bacteria were Gram-negative *Bacteriaceae*. No difference was noted between the effects of Gram-positive and Gram-negative bacteria on the mussels. Oehler (1916, 1920) reports that Gram-positive species are generally digested less readily than Gram-negative ones and that certain acid-fast bacteria and spores are not digested at all by flagellates or ciliates. After investigating the digestibility of numerous bacteria by soil protozoa Severtzova (1928) concluded that small non-sporulating rods and cocci are the best sources of food while the large spore-forming rods are not so useful, although the vegetative cells of the latter are readily ingested and digested.

Having found that mussels ingest bacteria and that they possess enzymes capable of digesting bacteria, experiments were next designed to ascertain if the mussels might derive any nutriments from the bacteria. For this purpose specimens 2–4 cm. long and weighing 2–5 gm. each were collected. The small mussels were selected because they require less space and less food and

normally grow faster than larger ones. After an initial quarantine and inanition period of two weeks they were placed in groups of three in 600 cc. of aged sea water. Each individual was marked with colored sealing wax and weighed bi-weekly. Three times each week enough washed bacteria were added to give 500 million per cc. of *B. marinus* or 5000 million *Rh. agilis*. The water was aerated in the dark at 14–15° C. and was changed once a month to obviate the accumulation of metabolic products in toxic concentrations. The mussels in 8 jars were fed exclusively with *Rh. agilis*, 8 others received *B. marinus*, and the mussels in the 8 control jars were not fed. The results are summarized in Table I.

TABLE I

AVERAGE WEIGHT OF MUSSELS AFTER BEING ON AN EXCLUSIVE DIET OF BACTERIA FOR DIFFERENT PERIODS OF TIME

Time	*Food received*	*Living specimens*	*Average weight*	*Mean gain in weight*
			gm.	*percent*
Initial	*Rh. agilis*	24	3.62	0
	B. marinus	24	3.65	0
	None	24	3.71	0
2 months	*Rh. agilis*	22	3.57	− 1.3
	B. marinus	21	3.72	1.9
	None	20	3.56	− 4.0
4 months	*Rh. agilis*	19	3.81	5.2
	B. marinus	21	3.86	5.8
	None	14	3.42	− 7.8
9 months	*Rh. agilis*	17	4.08	12.4
	B. marinus	21	4.04	9.7
	None	9	3.11	−16.3

The mussels fed on the red cocci gained an average of 12.4 per cent in weight in 9 months, those fed on the sporulating rods gained 9.7 per cent, and the fasting controls lost an average of 16.3 per cent. It is estimated that during the nine-month period covered by the experiment each mussel received at least 60 to 80 grams of bacteria or 6 to 8 grams of solid material, since the bacteria contain about 90 per cent water. This would indicate that between 5 and 10 per cent of the solid matter of the bacteria was assimilated by the mussels although it must be recognized that part of the weight gained by the mussels is calcareous shell substances taken from the sea water. Also numerous bacteria develop at the expense of the waste products of the mussels. However, probably much more than 10 per cent of the bacterial substance was assimilated because the gain in weight does not take into con-

sideration the energy which has been consumed by the mussels. Professor T. Kincaid at the University of Washington has maintained oysters for several months with nothing to eat except bacteria. The oysters appeared to develop normally and their glycogen content increased. Allen (1921) has emphasized the importance of nannoplankton, including bacteria, in the diet of freshwater mussels.

Although mussels have been maintained in the dark on an exclusive diet of bacteria for nearly two years, they do not thrive as well on such a diet as they do on the food they find in running sea water in the light. This may mean that the assorted dietary occurring in sea water is more nourishing than bacteria alone or that other conditions of the experiment were more favorable. There was no evidence that the bacteria themselves were injurious to the mussels in any way unless they were added in such great numbers that bacterial respiration vitiates the water. None of the 31 different species fed to mussels in concentrations ranging from 500 to 5000 million per cc. exhibited any toxic effects. In each case the mussels started to filter soon after the addition of the bacterial suspensions, freeing the water of the turbidity caused by the latter. Even the two acid-fast *Mycobacteria* and the *Actinomyces*-like rod were filtered from the water and many cells thereof were ingested although they were not digested. According to Fox (1936) the mussel is able to exercise a marked degree of discrimination with respect to the quality of food, selectively rejecting certain poisonous substances or closing its valves in their presence.

While the bacteria themselves are not injurious, the metabolites of rapidly reproducing cultures are quite toxic to the mussels. In the presence of sea-water peptone-broth cultures containing less than a hundred thousand bacteria per cc., the valves of the mussels remained closed and the animals ultimately died. That the peptone itself was not the toxic ingredient was proved by feeding peptone to the mussels. It was tolerated in concentrations as high as 2.0 per cent until the growth of bacteria vitiated the water. Similarly glucose, glycerol and asparagine, all of which promote the growth of bacteria when added to sea water, were tolerated by the mussels until the concentration of bacterial metabolites became high enough to force the mussels to close their valves and eventually die.

Some mussels survived two weeks in sea water containing 2.0 per cent peptone, glucose, glycerol or asparagine when refrigerated (0 to 4° C.) to retard bacterial activity. Under these conditions the mussels were also inactive but revived even when frozen in ice if the temperature was slowly raised again. Mussels could be kept active in the above mentioned nutrients at normal water temperatures (ca. 15° C.) by adding the equivalent of one part of acriflavine to 10,000 parts of sea water which inhibits bacterial multiplication but is tolerated by the mussels, thereby proving that it is bacterial metabolites and not peptone, glucose, glycerol or asparagine which are toxic.

EXPERIMENTS WITH SAND-CRABS

The sand-crab, *Emerita analoga*, is a detritus-eating crustacean which inhabits shallow bottoms. It feeds by extending its feathery setose antennae from the sand in which it buries itself and collecting whatever particulate organic matter that may come along. It was selected for a series of bacterial feeding experiments because of its abundance in the region and its adaptability to laboratory conditions.

The bottoms of liter jars were covered with clean sea sand to a depth of 25–30 mm. and 500 cc. of aged sea water was added. Ten sand-crabs measuring 8–10 mm. in length were placed in each jar. Enough of a washed suspension of bacteria was added to give around 500 million cells per cc. When disturbed or when toxic substances were added to the sea water the sand-crabs remained completely buried. Otherwise they extended their antennae always reaching for food. This they commenced to do soon after the addition of the bacterial suspensions slowly clearing the water of its turbidity. The cultural and microscopic examination of the fore-gut of the animals one or two hours after receiving a bacterial meal showed that they were fairly gorged with the bacteria.

The sand-crabs removed the bacteria from the water much more slowly than the mussels and their ultimate filtering efficiency was not nearly so great. In six to eight hours they reduced the number of suspended bacteria from over 500 million to less than a million per cc., whereas in control jars lacking sand-crabs the water remained turbid for a few days with several million bacteria per cc. in suspension. It seems incredible that the filtering mechanism of the crabs is sufficiently fine to enable them to remove particles from water as small as bacteria (diameter ca. 1.0 μ) but they appear to be equipped for collecting the bacteria on the feathery antennae from which they can be scraped off into the crab's mouth. It is highly probable that the sand-crabs have a tendency to agglutinate or clump the bacteria because direct microscopic observations revealed the presence of many more clumps of aggregated bacteria in jars of water containing crabs than in the uninhabited control jars. Mr. Weldon M. Lewis who is studying the food habits of *Emerita* in this laboratory suggests that bacteria may be clumped by being enmeshed in the gills from which they are raked off when the crab cleans its gills. The crabs also unquestionably ingest large numbers of bacteria adhering to the sand grains.

The bacteria ingested by the crabs were rapidly digested. Bacteria in different stages of lysis were found in the fore-gut of crabs when examined soon after being fed. If, however, they were transferred to sea water sans bacteria after a bacterial meal, only sand grains were to be found in the gut half an hour later. Enzymes which dissolve the bacteria were also demonstrated in the digestive tracts of the crabs by dissecting these organs from large specimens and using a technique similar to that described above for

mussels. However, due to the difficulties attending the extraction of the enzyme in appreciable quantities it has been feasible to test the extract on only three different species of bacteria, all of which were digested.

Are the bacteria utilized as food by the sand-crabs? Enough *Rhodococcus agilis* to give about 5000 million per cc. were added semi-weekly to four jars each containing ten crabs. Another four jars received a like suspension of *Bacillus marinus* and four others received *Flavobacterium boreale* Lundestad. The latter is a Gram-negative non-motile rod 0.5 to 0.7 μ by 1.6 to 2.6 μ occurring singly and occasionally in pairs. It grows readily on sea water agar with the production of circular, glistening, bright yellow colonies. The crabs in four control jars were not fed. The water was aerated by bubbling air through it and was changed bi-weekly. Also at bi-weekly intervals the crabs were recovered from the sand by washing the latter through a large mesh sieve. The survivors were counted and after being washed entirely free of sand were weighed collectively in tared bottles.

Whenever the water remained or became turbid in any jar it was examined for dead crabs and the survivors were weighed and transferred to fresh sea water. The casts of the specimens which had moulted were recovered and counted. Table II summarizes the results of such an experiment of five months' duration.

TABLE II

NUMBER OF SAND-CRABS SURVIVING AFTER BEING FED ON BACTERIA FOR 22 WEEKS. THE NUMBER OF MOULTED CASTS AND THE AVERAGE WEIGHT OF THE LIVING SPECIMENS IS ALSO GIVEN AFTER DIFFERENT INTERVALS OF TIME

Time	*Food received*	*Living specimens*	*No. of casts*	*Average weight*
				gm.
Initial	*Rh. agilis*	40	0	2.6
	B. marinus	40	0	3.1
	Fl. boreale	40	0	2.5
	None	40	0	2.8
4 weeks	*Rh. agilis*	36	0	2.6
	B. marinus	31	2	3.0
	Fl. boreale	35	0	2.7
	None	32	0	2.7
10 weeks	*Rh. agilis*	27	3	2.5
	B. marinus	29	10	3.4
	Fl. boreale	31	7	2.8
	None	17	0	2.3
22 weeks	*Rh. agilis*	12	9	2.9
	B. marinus	14	19	3.4
	Fl. boreale	9	14	2.7
	None	0	0	—

The significance of the average weight of the sand-crabs is to be questioned, due to the inaccuracies of weighing the animals and also because many specimens perished in the course of the experiment. However, the weights given in Table II are more likely to be too small after the fourth week rather than too large because more small than large specimens survived. Moreover, the weight of moulted casts could legitimately be added to the average weights since they represent part of the organisms. Nevertheless in spite of these sources of error, the experiment shows that by giving them careful attention sand-crabs can be maintained longer when fed bacteria than without food. Furthermore, the moulted casts from the bacteria-fed animals is indicative of their development. It may be significant that the sand-crabs fed on the two larger rods, *B. marinus* (1.3 x 8.1 μ) and *Fl. boreale* (0.6 x 2.1 μ) developed better than those fed on the smaller coccus, *Rh. agilis* (0.8 x 1.0 μ).

The sand-crabs are more sensitive to large doses of bacteria than the mussels. However, there was no evidence of the bacteria themselves being toxic in moderate concentrations. Only when they were present in sufficient numbers to vitiate the water by their metabolism were the bacteria injurious to the sand-crabs. The sand-crabs soon died in the presence of as little as 0.2 per cent of the bacterial nutrients, peptone, glucose, glycerol or asparagine. Under these conditons they could not be kept alive by refrigeration or selective bacteriostatic substances. Concentrations of acriflavine which were tolerated by the mussels were lethal for the sand-crabs.

Like the mussels, the crabs tolerated living bacteria better than heat-killed ones, probably because other bacteria which are always associated with the animals multiply at the expense of the organic matter from the heat-killed bacteria. Baier (1935) reports that in moderate doses heat-killed bacteria are just as nutritious as live ones. However, he records that young bacterial cells are more nutritious than old ones.

EXPERIMENTS WITH GEPHYREAN WORMS

The sipunculid Gephyrean worm, *Dendrostroma zostericola,* is a hardy animal capable of being maintained in the laboratory for a long time (Peebles and Fox, 1933). Normally it lives burrowed in mud which it ingests for the organic matter content. It extrudes its tentacles from its burrow as if the animal were also collecting planktonic organisms from the water for ingestion. The feeding habits of *Dendrostroma* lead one to believe that the abundant bacterial flora in marine muds, as well as those in the water near the bottom or adhering to particulate matter, may play an important role in the nutrition of this animal.

Three healthy specimens measuring 3–6 cm. in length by 4 to 6 mm. in width at the greatest diameter when contracted were placed in each of

several liter jars together with 60 cm. of sand and 500 cc. of aged sea water. Some of these were fed the red coccus, *Rhodococcus agilis*, some the yellow rod, *Flavobacterium boreale*, and some were not fed. Shortly after receiving such a bacterial meal the oesophagus and descending arm of the intestine were noted upon dissection to be actually colored red and yellow, respectively. Microscopic examinations revealed an abundance of the two types of bacteria along with much sand. Confirming the observations of Peebles and Fox (1933) the control worms which received no food had ingested very few sand grains, and only by cultural procedures could any bacteria be demonstrated in the oesophagus. No bacteria were found in the intestines of the starved worms.* The lack of bacteria in the ascending arm of the intestine is regarded as evidence that the ingested bacteria were digested by the worms.

Contrary to expectations the worms failed to gain weight even after being fed on bacteria for 14 weeks although they appeared to be in good condition. However, the starved controls lost more weight than those which were fed, thereby indicating a nutritive property of the bacteria. It is possible that the bacteria-fed specimens failed to gain weight because they were mature adults, the quantity of food may have been insufficient, the bacteria may not be a complete food, or other conditions may have been such that the food could maintain the worms although the latter were unable to grow.

It has been shown by MacGinitie (1932) that the echinoid Gephyrean worm, *Urechis caupo*, lived well in sea water on an exclusive diet of bacteria, *Pseudomonas* sp. He (1937) points out that mucus plays an important role in entrapping bacteria and other plankton organisms which *Urechis* ingests. The former observation has been substantiated and elaborated upon somewhat.

Adult specimens of *Urechis caupo* were placed in U-tubes 20 cm. long in which they fit snugly when contracted (diameter 8 mm.). They were immersed in sea water in 2-liter battery jars. The water was aerated and kept in the dark. After the worms became acclimitized to these artificial burrows they commenced to force water through the tubes. In some cases bacteria were added to the sea water to give suspensions of 500 to 5000 million per cc. and in other cases heavier suspensions of bacteria were placed with a pipette directly in the inlet of the U-tube. In either case there was evidence of the bacteria being removed from the water by the worms.

Using the latter feeding technique two worms were fed every other day with 5 cc. of a concentrated suspension of *Rh. agilis*, two others received a like amount of *B. marinus* and two controls were starved. One of the

* Although no bacteria were added to the control jars, there were always several thousand mixed microflora which presumably grow at the expense of the metabolic waste products of the worms.

starved worms died after 12 days. Its surviving companion had lost much weight and died after 19 days. At this time the worms fed on *Rh. agilis* had lost a little weight, while those fed on *B. marinus* had gained slightly. All of the worms were killed on the 28th day when they were inadvertently fed on toluol-preserved suspensions of the bacteria. Up until this time the worms exhibited no toxic effects from large doses of bacteria and they appeared to be well nourished.

ABUNDANCE OF BACTERIA IN THE SEA

If the mussels, sand-crabs, and Gephyrean worms used in the foregoing experiments are at all representative of other animals, there seems to be little question that bacteria, if sufficiently abundant in the sea, may play an important role in the nutrition of marine animals.

According to literature summarized by Benecke (1933) sea water in the photosynthetic zone contains only a few to a hundred bacteria per cc. although occasionally several thousand or more bacteria occur per cc. The bacterial population is more sparse at greater depths until the bottom is reached. This is essentially in accord with the more recent observations of Waksman et al (1933) in the Atlantic Ocean, and of ZoBell and Feltham (1934) in the Pacific. However, these counts have been obtained using the conventional plating procedures which detect only a small percentage of the viable bacteria. Bere (1933) found direct microscopic counts of bacteria in lake water to be 20 to 335 times higher than plate counts, and Waksman and Carey (1935) report direct counts of bacteria in sea water to be about 200 times higher than plate counts.

Even if there are actually 1000 times as many bacteria in sea water as indicated by plate counts, they would constitute a rather inappreciable mass at any one time. Assuming the average size of marine bacteria to be 1 cu. μ and if sea water contained as many as 100,000 per cc., this would be equivalent to only 0.1 mgm. of bacteria per liter. However, the rapidity with which bacteria multiply tends to compensate for their extreme smallness, and regardless of how they are enumerated, the count is merely an expression of the dynamic balance between their rate of multiplication and the rate of destruction of bacteria. Thus if the bacteria reproduce only once every three hours (and many reproduce much more often than this) and if they are eaten by marine animals at the same rate, 0.8 mgm. of bacteria would be produced per liter per day. In a year a liter of water may yield nearly 3 gm. of bacteria, or approximately 200 mgm. of organic matter. If only a fraction of this amount of bacteria is formed per year, cumulatively it is of considerable importance in the food cycle in the sea, particularly when it is taken into consideration that sea water contains only about 10 mgm. of organic matter per liter.

Viewed from a different angle, it seems doubtful if bacteria constitute a very large part of the dietary of animals which feed in sea water. According to Harding (1937) the ciliate, *Glaucoma pyriformis*, eats around 13,000 bacteria per hour and it was semi-starved if the concentration of bacteria was less than 600 million per cc. Based on calculations by Baier (1935) an average-sized protozoan living exclusively on bacteria would have to fish through over 10,000,000 times its volume of water containing 100,000 bacteria per cc. in order to consume an amount of bacteria equal to its own volume! A mussel or sand-crab weighing 5 gm. would have to filter at least 50,000 liters of such water in order to consume an amount of bacteria equivalent to its own weight. Our experiments indicate that a dynamic population of several million bacteria per cc. is required to maintain these animals. From their studies on the food of pelagic copepods and the quantity of water filtered per day, Fuller and Clarke (1936) concluded that bacteria are insufficient in number to serve as an exclusive diet of these plankton crustacea.

In bottom deposits where bacteria are much more abundant than in the overlying water, they may constitute an appreciable part of the dietary of mud-feeders. According to the literature and original work summarized by ZoBell (1938) the bacterial population or marine mud as determined by plating procedures ranges from a few hundred to several million per gram of wet mud, the mean being in the order of magnitude of hundreds of thousands of bacteria per gram. The total bacterial population may actually be 10 to 100 times higher than this considering that plate counts detect only a small percentage of the viable bacteria.

Bacteria probably multiply much more rapidly in bottom deposits than in water since there is more organic matter available there and most other conditions are more suited to their reproduction. If the cells reproduce only once every six hours, it is estimated that under favorable conditions several milligrams of cell protoplasm may be synthesized per day by the bacteria in the topmost five centimeters of a square meter of marine sediment. While some of the bacterial cells may undergo autolysis, the majority of them are probably consumed as food by bottom-dwelling animals at about the same rate as they are formed. Cumulatively bacteria may supply 2 to 30 grams of particulate animal foodstuff per square meter per year in fertile bottoms. There may actually be enough bacteria in some bottom deposits to serve as the exclusive source of food for certain bottom-dwelling animals although the bacterial diet is perhaps supplemented to a large extent by other types of food.

There are numerous animals which live in and on the upper layers of slime or mud on the ocean floor. Sometimes the organic matter of this slime consists of 5 to 10 per cent by weight of bacteria. The majority of the slime-feeders whose habits have been described exert some discrimination in the selection of food, but many of them take in the entire substrate, digesting

the usable portion and ejecting the other. Baier (1935) includes in this category certain *Nematodes, Tubificidae, Chironomidae,* mussels, slime-inhabiting *Crustacea, Rotifera,* ciliates and flagellates. He also mentions a group of bacteria-devouring animals which graze on solid objects like shells, rocks, water plants and the like. Certain snails, ostracods, copepods, and amoebae are representatives of this group. According to ZoBell and Allen (1935), the slimy film scraped from the bottoms of ships contained a few million to several billion bacteria per gram and it consisted of 8 to 9 per cent by volume of bacteria. They (1933) found as many as a million bacteria attached per sq. cm. on glass slides after 24 hours' submergence in the sea, and the number increased progressively with time until the surface became obliterated with sedentary animals which presumably settled there to graze on the bacteria. This is in agreement with the studies on freshwater bacteria by Henrici (1933).

DISCUSSION

Although there may not be many animals equipped with filtering mechanisms sufficiently fine to catch individual suspended bacteria, most animals could catch them when the bacteria are clumped in aggregates of appreciable size. Esterly (1916) points out that certain copepods as well as ostracods form pellets of minute particles including bacteria which are readily ingested. A consideration of even greater importance is that most marine bacteria are periphytes or epiphytes which grow attached to particles of organic matter, plankton or other solid surfaces (ZoBell, 1936). Not infrequently there is a mass of bacteria attached to a particle of organic matter or a plankton organism which is greater than the mass of substrate itself. Consequently an inestimable quantity of bacteria are ingested by virtually all types of animals and the foregoing feeding experiments as well as the observations of others warrant the conclusion that at least some of the bacteria are assimilated as food.

Luck, Sheets and Thomas (1931) cite many references to show that protozoa are nourished by bacteria, as do Doflein and Reichenow (1928) for the *Ciliate.* These latter workers conclude that bacteria make up a great part of the food of ciliates. According to Kofoid (1933) the multifarious marine ciliates, *Tintinoinea,* feed upon bacteria. Some 200 different plankton organisms were found by Voroschilova and Dianova (1937) to feed on bacteria in the Caspian Sea where an increase in bacteria resulted in an increase in plankton. Waksman and Carey (1935) believe that protozoa, copepods and other bacteria-consuming organisms are primarily responsible for the paucity of bacteria in sea water. Naumann's studies (Baier, 1935) on the plankton crustacean, *Cladocera,* the copepod *Diaptomus* and certain *Rotifera,* reveal that these animals possess a filtering mechanism capable of catching bacteria which constitute their main nourishment. Stuart, Mc-

Pherson and Cooper (1931) found that live bacteria satisfy the food requirements of the micro-crustacean, *Moina macrocopa*, but that alls pecies of bacteria do not serve equally well as food. *Artemia* were observed by Bond (1933) to thrive on bacteria. That bacteria are consumed as food for marine sponges has been shown by Pourbaix (1932). After observing the development of frog tadpoles on an exclusive bacterial diet, Burke (1933) concludes that probably a wide variety of vertebrates as well as invertebrates utilize bacteria as food.

Regardless of whether one-tenth or only one-millionth part of the food of marine animals consists of bacteria, cumulatively it is of quantitative significance. It is now the concensus of opinion that, contrary to Pütter's theory, dissolved organic matter directly plays a very minor role (Krogh, 1931) in the nutrition of marine animals. Few, if any, animals have a mechanism for ingesting more than a casual amount of dissolved organic matter but when it is converted into particulate organic matter by bacteria (Krizenecky and Podhrasky, 1927) the latter may be readily ingested by many types of animals. Pütter (1924) himself has recognized this as shown by his observations on the bacterial nutrition of copepods.

Bacteria are of importance in food cycles not only by converting dissolved organic matter into a particulate and consequently a utilizable form but they also attack much solid organic matter unfit as food for other organisms such as, for example, cellulose and chitin, mineralizing part of it and synthesizing assimilable bacterial cell substance from the rest. It is for this reason that Baier (1935) writes that it is not the decomposing plant susbtance but rather the bacteria, the cause of decomposition, which nourish the animals consuming it.

Besides the heterotrophic bacteria which merely transform organic matter, there are autotrophic bacteria which synthesize cell substances from carbon dioxide, water and minerals deriving their energy either from sunlight or from the oxidation of hydrogen sulfide, sulfur, ammonium, nitrite, methane or ferrous iron. Little is known concerning the abundance of such photo- or chemo-synthetic autotrophs in the sea although the occurrence of several types has been reported including various kinds of sulfur bacteria (Bavendamm, 1924), iron-oxidizing bacteria (Baier, 1937) and nitrifying bacteria (Benecke, 1933). According to Benecke (1933) purple sulfur bacteria sometimes occur in the sea in quantities sufficient to impart a reddish coloration to the water. Below the photosynthetic zone chemosynthetic bacteria are perhaps the only organisms which contribute to the food cycle in the sea. Over ninety per cent of the ocean is below the photosynthetic zone.

SUMMARY

It has been demonstrated that the sea mussel, *Mytilus californianus,* ingests and digests bacteria. Specimens have been maintained on an exclusive diet of bacteria for several months during which time they gained in size and weight. Most marine bacteria are not injurious unless they are present in such great numbers that their metabolic products vitiate the water.

Similarly bacteria sustain the growth of the sand-crab, *Emerita analoga,* to a limited extent. This crustacean is more sensitive to large doses of bacteria than is the mussel and it is not as efficient as the mussel in removing bacteria from suspension.

The Gephyrean worms, *Dendrostroma zostericola* and *Urechis caupo,* were found to eat bacteria and to derive nourishment therefrom.

It is doubtful if bacteria are sufficiently abundant in sea water to constitute an appreciable item in the diet of marine animals, but indications are that cumulatively they play an important role in food cycles by synthesizing cell substances below the photosynthetic zone and by converting waste or dissolved organic matter into a form which can be utilized by animals as food. In marine bottom deposits and as a constituent of the slime on solid surfaces bacteria may be sufficiently abundant to nourish certain animals.

The authors acknowledge the helpful suggestions and criticisms given by Dr. D. L. Fox and Prof. G. E. MacGinitie, as well as the technical assistance rendered by Miss Winifred Landon, Mr. George C. Ford, and Mr. Charles C. Teter.

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