The bacterial flora of the Atlantic ocean in the vicinity of Woods Holl, Mass.

A contribution to the morphology and physiology of marine bacteria.

H. L. RUSSELL.

WITH PLATE XXXVI.

In a previous paper[1] was recorded a series of observations upon the bacterial life of the Mediterranean which were made at the Naples Zoological station during the spring and summer of 1891.

This paper contained a number of facts that had been gathered from a study of the bacterial life of that region, but the area covered was too limited to allow any general conclusions to be drawn concerning the bacterial life of the sea. The importance of a more thorough biological knowledge of the micro-organismal life of the marine waters led to a desire to continue this line of research, so the opportunity offered through the kindness of Prof. C. O. Whitman, Director of the Marine Biological laboratory at Woods Holl, Mass., to carry on a similar line of work at that station was eagerly accepted.[2]

The thorough elucidation of the laws that govern the distribution of this class of organic life can only be made upon extensive data gathered not only under similar but widely diverse conditions. A comparative study of the bacterial flora on this side of the Atlantic with that of the Mediterranean was therefore of importance, so the plan of work this season was practically the same as that of the previous year. Frequent reference will of necessity be made to the previous communication and some of the results that will be detailed here will have been considered before to some extent, but as the conclusions then drawn were only provisionally suggested, the confirmation of them will substantially increase their basis for support.

The main object of the work at Naples was a study of the deep sea bacteria and the sea bottom as well as the water was also examined from the shore line down to a depth of 3,500^{ft}.

[1] Zeits. f. Hyg. XI. 165–206.

[2] The quantitative part of the work was done at this station while the more detailed study of the individual forms was carried on at the biological laboratories of the University of Chicago.

J

A continuation of the study of the deeper waters was precluded at Woods Holl by reason of the shallowness of the ocean in the vicinity of this port. The shallow continental platform which skirts the eastern edge of the U. S. is here at its greatest width and the broad shoals of Nantucket are even out of sight of land. The conditions however were favorable for the investigation of marine forms in general.

Woods Holl is situated at the extremity of a narrow neck of land that pushes southward from the southernmost point of the Cape Cod peninsula. This narrow land strip is continued seaward in the chain of the Elizabeth Islands and divides Buzzard's Bay, an almost land-locked sea, from Vineyard Sound. This latter body of water separates the mainland and the Elizabeth Island chain from an outer range of islands comprising Nantucket, Martha's Vineyard and others.

The soil of the mainland is of a sandy nature and the general aspect of the surrounding country is that of low hills such as usually characterize a glaciated region. As there are no rivers of any magnitude, and no large cities to add their filth to the ordinary land drainage, the factor of land contamination is here reduced to a minimum.

Both the bodies of water mentioned, Buzzard's Bay and Vineyard Sound, served as a collecting ground for this work. Both are subject to tidal changes; the Sound being swept from end to end by an especially heavy tide.

The physical characters of the sea-bottom of these two bodies of water differed considerably, that of the Sound being of a sandy or rocky nature, while the bottom of the Bay, excluding a narrow littoral belt which is more or less sandy is covered with a uniform sheet of blue or gray silt. No depth within a working distance of the laboratory exceeded 65ᶠ, while the 20-fathom line was out of sight of land. Consequently all samples secured were from comparatively shallow depths, although in some cases twenty miles from the main land, with the exception of a few that were taken on board of the U. S. F. C. steamer *Grampus* at a distance of about 100 miles from the coast.

Methods.

The methods used in securing the samples of mud and water to be analyzed were essentially the same as those that were employed at Naples. These methods have been described elsewhere, so that a detailed account of them again is unnecessary.[3]

The use of the water apparatus for the second season has confirmed the favorable results obtained during the preceding year. By means of it, a sample of water may be taken from any depth without the slightest contamination from the intervening water masses. The cheapness and ease with which this simple piece of apparatus can be made makes it all the more applicable for its purpose.

The culture technique was substantially the same as used at Naples, so that direct comparisons might be made. Sea water was usually used in the preparation of the agar and gelatine media.

Relation of bacteria to marine waters.

In considering the bacterial content of the sea, attention will first be directed toward the water itself, as a home for bacterial life. When tested by culture methods, the results of the analysis of waters taken at Woods Holl have been of a positive nature, with but two exceptions. Samples were secured under as different conditions as possible, ranging from the surface of the water to the ground layer, and from the shore line to a distance of twenty miles from mainland. The number of germs per unit of volume (1^{cc}) varied within wide limits but to a much less extent than in fresh waters. When extraordinary numbers of bacteria were found in the cultures, one species almost always predominated to a large degree. The possibilities of the introduction of a small bit of floating zoogloea that might happen to be in the water renders this fact easily explainable. If the sample secured contained a fragment of the gelatinous mass of germs, it would be broken up in the preparation of the cultures and the separated germs would develop as isolated colonies and thus raise the normal average.

The following table summarizes the results obtained from the analyses of nearly fifty samples of water that were taken. The figures in the table indicate the average of the whole number of colonies that appeared in all the cultures made from samples at different depths. Those samples that contained enormous numbers and all of one kind were excluded from this set of averages.

[3] Zeit. f. Hygiene, XI. 166. Botanical Gazette, XVII. 312.

DEPTH OF WATER IN FEET.	NUMBER OF BACTERIA PER CC. FOUND IN THE WATER AT DIFFERENT DEPTHS (IN FEET).									
	0	15	20	25	30	35	40	45	50	65
30	6	8	....	....	46	....	....	....	....	....
35	....	....	....	....	....	175	....	....	....	....
40	....	....	3	....	....	....	105	....	....	....
45	....	....	....	16	...	....	....	5	....	....
50	....	....	....	18	....	....	....	....	40	....
55	....	....	....	....	....	....	....	....	....	....
60	....	....	....	....	....	....	....	....	....	....
65	110	....	....	....	120	....	....	....	...	6

As the above table indicates, the limits of variation per unit of volume varied from a few germs to about 120. No especial difference in numbers can be noted in the various depths that may not be ascribed to local variations. The water seems to be peopled at all depths with bacterial life, and the deeper layers appear to be as rich as the more superficial. These results, on the whole, agree quite closely with those obtained at Naples. Although the depth there was much greater, no marked diminution could be detected between the superficial and the bottom layers. Almost every cubic centimeter of water subjected to analysis contained bacteria, usually not exceeding one hundred germs per unit of volume. Karlinski[4] in studying the waters of Lake Borke, one of the fresh water lakes in Bosnia, arrives at a somewhat different conclusion. He finds that the germ life is much richer at the surface and that there is a gradual diminution in numbers as samples from increasing depth are examined. His investigation, however, only extended to the depth of fifty feet. It is surprising that there should be so marked a decrease in numbers as he gives with such a slight change in depth. This is certainly not the case with marine waters already examined, as the analyses made at Naples and at Woods Holl fail to show any law of distribution in this manner.

Whether the great bulk of the ocean waters is inhabited by micro-organisms is not yet positively known, as no bacteriological examination has been made of water at great distances from land. In fact the knowledge that we possess of

[4] Cent. für Bakt. XII. 220.

this class of life in the open ocean is practically nothing, for scientific expeditions have as yet paid no attention to the investigation of these forms.

While we have no direct knowledge concerning their presence in mid-ocean, it is not unreasonable to suppose that they are present throughout the great mass of oceanic waters. The conditions for their development here are quite as good as are found in fresh water. True it is that many forms in fresh water are of undoubted land origin, but we recognize numbers of species as being so well adapted for development in water alone, that they have received the name of "water bacteria." As we look upon the sea as the original home of organic existence, it is not at all improbable that these primitive generalized types of life may have been dwellers in the deep from time immemorial.

Although positive proof concerning the universality of bacterial distribution in the ocean is yet wanting, we have the strongest circumstantial evidence and that is the presence of identically the same species on this side of the Atlantic that have been found in European waters. This point will, however, be considered later in another connection.

The gradual disappearance of bacterial life in fresh water lakes as the distance from the shore increases has led some to think that bacteria are present in sea water in only limited numbers. They regard the presence of micro-organisms as evidence of land contamination and where this disturbing factor is excluded, the normal number of bacteria is regarded as extremely small. That there is a marked diminution in germ life as we recede from the shore is undisputed, but this decrease soon reaches its minimal point and then the proportion remains fairly constant. Even where the condition for introduction of land-derived forms is as favorable as it is in the harbor of a large seaport, the point of minimal diminution is usually reached within a distance of three or four miles from land. With the ordinary coastal drainage the influx of fresh water forms does not affect the average content to as great a distance as this.

A comparison of germ life of salt with that of fresh water shows that the latter is usually much richer in bacteria. The principal reason for this is perhaps the closer proximity of fresh water to the soil layer, the upper strata of which are so rich in bacterial life. Every rain fall brings innumerable germs to the water level and thus raises the average greatly for the time being at least. Then another cause is the greater rapidity of multiplication in these waters on account of the higher temperature.

Comparisons can scarcely be made under equal conditions between the bacteria found in streams and springs and those inhabiting the ocean. The proportions existing between the waters of our great inland seas and that of the ocean would be much more reliable but as we have no data concerning the bacterial contents of these large fresh water masses, this can not at present be made.

In the light of our present knowledge, the assertion seems to be warranted that marine waters are not as rich in bacterial life as fresh water masses.

The question of the vertical distribution of bacterial life throughout large bodies of water has a direct relation to the problem of sedimentation. The specific gravity of bacteria cannot exceed very much that of water, especially saline solutions like the sea, yet it would not be unreasonable to suppose that these organisms would slowly tend to settle to the bottom in obedience to a universal law. Especially would this be likely with forms that are in a spore condition, as they are immotile and of higher specific gravity than active protoplasm.

But the question as to whether there is a "perpetual shower" of germ life on the sea bottom is not so easily answered. A number of opposing factors enter into the question and make it difficult to say what actually takes place under natural conditions. The experiments of Bolton, and Hüppe upon the settling of bacteria in tall cylinders do not give any positive answer, for the natural increase by growth is counterbalanced by the constant dying off of old forms.

Motility is another factor of the problem as those germs endowed with locomotor powers are easily able to overcome the effect of gravity.

Cramer[5] who has investigated both the superficial and deep waters of Lake Zurich is unable to note any marked difference in numbers between the surface and the ground layer.

This season's work substantiates the results of the previous year and leads us to the conclusion that the deeper layers are as rich in bacterial life as the surface. Whether this is true

[5]Die Wasserversorgung von Zürich. 1885.

for the abysmal depths of the ocean is hazardous to say. The diminution in temperature as the depth of the ocean increases will of course retard the growth of micro-organisms. The deepest point from which water samples were taken in the Mediterranean (3,200ft) showed thirty germs per cc. but the temperature in this instance was high for deep water as this sea has a constant temperature of about 55° F. below the 600 feet line.

Relation of bacteria to the sea-floor.

When we consider the bacterial flora of the sea-bottom, the results of the analyses of mud show a widely different condition. Micro-organisms are always present in very great numbers. Numerically, as regards the bacterial contents, the sea floor bears a similar relation to the superincumbent water masses that the superficial soil layers do to the atmosphere above. This is only true, however, from a numerical standpoint, for the water does not derive its bacterial life from the sea floor, while the germs in the air have their origin in the soil. In only two instances have I found at Woods Holl forms in the water that plainly showed that they were derived from the slime layers below. These cases are explainable, however, for a heavy tide was noted at the time the samples were taken and it is highly probable that the tidal current detached particles of mud from the bottom and thus the mud bacteria were included in the sample of ground water, although five or six feet from the bottom.

The analyses of the sea bottom that were made at Woods Holl this season covered nearly one hundred tests. Most of the samples were secured from Buzzard's Bay and Vineyard Sound and included material of widely varying physical characters. The area covered was not far from one hundred square miles and included all depths from the shore line to sixty-five feet.

In the ninety-five samples that were analysed from this locality, the average number of bacteria per cc. was about 17,000. In no case was a sample of mud tested by means of culture methods that did not yield bacteria although the limits of variation were wide.

In a few instances, the number of germs present were fully 100,000 per cc., but these exceptional cases are to be explained in the same way as in those samples of water that far exceeded the general average.

Not much of an idea can be gained from an average like this unless other conditions are taken into consideration.

As there was a wide difference between the physical character of the sea bottom at the various places from which samples were taken, the analyses have been arranged with reference to this point to see what effect the substratum had upon the presence of bacterial life.

The opportunity for a comparison of this nature was all the more favorable at Woods Holl on account of the slight variation in the depth of the water. All samples that were analyzed were secured at depths ranging from 25 to 65^{ft}, so that this factor was fairly constant. According to the analyses made at Naples, this element of depth entered very strongly into the problem of quantitative distribution, there being a marked decrease in numbers as the depth increased.

It has already been ascertained through the investigations of Fränkel[6] and Reimers[7] that virgin soil is much poorer in bacterial life than that which has been disturbed, and that in a general way, the bacterial contents of a soil are largely dependent upon the amount of organic material that is contained therein. As the sea bottom is practically undisturbed as far as the influence of man is concerned, a comparison of the germ life of different soil bottoms ought to yield natural results.

From a mechanical standpoint, a fine silt would offer better conditions for bacterial life than a coarser soil, as there is more room in the interspaces in which the bacteria may develop.

Whitney[8] has estimated the absolute empty space in sand to be 45 per cent. of its volume while that of clay is fully 25 per cent. more.

The majority of the samples that were taken from the sea bottom were either composed of a very fine silt or a fine quartz sand mixed with clay. In several instances, samples of pure "live" sand, as these shifting shoals are called, were also tested as to their bacterial life. Grouping the analyses according to the physical character of the bottom from which the respective samples were derived, it was found that thirty

[6]Fränkel: Zeits. f. Hygiene II (1887). 521.
[7]Reimers: Zeits. f. Hyg. VII (1889). 307.
[8]Whitney: Fourth Md. Agric. Rept. (1892). 281.

tests of the very fine blue and gray silt yielded on the average about 17,000 germs per cc. Thirty-five samples of a mixed quartz sand and clay gave an average of nearly 20,000 germs per unit of measure. The few samples of pure shifting sand were practically free from organic matter and contained only about 5,000 germs. Not enough tests were made to say whether this ratio would be maintained or not, but the difference between the sandy clay and silt is quite within the limits of ordinary variation. The species found in the pure sand did not differ from those growing in the clay soils.

Quantitatively, the marine mud in the vicinity of Woods Holl contains much less germ life than that of the Mediterranean in the vicinity of Naples. The Naples cultures made from mud taken at the depth of 150^{ft} or less, yielded usually from 200–300,000 germs per cc., while those made on this side of the Atlantic in only exceptional instances exceeded 50,000 germs while the average content was about 15-20,000 per unit of volume. Just what conditions bring about such a marked diminution is not easy to see. The figures given for the Naples analyses include only the samples taken at a distance of two miles or more from land. From the appearence of the cultures, this limit seemed to be outside of the influence of land contamination. The majority of sewage and fresh water forms do not find favorable conditions in the sea for their development, but it is highly improbable that all germs introduced in this way are destroyed. Many no doubt adapt themselves to their changed conditions and are able to live. At all events, the conditions at Woods Holl are in all probability more nearly normal than they are at Naples, as the possibility of the introduction of such enormous numbers as would be derived from a large city are in this case excluded.

This season's work has been carried on in much more northern waters but the difference in temperature is not great. The period of observation at Naples extended from April to July, the temperature of the water during this time varying from 60°–75° F. This year the work was carried on somewhat later (June–August), the temperature ranging from 55°–70° F.

Temperature is one of the more important factors that govern the distribution of micro-organisms, but it would seem in this case that the difference in bacterial contents of the two localities examined must be explained in some other way.

As yet, we do not possess sufficient knowledge concerning the distribution of bacterial life to satisfactorily explain this peculiarity.

Relation of the bacteria of the sea bottom to the superposed water masses.

The idea was advanced as a result of last year's tests that the high content of mud when compared with water was in part due to the growth of distinct species that were to be found *only* in the mud. This indigenous flora was in no way derived from the water masses above but had spread itself over the sea bottom in a way not at present thoroughly understood. This theory was based upon the fact as determined by cultures that at least 35 per cent. of the total bacterial contents of the Mediterranean mud in the vicinity of Naples was included in three species that were exclusively slime bacteria.

The work this season afforded an opportunity to test the correctness of this view on data from a widely different source.

The idea has already gained some ground that the soil bottoms of the oceans have derived their bacterial contents from the water mainly as a result of sedimentation.

This result is based upon the fact that while river water is usually rich in germ life, lake water is poor; therefore it is inevitable that the mud must have derived its bacterial life from the lake water by sedimentation.

Practically no data had ever been gathered on the richness of either lake or sea bottoms in bacterial life from a quantitative standpoint so that the conclusion was mainly an *a priori* one. The results of experimental sedimentation tests do not show that bacteria have any decided tendency toward deposition. The self-purification of polluted streams that used to be explained upon the theory of sedimentation is now accounted for largely in another way and we have no positive experimental knowledge that sedimentation of all these microscopic vegetative forms exists. The results of this season's observationsare entirelyin conformity with those of the previous year, and indicate beyond a doubt that a large proportion of the bacteria in the mud are inhabitants solely of this habitat.

The majority of the individual germs present are embraced in a few species. One form, Bacillus limicola, is very common and is almost always found in every culture that is made from the mud. Besides this predominating form there are several other species that are also exclusive mud dwellers. This indigenous mud flora, however, does not make up the entire percentage. In every sample analyzed there is to be found a goodly number of germs that are also inhabitants of the superincumbent water masses. These have not fallen to the sea bottom in a dormant condition, but are actively vegetating, as will be shown in the succeeding paragraph.

The actual stage of development in which marine bacteria are found.

The quantitative analysis of the ocean waters and the underlying floor by means of cultures gives us an approximate idea as to the number of individuals that are to be found therein, but these results do not in themselves tell us the actual condition of the bacteria—whether the germ life is in an actively vegetating condition, or is merely in a quiescent spore stage.

Are the waters of the globe filled throughout with bacterial life in full activity? Is the ocean bottom peopled with forms that are undergoing their cycles of development, or is it merely the resting place for the "perpetual shower" of organic beings from above whose active existence is at an end?

We have determined the presence on the sea bottom of indigenous species, so the most natural inference is that these forms limited to the mud layers must possess the means of carrying on their metabolic activity.

The question can be approached in two ways. One of which is by inferential reasoning and can only be relied upon to the extent of affording a check upon the other method. This is to determine the number of colonies day by day as they appear in the cultures. If the bacteria present are in both a vegetative and spore stage, the time of incubation before the different germs develop into colonies microscopically visible will be quite different. Those germs that are already in an active vegetative condition will immediately begin their growth and with most saprophytic species this will be manifested within thirty-six hours from the preparation of cultures. With those forms that are in a resting stage, the period of incubation will be considerably lengthened. Taking advantage of this fact, we can by counting the number of developing colonies in the cultures on successive days get an approximate idea as to the actual condition of the bacteria in the sample when it was taken. This method is of course only applicable under certain conditions, for if there is a great variety in the number of different species, the rate of development of the various forms may vary to such an extent as to impair the accuracy of this method.

The method, however, possesses a certain value in the case in hand as the number of species is not large and as it affords a check on the second method which is experimental.

The other method consists in destroying all forms that are in a vegetative state by sterilization at a low temperature. This temperature should not be high enough to injure the germs that are already in a dormant state but should be sufficient to kill all forms having protoplasm in an active condition. The quantitative determination of the bacteria in equal volumes of the sample of water or diluted mud before and after this sterilizing process affords us data for this problem.

Unequal distribution of the germs in the different cultures will materially affect this result, but if proper precautions are taken to thoroughly distribute the bacteria in the fluid before the control cultures are made, this element of error is materially reduced.

Samples of water as well as of mud were subjected to this method of differentiation and were sterilized for one hour at the temperature of 70° C. This temperature is considerably higher than any known form of active protoplasm can endure,[9] so that one may be absolutely sure that all colonies developing in heated cultures originated from sporiferous germs.

Ten series of tests were made with samples taken from the water at varying depths and in all but one of these the "heated" cultures developed bacteria in greater or less numbers. The percentage of sporiferous bacteria in the water varied widely and in two instances the cultures subjected to this partial sterilization showed nearly as many colonies as those untreated. Bacteria, in a spore condition were demonstrated in the superficial water layers as well as at the bottom and intervening depths.

Ten series of tests were also made upon the mud from the sea bottom. These likewise showed a varying percentage of the bacteria present on the sea floor to be in a resting condi-

[9] Except two or three thermophilous species, according to Miquel and Globig.

tion. As in the case with the water cultures the limits of variation with the mud bacteria varied widely. In one instance only (forty-five feet deep) were no bacteria found in spore condition.

These results accord in a general way with those made in the Mediterranean and show that while the water and underlying sea floor are filled with bacterial life, they are by no means in an entirely quiescent condition. Both water and mud are peopled with micro-organisms that are undergoing their cycle of development here as elsewhere.

(*To be continued.*)

University of Wisconsin.

BRIEFER ARTICLES.

Vacation collecting.—I have spent the month of August at Sakonnet Point, Little Compton, R. I. For one week I had the company of Mr. James L. Bennett, who I found had made an extended list of the plants of the region. Among the interesting species are Senebiera Coronopus in great abundance; Woodwardia angustifolia, quite plentiful but not in fruit; and all the queer abnormal varieties conceivable of Onoclea sensibilis.

There is a swamp wood near the house where I am stopping, full of Ilex opaca of large size. I. glabra, I. laevigata, and I. verticillata, also occur. There is a perfect tangle of Mikania scandens, growing high up into the trees. It is rich botanizing all through this swamp. Nowhere did I ever see more brilliant Lobelia cardinalis. We find over forty trees about here, the oak being especially well represented. On all the meadow lands near the sea one finds quantities of Anagallis arvensis. The splendid Hibiscus Moscheutos grows in the salt marshes. I have found no Sabbatias. As curious strays have been picked up now and then in the neighboring town of Tiverton, I am on the lookout for them here. I might say that Physostegia Virginiana is quite common on the roadsides.

In the middle of the swamp wood before mentioned, near a lovely brook, I found a boulder thus inscribed: "To the Memory of Awertronks, Queen of the Sakonnates, and Friend of the Whiteman." I have spent many happy hours in this secluded spot.

In the early summer I botanized extensively about Mt. Wachusett in company with Mr. J. F. Collins. Afterwards I explored the Taconics with the Harrison brothers, of Lebanon Springs.—W. WHITMAN BAILEY, *Providence, R. I.*