Nardia crenuliformis.
Odontoschisma Macounii.
Porella navicularis.
Ptilidium Californicum.
Radula arctica.
Radula Krausei.
Scapania albescens.
  Bolanderi.
  glaucocephala.
  Oakesii. —32.

17. Last of all are the ten species peculiar to Asia:

Calycularia laxa.
Diplophyllum plicatum.
Frullania Davurica.
Jungermania fertilis.
  guttulata.
Jungermania quadriloba.
  Sahlbergii.
Lophocolea reflexula.
Mylia verrucosa.
Porella grandiloba.
 —10.

De Pauw University, Greencastle, Indiana.

Bacterial investigation of the sea and its floor.[1]

H. L. RUSSELL.

No class of living organisms, animal or vegetable, have been found to be so ubiquitous in their distribution as bacteria, yet strange to say, no especial attention has been paid to the investigation of the marine waters of the globe from a bacteriological standpoint. True it is that the phosphorescent forms of the sea have been more or less thoroughly worked out, and here and there other isolated forms have been described, but the general subject of the bacterial flora of the sea has been left quite untouched. It is not my purpose here to enter into any elaborate discussion of this subject, but only to give a short résumé of work along these lines which I have been carrying out for the past two summers, and also to suggest some problems of interest in connection with this subject.

I fully recognize the futility of attempting to draw any general conclusions from a comparatively small number of tests, but while the results which I have to offer may be regarded as somewhat provisional and will require extended confirmation before they can be accepted as general biological facts, I trust they may possess some interest even in this tentative connection.

The results, which I can only briefly summarize here, were obtained at the Zoological Station at Naples, during the

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[1] Read before Section F, A. A. A. S., Rochester meeting, August, 1892.

spring and summer of 1891, and at the Marine Biological Laboratory at Wood's Holl, Massachusetts, during the past season.

These widely separated places, so different in many of their conditions, gave exceptional advantages for a comparative study along these lines.

Before detailing the results, I will state, in the briefest possible manner, the methods used in the work. First, in regard to the manner of procuring the material for examination: In securing this for a quantitative bacterial determination, it is necessary that the sample secured should be kept free from contamination, as far as possible, from the time it is originally taken until the growth of the cultures has been completed. To do this with material from surface-soil or air is not especially difficult, but when the material is to be derived from the ocean floor, or at varying depths in the water, the problem of keeping it uncontaminated during its withdrawal is by no means easy. The conditions must be such that the possibilty of contamination during withdrawal will be excluded. The apparatus which is quite universally used to collect samples of deep water for chemical and physical analysis is here of little use as it cannot be previously sterilized, but the following method which has been thoroughly tested for two consecutive seasons and has given most excellent satisfaction is believed to fulfil all the conditions necessary for the work.

It consists of a large sized test tube which is tightly fitted with a rubber cork having a single hole. The opening in the cork is closed by a glass tube which projects about three-quarters of an inch below the lower end of the stopper. The upper part of this small tube is bent at right angles to the long axis of the collecting tube and drawn out at a certain point to a finer caliber, so that it may be quickly sealed in an ordinary flame. The different glass parts are first sterilized by heat, the rubber corks being kept in mercuric solution, then rinsed in sterile water and finally dried on sterile filter paper. These are then tightly fitted together and a partial vacuum produced either by attaching the small tube to a vacuum pump or by expelling a portion of the air by heating the tube with a small amount of hot water or dry heat. The end of the small tube is sealed as the air is expelled. The vacuum tube may then be protected from gradual leakage by coating the cork with a mixture of beeswax and rosin.

To secure the samples of water from any desired depth, these vacuum tubes are attached to a holder by means of an ordinary clamp, the small drawn out glass tube being so arranged that the point of it lies near the connecting line that is fastened to the holder. When the holder with the vacuum tube has been sunk to the desired depth, a lead messenger is sent down over the connecting line and as it is caught on the top of the holder, the small glass tube is broken and the vacuum thus destroyed. The collecting tube fills quickly with water (usually from about two-thirds to three-quarters full) and then the apparatus is quickly hauled up. By virtue of the imprisoned air which can not possibly escape, owing to the projection of the small glass tube below the rubber cork and the rectangular bend in the tube, water is absolutely prevented from entering the tube after the first partial filling.

Cultures are made in the ordinary way by taking 1 cc. of the water after it has been thoroughly shaken up, mixing it with nutrient sea water gelatine and then plating it in Petri dishes instead of using the regulation Koch plate.

The apparatus which has been used to secure material from the sea bottom is theoretically imperfect, i. e., it does not fulfill one of the cardinal bacteriological canons—previous sterilization, but in its practical workings, I am satisfied that it delivers samples of the sea bottom quite uncontaminated from the water layers above. It consists of an ordinary iron tube (a gas pipe serves the purpose admirably) pointed at one end. The other end is fitted by means of a screw with a removable "sleeve," the upper end of which is closed by a valve. As the weighted instrument descends, the water passes through the pipe and as it strikes the sea floor, it is forced into the soil so that it is filled with a compact mass of material. As the instrument is withdrawn, the pressure of the water closes the valve from above so that no water enters the pipe during its withdrawal. The cohesive nature of the ocean slime is quite sufficient, except where pure "live" sand is present, to hold the mud column in the tube.

The mass of mud is removed from the tube by means of a piston rod, and from the center of this mass a known volume of the material is extracted by means of sterilized brass tubes. For this purpose a small sized cork borer is well suited. This known volume of mud is then diluted with a definite volume of sterile water and plated as in the other case. The only possible chance for contamination is from diffusion which might take place from the sides and lower end of the iron tube. The material is within this for so short a time however, that in so solid and dense a mass as the mud core, this element of error has, I believe, little or no effect.

Attention may now be directed to some of the problems which arise in connection with the investigation of marine bacterial life. Space will only permit a reference to one or two phases of the work, and I can only briefly recapitulate some of the results which have already been obtained.

First, in regard to the presence of bacterial forms in the sea. To determine the bacterial content of the sea, it is necessary for one to secure material outside of the contamination limit from the land. This is of course a varying distance, depending upon the configuration of the shore and other conditions. Fresh water or sewage germs discharged into the sea soon perish on account of the change in their nutritive medium. Of course any quantitative determination of the bacterial contents of the sea must exclude all samples taken within this limit. To my knowledge, the surface water of the sea has not been analyzed bacteriologically at any great distance from land, but samples taken from the coastal line outside of land contamination show that micro-organisms are invariably present in the water. The number per unit of volume varies naturally within certain limits, yet there is on the whole quite a constant average number per unit of measure in these surface waters.

Examination of the *superficial* water layers has always revealed the presence of micro-organisms and it may be interesting to note in this connection the vertical distribution of germ life in the ocean. Are the marine waters peopled *throughout* with bacteria, or is this life confined to the warmer surface waters of the ocean? According to the researches of the Challenger, the marine fauna is separated into a superficial and an abysmal zone, while the intermediate depths are quite deficient in animal life. Analyses of the water at Naples taken at different depths from the surface down to a depth of 3,200 feet showed that bacteria were present in *all* cases. No zonary distribution was to be observed in any case and the intermediate depths as well as the water immediately above the sea floor were found to contain germs in about the same proportion as at the surface.

The usual content of the sea water ranges from 10 to 150 germs per cc., while in exceptional instances the number per unit of volume exceeded this; but the fact that the individuals present were in these cases usually of a single species indicated that the large number was due to a bit of zoogloea rather than active vegetative forms. A comparison of salt with fresh water shows that on the whole bacterial life is less abundant in the sea than in fresh water. The higher temperature of the latter and its proximity to land masses, which are nearly always extremely rich in bacterial organisms, are sufficient to account for this increase.

A bacteriological examination of the sea bottom shows that it, too, is filled with bacteria.

Observation demonstrates that the sea-floor is infinitely richer in germ life than the waters above it. A quantitative examination of the ocean bottom shows a wide variation in its bacterial contents. Just what factors bring about this difference in numbers, I am unable, as yet, definitely to state, but it seems more than probable, that the variable physical character of the sea flora, the depth at which material is taken, and the influence of temperature are conditions which largely determine the presence of micro-organisms. As might be expected, it will require an extended series of data gathered under similar as well as diverse conditions before the question of distribution can be satisfactorily explained. I shall only attempt to submit certain facts which have been brought out by the work, leaving a definite explanation until more thorough investigation.

At Naples, the investigation of the sea bottom was made from the shore line to a depth of 3,500 feet. At the depth of 150 feet and two miles from land, the sea floor contained from 200,000–300,000 germs per cc. From this number, it fell very rapidly as the depth increased until at the depth of 700 feet only 25,000 germs per cc. were present. From this depth to the deepest point investigated (3,500 feet) the number of germs remained tolerably constant. When these results are graphically represented, they show a marked coincidence with the temperature curve of the Mediterranean at this point. The Mediterranean being a closed basin is not subject to the general oceanic circulation and the temperature of the great mass of its water remains at a constant point. The summer temperature of the surface ranges from 77°–82° F., but this falls rapidly to 55° F. at a depth of 600 feet, and from this point downward there is no change.

This season's work which has been carried on at Wood's Holl in much more northern and cooler waters shows that the bacterial content of the sea bottom is very much less abundant at this point than in the Mediterranean. In the vicinity of Wood's Holl I was unable to reach any great depth on account of the width of the shallow continental plateau which lies off southern New England and the middle Atlantic states. The number of bacteria per unit of volume was found to be, under similar conditions very much less than at Naples. The germ contents of the slime from Buzzard's Bay averaged from 10,000 to 30,000 germs per cc. This is scarce more than a tithe of what was present in the Mediterranean mud at equal depths.

When we find the mud so much richer in bacteria than the water, the question naturally arises, to what are these results due? Is the ocean bottom merely covered with the spores of the water bacteria that have finished their cycle of development, and then like the remains of the foraminifera slowly sunk to the bottom, or is bacterial life here present in its fullest activity? The answer to these questions may be sought in two ways. Qualitative analysis of the water and the underlying mud will demonstrate whether the species found in the two habitats are analogous or not. If we find the deposit made up entirely of species similar to those found in the water above, even though they may be very much more numerous, it is at least presumptive evidence that the mud owes its bacterial flora to the superimposed water masses. On the contrary, the presence in the mud of species which are *only* to be found in this habitat is evidence that the ocean bottom is filled with forms which are indigenous to this stratum. Qualitative analysis of the Naples mud showed three very prevalent forms which made up at least thirty-five per cent. of the entire bacterial content of the sea slime. These were wholly indigenous and were not found in any cases in the samples of water taken at any depth. A similar result has been reached in the work at Wood's Holl. Two species are most prevalent in the water, together with two or three other forms that are occasional inhabitants. Now the mud contains the two prevalent water forms, it is true, but in addition to this, there is another common form that usually makes up from thirty to fifty per cent. of the whole number present, and is an indigenous slime bacillus. Besides this species there are two or three other species that are exclusively mud inhabitants although they are by no means so common as the one previously mentioned. I say exclusive, but this is not entirely true, for in two or three cases water cultures made at Wood's Holl have revealed species which had been supposed to be indigenous slime forms. These apparent exceptions can, however, be satisfactorily explained, for they were taken at localities where the tidal currents were strong and there is scarcely any doubt that they were detached from the mud by means of these currents much as the wind detaches bacteria from the soil and carries them about in the air.

The presence of these *indigenous* mud forms necessarily implies that they exist in a vegetative condition, but this can also be experimentally determined. Samples of the mud were taken and treated in the ordinary way in which cultures were prepared. The diluted material was then heated at a temperature sufficiently high to kill all the vegetative forms (80°C) but not enough to destroy the vitality of the spores. Cultures were then immediately made from the heated material and the actual condition of the individuals as they existed in the sample used, could thus be ascertained. These two sets could then be directly compared and the difference in the number of colonies gave the approximate number of vegetative forms actually present in the water or mud. This proportion is often a widely variable one but the analysis of a score or more samples show that the mud bacteria as well as the water forms are in a large degree in a vegetative condition, even under such adverse conditions for their development as those that are found at the bottom of the deep sea.

Mention has only been made so far, of the distribution of marine bacteria in general, but the vertical range of the different species also shows some interesting features. This bathymetrical range, *i. e.* the maximum and minimum depth limits of growth, which each species possesses, varies in different cases.

Great difference in depth means such a marked change in the environment of any single species that one might reason *a priori* that the same species would not be able to adapt itself to such widely different conditions. It is a well authenticated fact that such environments have brought out specific modifications in the faunal life of the sea.

Of the three most common mud forms found at Naples, the maximum depth limit of growth was not attained at the depth of 3500 ft. One of the three species *(Cladothrix intricata)* had nearly disappeared from the cultures, so that it was reasonable to suppose that the bathymetrical range had been almost reached. The other two species were at this depth sufficiently numerous to indicate that the maximum point of development had not been attained. This fact is of especial interest when we consider it in the light of the pressure experiments which have been carried out on bacteria.

Our knowledge of the action of high pressure upon bacterial metabolism is as yet imperfect, but there are several forms which seem to bear an increase of pressure of upwards of 100 atmospheres without material change.

A comparative study of the Mediterranean forms and those found on the New England coast gives an opportunity for a direct comparison from a specific as well as from a numerical standpoint.

The work during the present season has been mainly confined to Buzzard's Bay and Vineyard Sound off the Massachusetts coast, but through the kindness of Prof. Wm. Libbey, Jr., of the U. S. Fish Commission, samples of the mud were obtained about 100 miles from the shore at the depth of 100 fathoms. They were taken by the schooner Grampus on the edge of the great continental platform, which is skirted by the Gulf Stream. The samples are the farthest from land that have ever been analyzed bacteriologically, and give substantial evidence that the ocean bottom is peopled with bacterial life, to at least this distance from shore. Another interesting feature was determined by these analyses. The two prevalent slime species at this point were found to be the same as those taken from near the shore at Woods Holl. This proves a geographical distribution of the common mud species for at least 100 miles from land. A comparison of these forms with those at Naples shows a marked dissimilarity. The number of indigenous forms in the water and mud is not especially large in either case. One of the most interesting species found in the Mediterranean is an endosporous, pseudo-branching form, Cladothrix intricata, which was there quite frequent, but a rare species on the Atlantic coast. This indicates that this species, at least, is quite cosmopolitan in its distribution. Aside from this form, the other species were quite unlike, although they possess some similar characteristics. The bacteria that are so universally present in sea water and mud seem to be quite peculiar to this habitat. Of course many land and fresh water forms are carried into the sea by drainage, but sooner or later, most of them succumb to the changed conditions of their existence.

With this *introduced* or *adventive* flora, we are not especially concerned, but aside from this, there are these certain well defined species, that seem to be indigenous to this particular habitat. By long residence in salt water, some of them have become so modified, that they grow much more luxuriently upon media made from sea water than upon that which contains only the normal amount of salt. In one of the species isolated at Naples, this specialized saprophytism was as well marked as in the case of certain pathogenic species which are cultivated upon artificial media with only the greatest difficulty. Time will not permit any further discussion of this question of marine microbiology and these disconnected statements will be closed with a few suggestions as to the more important problems presented in this line of work.

Aside from the subject of geographical and vertical distribution of bacterial life and the forces which produce these results, there are various problems which possess a morphological as well as a physiological interest. For example, the inner structure of the bacterial cell—the relation of the karyoplasm to the cytoplasm and the cell membrane—a subject which at the present time in this group is imperfectly understood can, I believe, be better demonstrated with marine species of bacteria than the great majority of other forms. As a rule, the individual cells are relatively large and the protoplasm instead of being homogenous is highly granular.

Besides these morphological questions, there are many of a physiological character, such as the relation of bacteria to phosphorescence;their connection,if any,with deep-sea decomposition;the influence of high pressure incident to depth; and the changes in their oxygen supply, which might be profitably considered.

Much of this class of work can be best done under the auspices of the government, either by the Fish Commission or the Coast Survey, as these departments are already provided with the necessary outfit of vessels fitted with suitable dredging apparatus, etc., for deep sea work. Unfortunately, the methods of work preclude the use of preserved material, as this subject can only be prosecuted by means of culture work. Not only would such a department of research upon our scientific exploring expeditions add greatly to our knowledge of bacterial life, but the lower forms of fungi could be investigated as well.

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A peculiar case of plant dissemination.

EDWARD L. BERTHOUD.

Studying lately with intense interest "Island Life," by Alfred Russell Wallace, and his remarks upon the dissemination of plant life everywhere, both on continents and islands, it brought to my mind what many years ago I had observed during a long residence, and numberless scouts, excursions, surveys and pleasure trips I have made in the region included between the Missouri river and Great Salt Lake, and from the $34\frac{1}{2}°$ N. latitude to that of Eau qui Court in Dakota, and Sun river in Montana.

As these may be of interest and some value in the determinations of geographical botany, and have a bearing in the elucidation of geological botany, I will briefly give the more salient points of these observations. I can show to some extent that between the Missouri river and the Rocky mountains, the American buffalo has been an efficient agent in plant dissemination. Until within twenty-six years the buffalo was known to range from Peace river and Athabaska valley to central Texas. Very much as our Indian tribes are known to do, the buffalo uniformly followed trails in their annual migrations from north to south, or *vice versa*, very rarely deviating from them, whether across prairie or woods, or over spurs of the Rocky mountain range, on their migrations through South, Middle, North Park and Laramie Plains. And when in the spring the former countless herds from Texas moved north across the Arkansas, Smoky Hill, Republican and Platte rivers, the same trails were used, the same river fords crossed, and, following the best ground for their migrations, their sagacity or instinct (if you choose so to call their