THE CONCENTRATION OF SEA-WATER AS AFFECTING ITS BACTERIAL POPULATION

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In connection with certain studies on the relation of marine bacteria to the precipitation of $CaCO_3$ in the sea, carried out by the author at the Tortugas Laboratory of the Carnegie Institution of Washington in 1922, a brief study was made of the influence of dilution of sea-water on its bacterial population. The facts obtained in this study appear to be of sufficient interest to warrant their publication and to stimulate further study of the subject. They are, therefore, set forth below.

In the first part of this study, I determined the influence of dilution on the mixed flora of the sea-water occurring near the wharf of the laboratory where, of course, there is much contamination from shore flora. The sea-water just described was plated on four different media, made up as follows:

No. 1. One gram calcium lactate, 1 gram peptone, 0.5 gram potassium nitrate, 1000 cc. sea-water.

No. 2. Same as no. 1, but water supplied was made up of 750 cc. sea-water and 250 cc. distilled water.

No. 3. Same as no. 1, but water supplied was made up of 500 cc. sea-water and 500 cc. distilled water.

No. 4. Same as no. 1, but water supplied was made up of 250 cc. sea-water and 750 cc. distilled water.

Appropriate dilutions were made for the platings and after four days' incubation at laboratory temperature (approximately 28°C.), the final counts were made with the following results:

	Number of bacteria per cubic centimeter sea-water
Medium 1	960,000
Medium 2	730,000
Medium 3	640,000
Medium 4	680,000

On medium 1, sea-water from 1 mile out to sea showed only 100 to 200 bacteria per cubic centimeter.

It is clear from the foregoing data that dilution of sea-water close to shore, or what should amount to the same thing, reduction of the salt content of the medium on which it is plated, does result in depressing the numbers of organisms, the more so as sea-water is more and more diluted until equal proportions of sea-water and distilled water are employed. Still greater dilutions beyond that point, however, do not greatly depress the total number of organisms as judged by a bacterial count. It occurred to me, however, that this may be only a partial truth, inasmuch as certain organisms belonging normally to the shore would have greater opportunities for development with increasing dilution of the medium, whereas others belonging normally to uncontaminated sea-water would be depressed thereby. For that reason, I determined to study the influence of dilution of a sea-water medium like that above described on the growth of a pure culture of a sea-water bacterium found in pure ocean water as a normal inhabitant in tropical seas. For that purpose, I chose an organism which has been variously named (in my opinion, incorrectly) *B. calcis* (Drew), and *Ps. calcis* (Kellerman and Smith). On certain kinds of media, this normal marine bacterium, in common with other normally occurring bacteria in tropical sea-water, precipitates $CaCO_3$ from the medium. Other studies on this organism have been published elsewhere by the author[1] and a further communication on the subject will soon be forthcoming.

Five different media were employed in plating out the pure culture of the organism in question. They were as follows:

No. 1. Two grams calcium lactate, 1 gram peptone, 0.5 gram potassium nitrate, 1000 cc. filtered sea-water.

No. 2. Same as no. 1, but with $\frac{7}{10}$ sea-water and $\frac{3}{10}$ distilled water, making up the 1000 cc. of water used.

No. 3. Same as no. 1 but with $\frac{5}{10}$ sea-water and $\frac{5}{10}$ distilled water.

No. 4. Same as no. 1, but with $\frac{3}{10}$ sea-water and $\frac{7}{10}$ distilled water.

No. 5. Same as no. 1 but with $\frac{1}{10}$ sea-water and $\frac{9}{10}$ distilled water.

[1]Publication 340, Carnegie Inst. of Wash., pp. 179–191.

Appropriate dilutions were made in all the platings but the counts were rendered easy on the second dilution of the suspension, namely 1:10. The results of the counts which represent averages of very closely agreeing replicates were as follows:

	Number of colonies from a given amount of suspension
Medium 1	270
Medium 2	200
Medium 3	150
Medium 4	None
Medium 5	None

The foregoing data seem to furnish unequivocal evidence of the effect of dilution of the sea-water medium on the ability of a typical marine bacterium to form colonies on suitable artificial media. The dilution of sea-water to the point of cutting its concentration into two seems to result in a depressing effect on the power of the organism in question to develop on the plates, but the depressing effect grows gradually; further dilution of the sea-water, in accordance with the scheme given above, proves to be completely destructive to the cells. Moreover, the appearance of the colonies on each medium described above is quite characteristic. On medium 1, there is a preponderance of large surface colonies, but also many deep colonies appearing as opaque dots at the bottom of the medium. On medium 2, there are very few surface colonies, and no large ones, but numerous deep colonies with denser nuclei than those appearing in the colonies of medium 1. On medium 3, there are no surface colonies, and the deep colonies are all very small, appearing as dense dots deep in the medium. On the other media, as indicated above, there were no colonies.