Stream Pollution

SURVIVAL OF COLIFORM ORGANISMS IN PACIFIC OCEAN COASTAL WATERS *

By Isadore Nusbaum and Richard M. Garver

Respectively, Water Pollution Control Engineer,† San Diego Regional Water Pollution Control Board, San Diego, Calif., and Lt. J.G., M.S.C., USN, U. S. Navy Preventive Medicine Unit No. 5, U. S. Naval Hospital, San Diego, Calif.

This study was made to obtain information on the viability of coliform organisms in saline waters of the Southern California Coastal Area. Specifically, the investigations were performed on the waters of San Diego Bay and Pacific Ocean bordering the coastline of San Diego County, Calif. Forty-one million gallons per day of undisinfected, partially treated municipal wastes are discharged into San Diego Bay. The coastal waters of Southern California daily absorb the liquid wastes of an estimated 6,000,000 people. Almost all of these wastes are discharged in areas contiguous to beaches, industrial and military installations requiring a "bacterially safe" water.

Historical Background

Adequate reviews of the literature on the viability and dispersal of fecal bacteria in the sea may be found in "Marine Microbiology" by ZoBell (1) and the studies of the Woods Hole Oceanographic Institution (2) (3) (4). It is of interest to note that many of the reports are contradictory. Ketchum (3) notes that results have been reported varying from death rates far more rapid than those found in fresh water to information that sea water is neither antiseptic nor inimical to enteric bacteria. Peculiarly enough, despite the work of many investigators showing that enteric organisms may persist for long periods in autoclaved sea water, statements are still to be found in recent reports (5) (6) that sea water, per se, is detrimental to the existence of these organisms.

Some studies (7) have shown such an extremely rapid reduction of enteric organisms in sea water that doubt was expressed as to the public health significance of waste discharges into the sea. On the other hand, Buttiaux and Leurs (8), following a series of optimistic reports by several French investigators, cautioned against undue reliance on the so-called antibiotic effect of sea water on the bacteria in municipal wastes. Although these two investigators hesitated to generalize on their findings, it was shown that under the conditions used *S. typhi, S. paratyphi, B.* and *S. enteritidis* persisted with little change in numbers for periods in excess of 24 hr. At the same time, controls using spring water showed reductions of 75 to 100 per cent in the numbers of organisms in 22 hr. and 93 to 100 per cent in 44 hr.

It is to be expected the circumstances influencing the existence of enteric organisms in an aqueous media would, or might, vary considerably with the locale. Some observers have

* Presented at 1955 Annual Meeting, California Sewage and Industrial Wastes Assn.; Riverside, Calif.; April 27–30, 1955.

† Presently Sanitary Engineer, USPHS, Detroit, Mich.

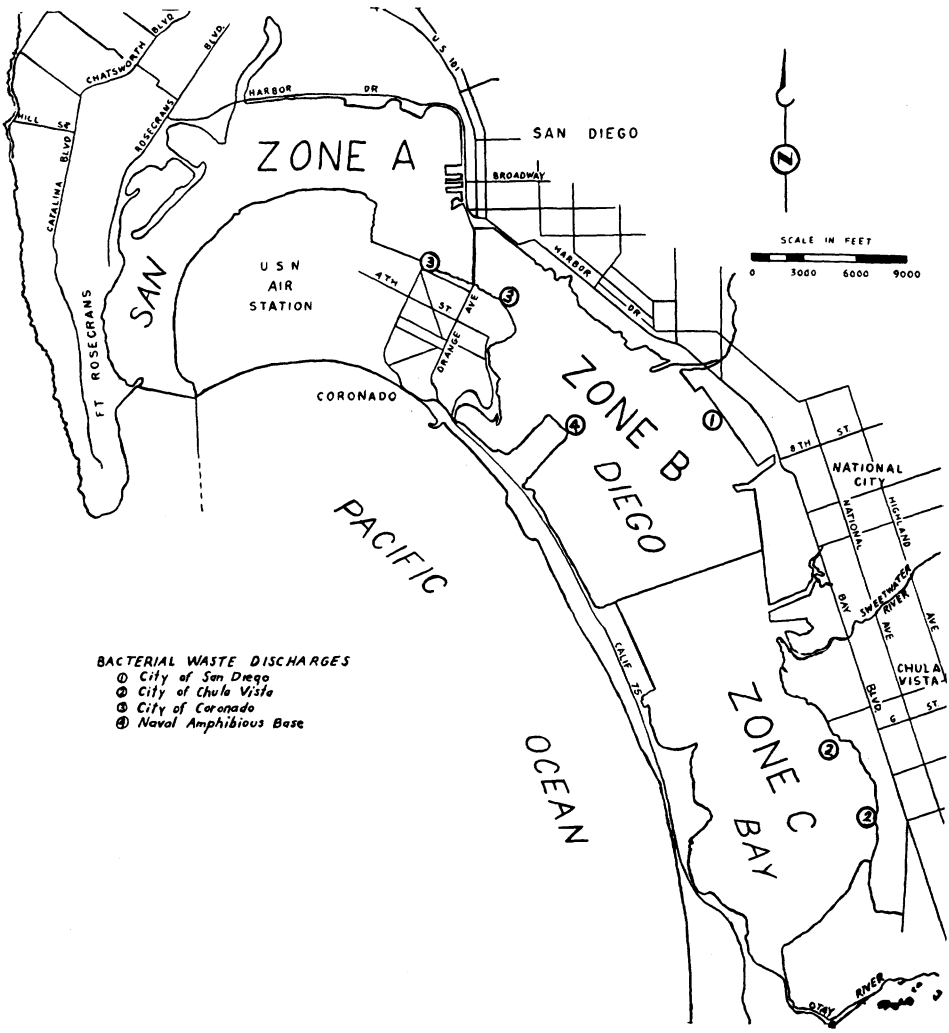

FIGURE 1.—Map of San Diego Bay, Calif., showing sources of bacterial pollution.

demonstrated an apparent difference in the viability of enteric organisms not only in different bodies of saline water but also in adjacent areas of the same body. However, this effect (5) (9) has been frequently noted for fresh water as well as for sea water.

San Diego Bay, the waters of which were used for a large part of this study, is shown in Figure 1. The only fresh water normally discharged into the bay is in the form of wastes. The rivers and streams shown are dry throughout most of the year. A company extracting salt from sea water is located at the extreme south end of the bay. Sources of waste containing significant bacterial populations are shown in Figure 1. Untreated sewage is also discharged from large Naval vessels moored in the bay. The south section of the bay, zone C, is shallow with an average depth of about seven feet with large portions exposed at low tide. Approximately 95 per cent of the wastes are discharged to the middle bay, zone B.

The mean tidal range in San Diego Bay is 4.2 ft. with an average of 1.9 tides per day. The tides are characterized by diurnal inequality. The effect of the tides with respect to waste

disposal is felt principally in the north end of the bay, zone A.

There are no extreme differences in the salinity of the bay. Vertical stratification of the waters of San Diego Bay where it exists is mainly thermal. Water temperatures vary from a low of 12° C. in the winter to a high of 24° C. during the summer.

Investigational Techniques

Samples of bay and ocean water were filtered through cotton to remove gross extraneous matter and placed in 1-gal. glass jugs which were incubated in a running water bath in the laboratory. The temperature of the water bath was maintained within 1° to 2° C. of the bay temperature at the time of sampling. The water samples were inoculated with either washed suspensions of a laboratory culture of *E. coli* or with one part per 100 of cotton-filtered San Diego Sewage Treatment Works primary tank effluent. In some cases San Diego Bay waters naturally contaminated were incubated without further inoculation. Every effort was made to duplicate normal environmental conditions within the limits of an in vitro experiment.

A portion of each sample was removed daily and the coliform organism population was determined by the presumptive method with five tubes in each of three or more serial dilutions using double strength lactose broth. Ninety-nine per cent of the several hundred individual determinations were confirmed in brilliant green lactose bile broth so additional confirmations were not made. The ocean waters in the San Diego area are virtually free from coliform organisms except where some source of contamination is near. Except for waterfowl, municipal and ship wastes are the principal source of coliform organisms in San Diego Bay. Inoculated samples of bay and ocean water were also placed in cellophane dialysis tubing and suspended in San Diego Bay. Determinations were made using autoclaved sea water, prepared B.O.D. water, tap water and stored sea water.

Total bacterial counts were obtained during the course of some experiments by inoculating solid sea water media (1). Lactose broth was also prepared using sea water instead of distilled water for some of the experiments.

The membrane filter was investigated for use in this work and discarded for several reasons. The most important reason was that it would have taken a research project merely to establish its suitability for the study. The modified E.H.C. Endo medium did not give results comparable with the fermentation tube method. In a recent investigation, which has not yet been reported, another laboratory using E.H.C.-B.G.F. medium isolated three different types of colonies, only one type of which showed sheen. However, all three types of colonies confirmed for the coliform group. The sheen colonies were later determined to be *E. coli*. Another colony type was found to be *Aerobacter aerogenes* and the third type was another coliform variant. The source of these organisms was primary sewage treatment plant effluent. Strict interpretation of the membrane filter results as compared to the M.P.N. fermentation tube data was impossible without a great deal of additional information.

Experimental Data

ZoBell (1) has stated that artificial, synthetic, diluted or autoclaved sea water does not necessarily simulate natural sea water and the biological properties of the latter may vary greatly. Any sample of natural sea water that has been removed from its source and placed within the artificial boundaries of a container does not simulate natural conditions. In fact, the authors were unable to determine that what occurred under controlled experimental conditions exactly dupli-

cated the phenomenon occurring in the bay or ocean.

A decision that had to be made was whether to use sewage directly as a source of the bacteria or to use a laboratory culture of *Escherichia coli.* Another decision concerned the numbers of bacteria to be inoculated in the water. Although these points may seem trivial, it did not seem logical to compare in vitro experiments in which neither the quantity nor the quality of the bacteria population was similar with in vivo results. For example, the sewage contained at least three different groups of coliform organisms, whose viability might not be at all comparable to a specific *E. coli* laboratory strain, together with innumerable other bacteria and suspended and dissolved organic and inorganic matter. Coliform bacteria in the primary sewage varied from 200,000 to 800,000 cells per milliliter and after immediate dilution through the existing sewage outfalls, counts of 5,000 to 30,000 cells per milliliter were found; whereas in many investigations bacterial populations of the indicator organism of from 10^7 to 5×10^8 cells per milliliter have been used. Although theoretically the death rate of the organisms under consideration should not vary because of initial concentrations, actually it was decided not to use pure cultures in extremely high concentrations for the general purposes of this work. The initial lag periods for *E. coli* suspensions of about 10^7 cells per milliliter in sea water and *E. coli* or sewage dilutions of about 5,000 cells per milliliter appeared to be the same, but the death rate in the subsequent phase appeared about twice as rapid for the former.

An initial lag period, during which the coliform population remained somewhat stable, lasted from less than 24 hr. to four days when natural sea water was incubated in 1-gal. glass jugs in the laboratory. The length of the lag period varied in general with the season of the year, although this property was not consistent during any season. The lag period and the coliform variability was similar to that found by Vacarro, Ketchum et al. (2) (3). The existence of this lag period takes on far more significance when one considers that it is almost impossible to locate an outfall in the populous regions where under general or unfavorable current conditions the diluted effluent will not reach sensitive areas within 24 to 96 hr.

A number of parallel experiments were run using natural sea water, dechlorinated tap water and a standard buffered dilution water. All the samples were inoculated with filtered primary sewage effluent. The results in Table I are typical of a series of these determinations:

TABLE I.—Comparative Study of Incubated Samples Using Different Dilution Waters

Inoculation Period (Days)	Natural Sea Water (M.P.N.[1])	Dechlorinated Tap Water (M.P.N.[1])	Buffered Dilution Water (M.P.N.[1])
Initial	490,000	330,000	140,000
1	170,000	240,000	3,300
2	220,000	240,000	1,100
3	33,000	240,000	950
4	4,900	240,000	400
5	1,400	70,000	700
7	310	13,000	790
8	490	3,300	1,100
9	330	13,000	490

[1] Coliform per 100 ml.

These data revealed, that natural sea water cannot be compared with a treated chlorinated tap water or a synthetic dilution water. If a comparison is to be made it should be made with natural fresh water. However, the data did show that sea water is not alone in demonstrating rapid reductions in bacterial concentrations on storage. This phenomenon is almost as old as bacteriology. There have been many reports of the rapid decrease in coliform organisms in natural fresh waters. The excellent studies of the U. S. Public Health Service on the Ohio River Basin are typical. In a discussion of the via-

bility of coliform organisms in water by Prescott, Winslow and McCrady (10) some figures are given on the change of coliform organisms in the Ohio River between Cincinnati and Louisville. Strangely enough the death rate demonstrated by the Ohio River data is almost identical with some other information in the same reference from the Massachusetts Department of Health on the purportedly rapid rate at which the coliform group dies in sea water.

The data in Table I on the buffered dilution water are perhaps indicative of the difficulties one may encounter. The extremely rapid initial change in bacterial counts was surprising. The buffered water was identical with standard B.O.D. dilution water. The distilled water used to prepare the dilution water was stored in a stainless steel tank containing a brass fitting and soldered joints. Although traces of the metals could not be detected in the distilled water, it was probable that enough had been dissolved to establish some oligodynamic action. Distilled water from another source was used and the difficulty disappeared. It would not be unusual to find substances with an oligodynamic action in natural waters or from the wastes discharged.

Autoclaved Sea Water

Autoclaved sea water inoculated with washed suspensions of *E. coli* exhibited the same marked change on the viability of the test organism which had been reported by others (2) (3). Table II represents one of a series using an inoculum of sewage in autoclaved and reaerated autoclaved sea water.

There was no practical difference between the results obtained on the autoclaved and the reaerated sea water. Although dissolved oxygen determinations were not made during the course of the experiment, the autoclaved water contained no initial dissolved oxygen and the reaerated water was saturated with oxygen. It appears that autoclaved sea water is a better habitat for the coliform group than fresh water and would probably make an excellent dilution water. It has been frequently reported that the enteric organisms will not propagate in sea water media. All of that work appears to have been done with solid media. Lactose broth was prepared using distilled water, sea water and preautoclaved sea water and inoculated with effluent from a primary treatment sewage plant. Two determinations using the same sewage were run on each broth. Specifically it is believed that the normal saline constituents of sea water are not antagonistic to enteric organisms. Table III shows the comparative results of incubated sea water, preautoclaved sea water and standard lactose broth.

TABLE II.—Comparison of Incubated Samples of Autoclaved Sea Water

Incubation Period (Days)	Natural Sea Water (M.P.N.[1])	Autoclaved Sea Water (M.P.N.[1])	Reaerated Autoclaved Sea Water (M.P.N.[1])
Initial	460,000	1,300,000	350,000
1	230,000	540,000	230,000
2	4,900	1,600,000	540,000
3	2,700	1,600,000	540,000
4	3,500	540,000	350,000
5	700	350,000	540,000
6	1,700	920,000	140,000
7	700	350,000	350,000
8	230	920,000	350,000

[1] Per 100 ml.

Temperature

An attempt was made to determine the effect of temperature on the viability of coliform organisms in natural

TABLE III.—Comparative Study of Incubated Sea Water and Preautoclaved Sea Water

Lactose Broth Distilled Water (M.P.N.[1])	Lactose Broth Sea Water (M.P.N.[1])	Lactose Broth Preautoclaved Sea Water (M.P.N.[1])
49,000,000	49,000,000	23,000,000
23,000,000	17,000,000	23,000,000

[1] Per 100 ml.

sea water under laboratory conditions. Samples were incubated at 5° C., 18° C. and 30° C., although it would be rather unusual to find sea water temperatures of 5° C. in Southern California. There was little difference between the samples incubated at 18° C. and 30° C. These samples showed an initial population stability of one to three days followed by a rapid decrease. The 5° C. samples showed insignificant changes in coliform counts for periods up to 9 days.

It would be impossible to say whether this reduction in mortality is due to reduced effectiveness of the postulated antibiotic activity or to the reduced metabolism of the organisms at low temperatures. It has been found frequently that some substances appear to be less toxic at low temperatures due to the reduced metabolic activity of the test organism.

Sedimentation

Experiments to determine the effect of sedimentation were conducted in the laboratory with inconclusive results. It was impossible to simulate natural conditions affecting flocculation and sedimentation. Filtered sewage used for an inoculum did not contain sufficient particulate matter for agglomeration.

Sea Water Samples

Additional experiments were planned using inoculated samples of sea water in cellophane dialysis tubing suspended in the bay.

The results showed a definite increase in the initial lag phase and there appeared to be some increase in coliform bacteria numbers. Several typical examples are shown in Table IV.

Total bacterial counts were also determined on solid sea water media. The total counts in the dialysis tubing were as much as 1,000 times greater than the total counts in the laboratory incubated samples. It is quite apparent that the container in which the experiment is conducted is of paramount importance. It should also be apparent from these data and other sources that while certain changes which take place in the laboratory are of the same order as the natural phenomena, they may not even be related.

A non-filterable, heat labile material which demonstrates a considerable effect on enteric bacteria in in vitro experiments appears to be present in natural sea water. This substance has been accredited to other organisms in the sea and to bacteriophage. Peculiarly enough, samples taken in the ocean in areas virtually free of bacteriophage and having low total bac-

TABLE IV.—Bay and Laboratory Incubation of Inoculated Sea Water Samples

Days Incubated	Sample 1		Sample 2		Sample 3	
	Lab. Incub. In Glass (M.P.N.)	Bay Incub. Dialysis Tubing (M.P.N.)	Lab. Incub. In Glass (M.P.N.)	Bay Incub. Dialysis Tubing (M.P.N.)	Lab. Incub. In Glass (M.P.N.)	Lab. Incub. Dialysis Tubing (M.P.N.)
Initial	5,400,000	5,400,000	330,000	330,000	170,000	170,000
1	3,500,000	9,200,000	170,000	490,000	220,000	490,000
2	130,000	24,000,000	26,000	3,500,000	33,000	790,000
3	17,000	3,500,000	11,000	1,100,000	7,000	1,700,000
4	11,000	700,000	1,300	49,000	—	—
5	4,900	1,700,000	3,300	49,000	3,300	790,000
6	11,000	1,400,000	490	23,000	1,700	2,400,000
7	—	—	—	—	200	1,300,000
8	—	—	—	—	310	79,000

teria and phytoplankton populations demonstrated the antibiotic action in vitro. Whereas autoclaved sea water samples placed in glass containers and inoculated with sewage undoubtedly containing large numbers of bacteriophage showed little change.

San Diego Bay Factors

Available bacteriological data on San Diego Bay, much of which had been obtained at the same time as this study, was re-examined. Bacterial pollution did not appear to exhibit a seasonal pattern despite the fact that in vitro tests showed a much greater antibiotic action in summer than in winter. The coliform organisms disappear rapidly in the large shallow south section of the bay contiguous to some of the heaviest pollution. In vitro tests comparing water from this end of the bay with other sections and with ocean water showed no marked difference in antibiotic action. During the extreme low tides of the area (1.0 to 1.9 ft. below datum) M.P.N.'s as high as 24,000 per 100 ml. have been found and can be traced as far as six miles from the San Diego Sewage Treatment Works outfall. At other times and tides high coliform concentrations are not found more than one to two miles from the outfall.

The flushing characteristics of San Diego Bay are not well defined, but the major changes in coliform population are taking place within the bay. For example, the mean M.P.N. in the bay, if the sewage were dispersed uniformly, would be 35,000 for each day's discharge. This is based on a rough calculation of the mean tide volume of the bay and assuming 41 m.g.d. of sewage plant effluent containing an average M.P.N. of 50,000,000. Presumably it would be much higher based on available information on the initial lag period. The average M.P.N. in the central section of the bay may approach this value at times, but it is generally lower. In the south and north sections of the bay it is much lower. At Ballast Point, the entrance to the harbor, the mean M.P.N. on an ebbing tide is about 100.

In general, the following have been accredited with having some effect on bacterial numbers in sea water: antibiotic action due to some organic substance, bacteriophage, salinity, sunlight, flocculation and sedimentation, adsorption, predatory and filter feeding organisms, and dilution. The authors doubt that bacteriophage, salinity, or sunlight have any special significance. It is extremely doubtful that the germicidal effects of solar radiation penetrate beyond 5 to 60 cm. ZoBell (1) has stated that if there is any direct effect of sunlight on bacteria in sea water, it is obscured by other factors.

Flocculation and sedimentation are probably important factors. Mud samples from the bay bottom have shown coliform densities of from two million per 100 ml. to more than 2.4 billion per 100 ml. of wet mud. In general the counts varied inversely with the distance from the outfall.

In a bay such as San Diego Bay, which is large and shallow with many surfaces for the development of filter feeding organisms, large numbers of bacteria must be ingested daily. Organisms such as mussels, clams and tunicates may filter 6 to 10 gal. of water per day. With the tremendous population of these animals found in San Diego Bay, particularly in the central and south sections, it would appear that many bacteria would be removed.

Adsorption of the bacteria on suspended matter in the water may be an important factor. Weiss (11) has reviewed available information on adsorption and demonstrated, in vitro, such effects. Additional data, particularly on sea water, is necessary.

Summary and Conclusions

A high degree of uncertainty still prevails with respect to the viability of enteric organisms in sea water.

Even less is known on the fate of pathogens in the sea than on the tracer organisms commonly used.

Existing information must be re-evaluated with respect to the environment in which the experiments were conducted. These studies have shown that significant differences exist with respect to the treatment given the waters used, the source of the test organisms and the container in which the experiments were conducted. Sea water alone is not antagonistic to coliform organisms because of its salinity.

Under the conditions of the experiments in this study, coliform organisms have been found to persist in sea water for relatively long periods. Additional data are required comparing the effects of dispersal of bacteria containing wastes into fresh as well as sea water. It has been demonstrated in many instances, that the viability of enteric organisms in fresh water is not appreciably greater than sea water and may actually be less.

Acknowledgments

The laboratory work was done under the direction of Captain V. C. Tipton, U. S. Navy Preventive Medicine Unit No. 5. The authors acknowledge with appreciation the suggestions and criticism of Harold E. Miller, Executive Officer, San Diego Regional Water Pollution Control Board, Professor C. E. ZoBell and Dr. Carl Oppenheimer of the Scripps Institution of Oceanography and many others. They acknowledge with appreciation the assistance of E. P. Sweeney, HM 1, USN, Research Assistant.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the U. S. Navy Department or the Naval Service at large.

References

1. ZoBell, C. E., "Marine Microbiology." Chronica Botanica Co., Waltham, Mass. (1946).
2. Vacarro, F. R. et al., "Viability of *Escherichia Coli* in Sea Water." *Amer. Jour. of Pub. Health*, **40**, 1257 (1950).
3. Ketchum, B. H., Carey, C. L., and Briggs, M., "Preliminary Studies on the Viability and Dispersal of Coliform Bacteria in the Sea." *Limnological Aspects of Water Supply and Waste Disposal*, Amer. Assoc. Adv. Sci., Washington, D. C. (1949).
4. Ketchum, B. H., Ayers, J. C., and Vacarro, R. F., "Processes Contributing to the Decrease of Coliform Bacteria in a Tidal Estuary." *Ecology*, **33**, 247 (1952).
5. Allen, L. A., "Faecal Bacteria in Polluted Water." *Proc. Soc. for Water Treatment and Examination* (1953).
6. Gilcreas, F. W., and Kelly, S. M., "Significance of the Coliform Test in Relation to Intestinal Virus Pollution of Water." *Jour. New Eng. Water Works Assn.*, **68**, 255 (1954).
7. ZoBell, C. E., "Bactericidal Action of Sea Water." Soc. Expt. Biol. and Medical Proc., **34**, 113 (1936).
8. Buttiaux, R., and Leurs, Th. "Survie des Salmonella dans l'eu de mer." *Bull. de l'Acad. Nat. de Medecine*, **137**, 457 (1953).
9. Kehr, R. W. et al., "A Study of the Pollution and Natural Purification of the Scioto River." USPHS Publ. No. 276 (1941).
10. Prescott, S. C., Winslow, C. E. A., and, McCrady, M. H., "Water Bacteriology." John Wiley and Sons, Inc., New York, N. Y. (1931).
11. Weiss, C. N., "Adsorption of *E. Coli* on River and Estuarine Silts." THIS JOURNAL, **23**, 2, 227 (Feb., 1951).