BACTERIA IN THE INSHORE ENVIRONMENT AT MANDAPAM

By N. K. Velankar

(Central Marine Fisheries Research Station, Mandapam)

Investigations by marine bacteriologists during the course of the last seventy years in different parts of the world have shown the existence of a biochemically versatile bacterial flora autochthonous to the marine environment. Marine bacteriological studies have been carried out mostly with the view to understanding the role of bacteria in the economy and ecology of the sea, though considerable data on bacteria from marine sources have also been published as a result of studies relating to marine fish spoilage. The extensive literature on marine bacteria, however, contains few records of observations made over any considerable period in any one area of the ocean. Changes occurring in the magnitude and nature of the bacterial population of the sea in relation to the seasons and to other factors such as the abundance and composition of the planktonic organisms present, can be evaluated only through observations over a long duration.

Seasonal changes in the bacterial population are likely to be of a more pronounced character in the inshore environment compared to the waters of the ocean remote from land. With a view to determining the pattern of bacterial distribution, qualitative and quantitative, in the inshore environment near Mandapam studies were carried out during the course of four years (1950 to 1953) on the waters from the surface and from near the bottom, on plankton and on bottom muds. The results are reported and discussed in this paper.

Material and Methods

Samples were collected about two miles off the shore in the Gulf of Mannar and Palk Bay near Mandapam. In the Gulf of Mannar the location of collection was midway between the mainland and the chain of coral islands. In both the regions the waters are shallow, not exceeding 2½ fathoms in depth. During the year 1950 two samples were taken weekly from the Gulf of Mannar. From May 1951 onwards two samples from the Palk Bay and one sample from the Gulf of Mannar were collected weekly. All the collections were made between 6 and 7 A.M. Surface-water samples were collected in sterile 500 c.c. glass bottles. Samples of the water from depth were obtained with a Casella type (Thresh *et al.*, 1944) bottle in which a glass bottle was placed in the metal container, a glass tube was used for the inlet, the inlet and the outlet being connected with a piece of rubber tubing

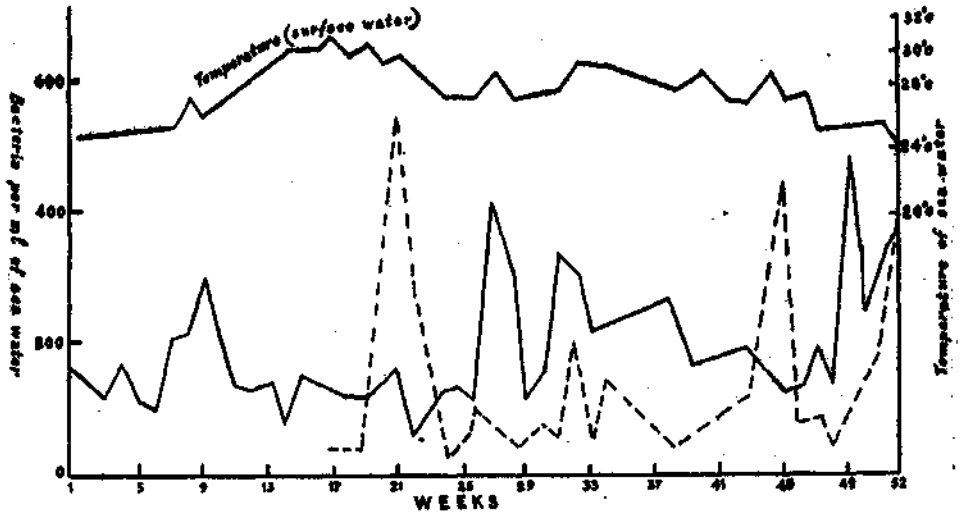

FIG. 1. Bacterial population of sea-water during 1950 (Gulf of Mannar). Surface sample: Continuous line. Depth sample: Broken lines.

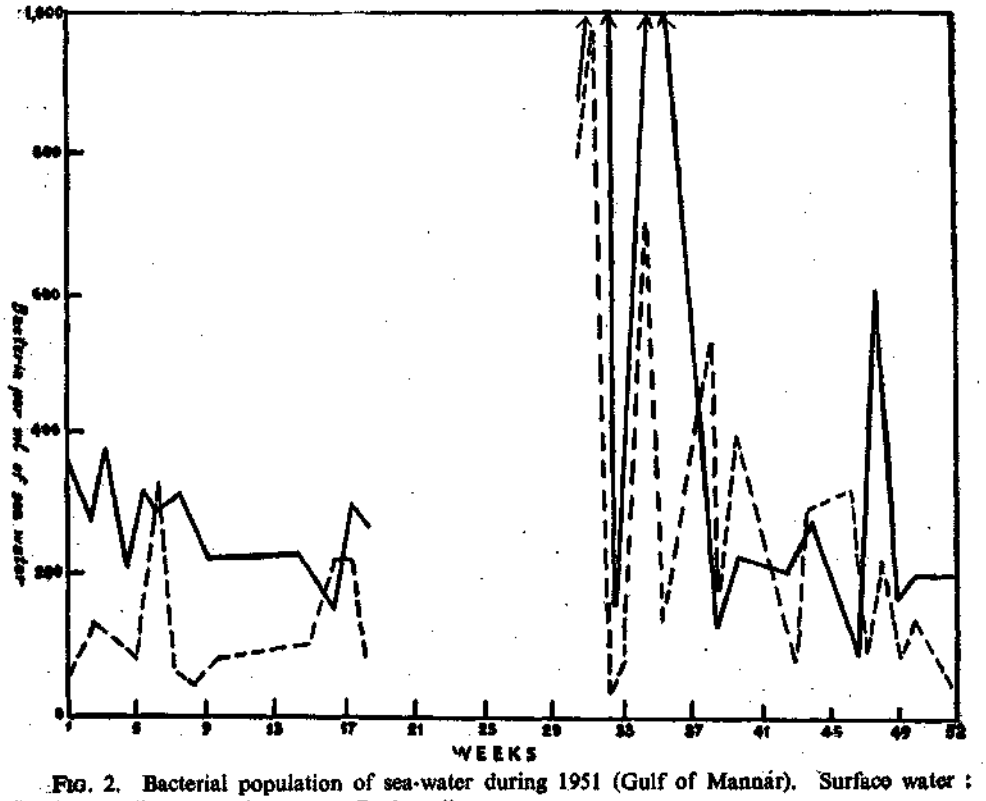

FIG. 2. Bacterial population of sea-water during 1951 (Gulf of Mannár). Surface water : Continuous line. Depth water: Broken line.

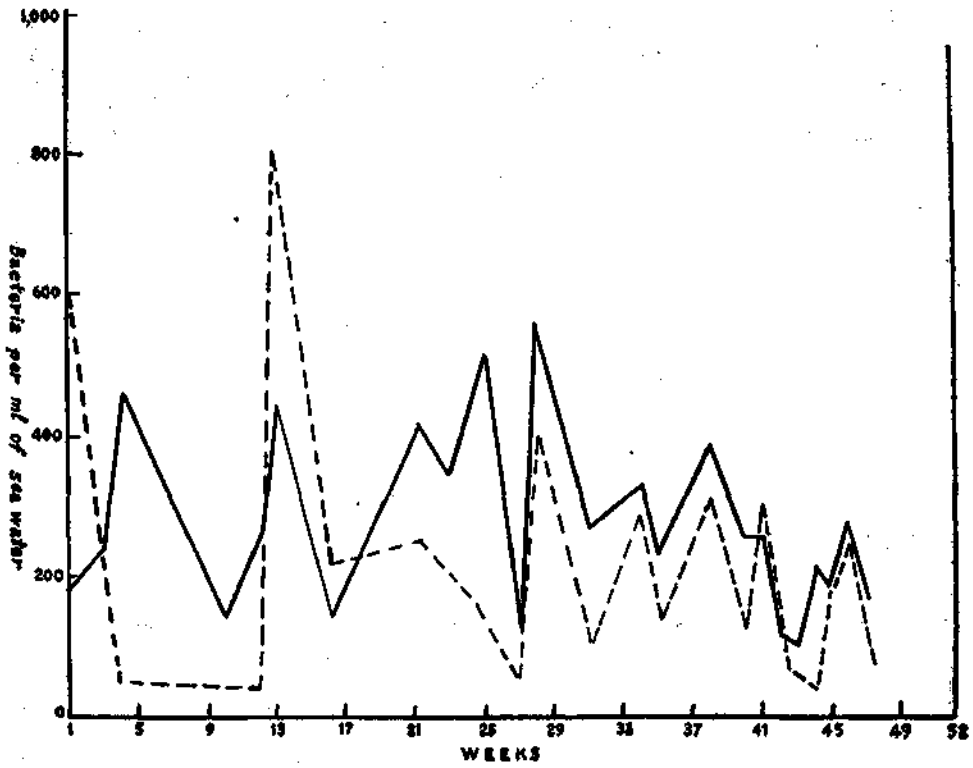

FIG. 3. Bacterial population of sea-water during 1952 (Gulf of Mannar). Surface water: Continuous line. Depth water: Broken line.

which could be removed with a jerk by an attached rope while sampling. The completely assembled apparatus was sterilised in the autoclave before use. This method of sampling was found satisfactory for these investigations. Plankton collections were made by fifteen minute surface hauls, using an organdie net, and the plankton was transferred to sterile 500 c.c. glass bottles for transportation to the laboratory. The time interval between collection of the samples and the plating for bacterial counts was reduced to the minimum as far as possible in order to minimise the chances of bacteria multiplying during the interval, particularly through presence of decaying material. Bottom mud samples were obtained with a Petersen type grab. Mud collections were made once weekly, mostly from the Palk Bay.

Bacteria in the samples were enumerated by plate counts on sea-water agar ("Medium 2216" Zobell, 1946). Autoclaved sea-water was used for making suitable dilutions in the case of the mud and plankton samples. Colonies were counted after one week's incubation at room temperature. Inoculations of suitable dilutions of the samples into standard selective media were made for demonstrating the physiological groups of bacteria.

Results

The surface bacterial population in the Gulf of Mannar remained generally between 200 and 300 per ml., varying from less than a hundred to 850 per ml. Excepting in a very few samples the bacterial population did not fluctuate to any large extent. During the year 1950 the bacteria were less numerous than during the succeeding two years. Though no well-defined seasonal cycles are observed, the trends in the changes in the surface bacterial population in the three years appear to be similar (Figs. 1–3).

The population of the water from near the bottom was usually less than the surface population, the former being often below 100 per ml. The bacterial count of the surface and depth samples often differed considerably. In general the changes in the depth population paralleled those in the surface-water. Qualitatively the bacteria in the depth samples often differed to some extent from the surface flora (see following).

The distribution of bacteria in the Palk Bay is shown in Figs. 4 and 5. During the year 1951 the population changes show trends similar to those in the Gulf of Mannar, but during 1952 the distribution showed no definite pattern.

The monthly average bacterial count per ml. of sea-water varies within a very limited range (Table I) indicating the existence of a fairly constant level of population in the inshore waters.

Bacterial population in association with plankton was of the order of 100 to 1,000 times that in the unstrained sea-water (Tables I and II). Considering individual samples, the bacterial count was generally high when phytoplankton, particularly diatoms, was abundant; in samples consisting almost entirely of zooplankton, bacteria were less numerous; when the total bulk of plankton was low the bacterial count was also low (Table III). The bacterial count of plankton samples was low during the months of January and February (Tables I and II) and during these months zooplankton was predominant both in the Gulf of Mannar and in the Palk Bay.

Aerobic bacterial counts of the muds varied from a few thousands to over a million per g. (wet basis). The mud, which was of a coarse character, had a moisture content varying from 27 to 33%. The bacterial count of the muds was generally lower during 1952 than during 1951 (Table IV).

Anaerobic counts employing the Sprays dish technique were also made on some of the mud samples. The anaerobic count was often lower than the corresponding aerobic count.

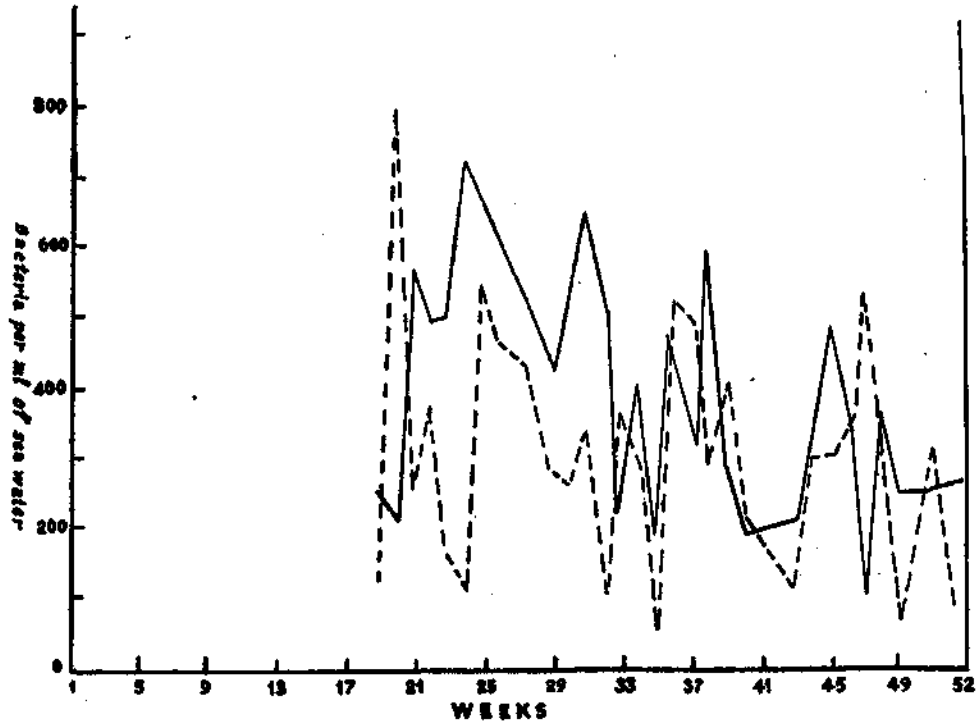

FIG. 4. Bacterial population of sea-water during 1951 (Palk Bay). Surface sample: Continuous line. Depth sample: Broken line.

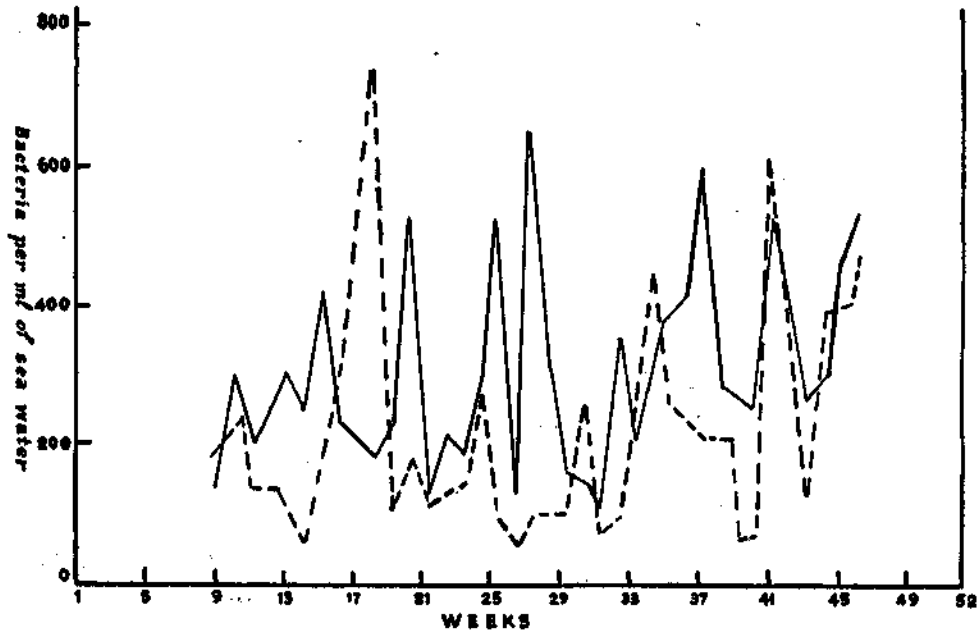

FIG. 5. Bacterial population of sea-water during 1952 (Palk Bay). Surface sample: Continuous line. Depth sample: Broken line.

TABLE I

Monthly Average Bacteria per ml. of Surface and Depth Water Samples, and per ml. of Plankton (Gulf of Mannar)

Month	1950			1951			1952			Average count for the different months during the three years		
	Surface	Depth	Plankton (in thousands)	Surface	Depth	Plankton (in thousands)	Surface	Depth	Plankton (in thousands)	Surface	Depth	Plankton (in thousands)
January ..	132	..	58·0	243	74	111·0	298	291	78·0	224	183	82·0
February ..	157	..	34·0	271	119	60·0	..	..	..	214	119	42·9
March ..	169	..	42·3	..	..	..	278	289	112·0	149	289	77·0
April ..	107	164	148·0	235	146	142·0	133	212	70·0	158	176	120·0
May ..	141	351	58·0	..	..	..	412	252	150·0	276	302	104·0
June ..	106	100	40·0	..	..	..	402	155	201·5	254	128	121·0
July ..	257	53	30·0	861	880	139·5	307	176	67·6	475	370	79·0
August ..	296	92	83·0	848	260	59·0	262	201	189·0	469	184	110·0
September ..	216	106	95·0	240	350	86·5	372	306	121·0	276	254	100·0
October ..	191	116	15·6	273	460	196·0	172	143	172·5	212	240	128·0
November ..	166	111	41·0	247	183	133·0	199	119	171·0	204	137	118·0
December ..	356	209	73·0	180	79	79·0	239	217	159·0	258	166	104·0

TABLE II

Monthly Average Population of Bacteria in the Surface and Deep Water and Plankton in Palk Bay (May 1951–April 1953)

Month		Surface-water per ml.	Deep water per ml.	Plankton (in thousands per ml.)
May	1951 ..	384	366	119·0
June	,, ..	621	385	224·0
July	,, ..	509	298	200·0
August	,, ..	362	284	199·0
September	,, ..	342	404	67·4
October	,, ..	275	224	397·0
November	,, ..	315	326	61·2
December	,, ..	220	216	104·0
January	1952 ..	338	100	10·2
February	,, ..		No samples	
March	,, ..	223	134	55·6
April	,, ..	280	178	650·0
May	,, ..	235	196	570·0
June	,, ..	267	152	291·0
July	,, ..	309	138	428·0
August	,, ..	274	231	116·0
September	,, ..	428	187	156·0
October	,, ..	300	215	143·0
November	,, ..	427	459	463·4
December	,, ..	155	100	253·0
January	1953 ..	186	65	26·3
February	,, ..	192	138	83·6
March	,, ..	170	74	93·0

PHYSIOLOGICAL GROUPS

Nitrifying bacteria.—Surface and depth water samples in 10 ml. quantities, when inoculated into standard ammonium sulphate medium, did not produce nitrification in three months incubation period. Inoculations of 1 ml. of plankton samples produced nitrite in ammonium sulphate medium in about 15 days at room temperature.

TABLE III

Bacteria in Association with Plankton (Palk Bay)

Bacteria (in thousands per ml. of plankton)	Phytoplankton (cells per ml.)	Zooplankton (animals per ml.)	Date	
236·0	70,060	1,510	21- 3-1952	High phytoplankton
over 1 million	125,325	676	26- 5-1952	
160·0	46,816	926	30- 5-1952	
over 1 million	92,624	742	13- 6-1952	
208·0	99,970	1,153	16- 6-1952	
800·0	277,984	1,288	29- 6-1952	
87·0	92,228	1,515	1- 8-1952	
over 1 million	596,360	14,407	15- 9-1952	
312·0	207,258	504	13- 9-1952	
25·0	21,668	884	3-11-1952	
15·80	46	1,037	24- 3-1952	High zooplankton
32·40	56	3,382	18- 4-1952	
240·0	60	1,389	2- 5-1952	
76·50	nil	3,511	12- 5-1952	
4·1	18	3,052	23- 6-1952	
32·0	72	2,112	30- 6-1952	
29·50	14	2,335	2- 7-1952	
130·0	6	3,715	19- 7-1952	
9·20	nil	2,850	21- 7-1952	
228·0	8	1,386	25- 8-1952	
13·0	nil	1,094	29- 9-1952	
5·10	36	2,381	3-10-1952	
13·0	61	131	18- 1-1952	Mixed type plankton
7·40	10	69	21- 1-1952	
8·10	1,720	1,030	10- 3-1952	
24·70	1,172	754	14- 3-1952	
10·90	130	112	21- 3-1952	
155·0	4,462	1,066	19- 5-1952	
7·06	2,374	955	2- 6-1952	
18·40	1,520	2,475	25- 7-1952	
228·0	3,048	1,049	28- 7-1952	
25·0	6,672	1,197	8- 8-1952	
30·0	605	534	18- 8-1952	
87·0	1,271	646	22- 8-1952	
80·0	3,208	2,973	12- 9-1952	
266·0	7,185	1,388	19- 9-1952	

Bottom muds from Palk Bay and the Gulf of Mannar, when inoculated directly into ammonium sulphate medium, produced nitrite in about 10 days. After further incubation for about six weeks the nitrite disappeared and

TABLE IV

Bacterial Counts per g. of Mud (Wet Basis)

Month	1951 Bacterial count (×100)		1952 Bacterial count (×100)	
	Aerobic	Anaerobic	Aerobic	Anaerobic
May ..	707·0	14·4	151·0	17·5
	48·3	21·5	70·0	13·1
	246·0	25·0	77·1	10·1
	46·0	40·0		
June ..	84·0	..	35·0	30·0
	1,500·0	15·0	37·0	21·0
	94·0	12·6		
July ..	1,100·0	..	27·6	12·3
	390·0	10·0	60·2	19·5
	201·0	90·0		
August ..	569·0	1·0	20·3	..
	46·9	5·4	37·0	..
	310·0	54·0	4,800·0	..
	3,300·0	114·0	126·0	..
	38·5	..		
September ..	92·0	14·0	1,100·0	19·8
	59·2	20·0	67·0	5·9
	110·0	15·0	15·0	14·0
October ..	35·0	21·0	46·7	42·8
	200·0	38·0	45·7	11·0
			29·2	..
November ..	70·0	65·0	57·7	41·0
	93·0	17·0	31·0	7·2
	1,095·0	312·0	38·1	3·9
December ..	56·0	44·0	17·8	..

the presence of nitrate could be detected. Using enriched cultures from the mud sources nitrite was produced in standard ammonium sulphate medium in 8 days, which disappeared after a further period of 10 days. When the enrichment cultures were added to sodium nitrite medium the nitrite disappeared in about 12 days and the presence of nitrate was observed.

TABLE V

Occurrence of Denitrifiers in Sea-Water, Plankton and Mud

(Positive samples expressed as % of the total samples)

	Surface-water Dilution		Water from near the bottom Dilution	Plankton Dilution		Total number of samples
	1 ml.	1/10 ml.	1 ml.	1/100 ml.	1/1,000 ml.	
Palk Bay ..	52·6	19·6	21·0	44·1	22·4	143
Gulf of Mannar	56·9	21·5	40·1	56·9	18·5	65

Muds (Palk Bay)

Denitrification positive with inoculations in dilutions of				Total number of samples
1/100	1/1,000	1/10,000	1/100,000	
71·1	48·8	33·3	11·1	45

NITRATE REDUCERS

Bacteria which reduce nitrate to nitrite were present extensively in the sea-water and mud. They occurred to the extent of 10 to 100 per ml. of sea-water, 1,000 to 10,000 per ml. of plankton and 10,000 to 100,000 per g. of mud.

Denitrifying bacteria which reduce nitrate and nitrite to free nitrogen also occurred generally in the inshore environment, but to a much lesser extent, *i.e.*, 1 to 10 per ml. of sea-water, 100 to 1,000 per ml. of plankton and 1,000 to 10,000 per g. of mud. They were present in over 50% of the surface-water samples and in about 30% of the water samples from near the bottom. Their incidence fluctuated during the year, but showed no marked seasonal trends; however, some increase in their incidence appeared towards the end of February after a period of comparative paucity during each of the three years. During some periods extending up to six weeks denitrifying bacteria were absent in the sea-water and plankton. No periodicity was observed in the case of their disappearance also. A number of strains of denitrifying bacteria were isolated from sea-water and mud; all were

gram negative non-sporing motile rods with polar flagellation. In media containing sodium nitrate and calcium acetate, these bacteria precipitated calcium carbonate accompanied by denitrification. The denitrifiers could grow in sea-water enriched with algæ alone as the source of energy and reduced nitrate to free nitrogen.

Nitrogen fixers.—Using sea-water enriched with mannitol, bacteria of the *Cl. pastorianum* type were found generally occurring in the mud and sea-water. Occasionally typical azotobacter cells were found in the pellicle usually present on the mannitol medium.

Sulphate reducers.—The nitrogen-deficient mannitol medium when inoculated with mud or plankton was often found to be blackened owing to the formation of H_2S. Enrichment cultures were obtained from this source and these were found to produce H_2S in synthetic media containing sulphate alone as the source of sulphur. The enrichment cultures showed the presence of small, gram negative curved rods as the dominant organisms.

Agar digesters.—Agar digesters occurred normally in the inshore environment as found by the appearance of softened areas of the agar on flooding the counting plates with iodine, and also by the presence of depressed colonies on the agar. Those which produced depressions liquefied agar very rapidly; these were morphologically distinct in being elongated ($0{\cdot}6 \times 10\,\mu$) rods. These were common in the Palk Bay samples, but rare in the Gulf of Mannar waters. Their existence is probably associated with the relatively richer phytoplankton in the former environment.

Luminous bacteria.—Luminous colonies were usually present on agar plates inoculated with sea-water and plankton, occurring to the extent of 5 to 8%. They were more common in the surface-water than in the depth samples. Mud inoculated plates showed luminous colonies only infrequently.

Coliforms

The incidence of coliforms was examined by the Most Probable Number method. They were present in fairly large numbers (50 to 100 per 100 c.c.) up to a distance of about 400 yds. from the shore, but were generally absent in the usual sea-water samples collected two miles off the shore. Coliforms were not found in any of the depth water samples examined. On the occasions when coliforms were found in the surface-water samples the total bacterial count of the water was significantly high, indicating chance pollution probably of local character. These observations stress the probability of an adventitious occurrence of coliform in the sea-water.

Bacterial Flora of the Environment (Qualitative)

The colonies appearing on the sea-water agar plates used for bacterial counts were mostly smooth, moist, shining often with bluish iridescence, and varied greatly in opacity. A greater variety of pigmented colonies was found in the surface-water plates compared to the plates inoculated with water from near the bottom. Pigmented colonies were very rare on mud inoculated plates.

Over fifty strains, from colonies of various types noticed frequently during the quantitative work, were isolated. The majority of the strains were achromic, nonsporing, gram negative, motile rods with polar flagellation. A few *Bacillus*, *Micrococcus* and *Sarcina* spp. were isolated. The chromogens produced yellow, orange, red, pink, violet, flesh coloured and dark pigments. Two *Nocardia* spp. and one black pigment producing yeast-like organism were also isolated.

All the strains were aerobic. Some could grow in fresh-water media in which their growth was poor compared to that in sea-water media. The gram positive types, *i.e.*, *Bacillus*, *Micrococcus* and *Sarcina* spp. showed luxuriant growth on fresh-water nutrient agar compared to sea-water agar. All the strains showed better growth at room temperature than at either 37° C. or at 2° to 5° C. Very few showed visible growth near 0° C. during 4 weeks' incubation. 80 % of the isolates reduced nitrate to nitrite, 66% liquified gelatin, 68% peptonised milk, and 56% produced acid from dextrose and other sugars. Gas was not produced from dextrose or other carbohydrates by any of the strains. The chromogenic strains were particularly weak in saccharolytic activity. Starch was hydrolysed by a large number of the strains. A few produced indol.

Discussion

Very large numbers of bacteria are associated with plankton and muds in the inshore environment while few are present in the sea-water. The bacterial population of the sea-water appears to be fairly constant numerically and is comparable to the bacterial population found in the sea-water at La Jolla, California, by Zobell (1946), and in the Australian coastal waters by Wood (1953). Trends in the bacterial counts of the sea-water during three successive years in the Gulf of Mannar station are similar and are probably of seasonal character.

Quantitative and qualitative bacteriological differences were observed in water from the surface and from near the bottom. The lower bacterial population of the water from depth is probably due to adsorption of

bacteria by the muds. Qualitative differences such as the paucity of chromogens and denitrifiers, and the absence of coliforms in the depth samples as compared to the surface-water, are presumably due to ecological differences. The paucity of chromogenic bacteria in water from depth appears to be common to the aquatic environment, since Taylor (1942) noticed similar distribution of chromogens in fresh-water lakes.

There appears to be no relation between the temperature and bacterial population in the sea-water (Fig. 1). Wood (1953) recorded higher counts during the winter months than in the summer months, but he considers the inverse relation to be more apparent than real, since extraneous factors such as rainfall might be significant. The author (1950) recorded higher bacterial counts in the surface sea-water off Madras during periods of rainfall. However, in the Mandapam area, where most of the rainfall occurs in the short north-east monsoon season, no significant increase in the bacterial population of the sea-water during the rainy season was observed.

Association of bacteria in large numbers with plankton, particularly phytoplankton, has been noticed. The vertical distribution of diatoms and bacteria in the sea-water has been observed to be parallel by Zobell (1942). According to Waksman *et al.* (1933 *a* and 1937) no direct quantitative relation between bacteria and diatoms exists, though the living as well as the dead diatoms serve as substrata to grow on; bacterial maxima may therefore follow the diatom maxima in an environment. Such a sequence is seen in Fig. 6 (May to December 1951, Palk Bay). During this period

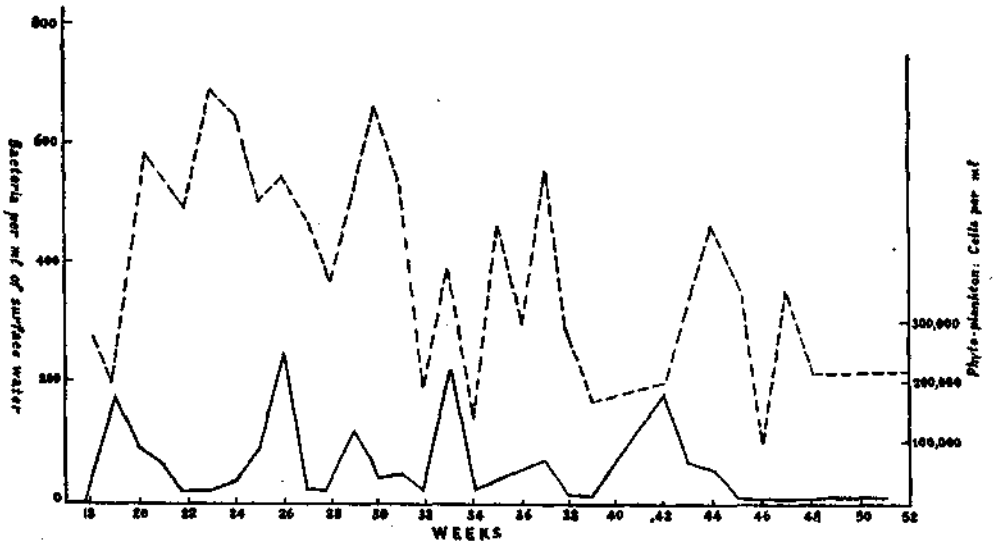

FIG. 6. Bacteria and Phytoplankton. (Palk Bay, May 1951–December 1951). Phytoplankton: Continuous line. Bacteria: Broken line.

the magnitude of phytoplankton present was high. The time interval between any two successive diatom or bacterial peaks is seen to be almost the same, *i.e.*, seven to eight weeks. Brandt *et al.* (1937), in their experiments *in vitro* on the degradation of plankton to ammonia and the regeneration of nitrite and nitrate, found the formation of nitrite to take place in about seven weeks. It would be interesting to associate this interval of time with the process of regeneration of the nutrients through the degradation of plankton in the sea, though any inference on present evidence alone is not permissible. It is possible that the relation between bacteria and diatoms would be noticeable only under very favourable conditions such as the prevalence of a high plankton level along with the negligible influence of extraneous factors.

In this investigation no periodic fluctuations or decided trends in the bacterial population of the muds were observed. Since the source of food for the mud bacteria is the plant and animal residues that sink down from the overlying waters, variation in the plankton abundance would be expected to influence the level of available nutrition and consequently the bacterial population of the mud. The prevalence of lower bacterial population during 1952 as compared to that in 1951 is possibly attributable to a lesser standing crop of plankton during 1952. According to Wood (1953) direct microscopic counts are more representative than plate counts in the case of the muds. This might account for the absence of decided trends in the mud bacterial population during the present work. The fact that anaerobic counts were often lower than the corresponding aerobic counts emphasises the aerobic nature of the majority of the bacteria in the muds. Wood (1953) found no difference in the anaerobic and aerobic counts of the muds he examined, and according to him there are very few obligate anaerobes in the mud from near the surface.

The presence, in association with plankton, of bacteria which oxidise ammonia to nitrite, in the muds, of bacteria which oxidise ammonia to nitrite and to nitrate, and of denitrifiers, sulphate reducers and agar digesters in the sea-water, mud and plankton, has been shown in the present studies on the inshore environments. These bacteria were found in other marine environments by previous workers (Waksman *et al.*, 1933 *a* and *b*); Carey, 1938). According to the present consensus of opinion loss of combined nitrogen from the sea through bacterial denitrification is not significant. Certain features of the environment under present investigation, *i.e.*, shallow waters such as would permit the accumulation of undegraded plant and animal residues at the bottom and the tropical temperature, appear likely to favour the denitrification process.

Though the quantitative significance of the bacterial processes involving the transformation of the nitrogen compounds etc. in the sea cannot be assessed in the light of present knowledge, the presence and wide distribution of these bacteria in the marine environment is *per se* significant. On purely quantitative considerations the overall significance of bacteria in the marine environment would itself appear to be doubtful, in view of the paucity of their numbers in the sea-water. Wood (1950) has suggested, as a hypothesis, two possible ways in which a relatively small bacterial population could effect chemical changes out of all proportion to its magnitude in the sea:

(i) Bacteria may produce non-specific organic or inorganic catalysts and these may be adsorbed by particles in the sea. These would be cumulative and not related to the actual population of living bacteria.

(ii) Bacteria may produce conditions in the sea favouring certain reactions and these reactions may be catalysed by substances present in the substrate.

Qualitatively the bacterial flora found here resembles to some extent the flora from the environs of La Jolla, California, described by Zobell *et al.* (Zobell and Feltham, 1934; Zobell and Upham, 1944) as well as the Australian sea-water flora described by Wood (1953). The preponderance of non-sporing, gram negative motile rods is common; so also is the absence of bacteria producing gas from sugars and the paucity of gram positive organisms. Wood (1953) found micrococci to be next in abundance to the gram negative rods in the sea-water. Micrococci are relatively rare in the Mandapam environment. The gram positive non-sporing rods classified by Wood (1953) as *Corynebacteria* and found by him to be abundant in estuarine muds and to some extent in the sea-water, were not encountered in the present investigation. The chromogenic flora found here is very similar to that described by Zobell and Feltham. Though it was not possible to observe the flagellation of all the motile strains isolated, all those non-sporing motile rods, which were successfully stained, showed polar flagellation. Wood has remarked on the rare occurrence of peritrichous flagellation in his non-sporing strains. Zobell and Upham also found polar flagellation to be more general in their sixty isolates.

The use of comparable procedures in the investigations by Zobell, Wood and the author renders possible a comparison between their findings. The heterotrophic bacterial flora of the inshore environment does not differ markedly from the other marine environments, qualitatively or quantitatively.

Summary

The quantitative distribution of bacteria in the sea-water, in association with plankton, and in the bottom muds of the Palk Bay and the Gulf of Mannar at Mandapam, at a distance of two miles from the shore was investigated during a four-year period (1950–53). Bacteria were present in the sea-water to the extent of a few hundreds per ml., determined by plate counts on sea-water agar; they were more numerous in the water from the surface than from near the bottom. Bacteria were present associated with plankton in large numbers, ranging from a few thousands to over 500,000 per ml. of plankton, and appear to be influenced numerically by the nature of the plankton. Plate counts of the muds ranged from a few thousands to over a million per g. (wet basis). Anaerobic counts were often less than the aerobic counts.

Numerical changes in the sea-water bacterial population showed trends which are probably seasonal. The presence of nitrifying, denitrifying, nitrogen fixing, agar digesting and sulphate reducing bacteria in the sea-water and/or muds is reported. Gram negative non-sporing motile rods predominated in the bacterial flora. The normally occurring heterotrophic bacterial flora of the environment is briefly described.

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