

Occurrence & Distribution of *Vibrio parahaemolyticus* (Sakazaki *et al.*) & Related Organisms in the Laccadive Sea

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Distribution of *V. parahaemolyticus* and related organisms in the water column up to 200 m deep showed a fluctuating trend. Typical *V. parahaemolyticus* was not isolated from water samples collected at 200 m. However, zooplankton samples from all stations yielded *V. parahaemolyticus*. This study showed that *V. parahaemolyticus* occurs in offshore waters but in low numbers.

Halophilic bacterium *V. parahaemolyticus* has been isolated from different environments and a variety of marine animals¹. While the incidence of *V. parahaemolyticus* in inshore waters of India is well documented, its distribution in offshore waters is not known¹⁻³. The present study pertains to the occurrence and distribution of this bacterium and related forms in Laccadive Sea where a number of coral attols exist.

Materials and Methods

Sampling was done on board *R V Gaveshani* during 116 cruise (10 Feb. to 3 March 1983). Seven stations were selected for sampling (Fig. 1). Water samples from different depths (surface, 10, 40, 100 and 200 m) were collected with ZoBell's bacteriological samplers and transferred to presterilized glass bottles (500 ml). Plankton samples were collected with a Heron-Tranter (HT) net (300 μ m mesh), hauled vertically from 200 m depth. The plankton samples were immediately transferred to sterile widemouth glass bottles.

Depth, water temperature and salinity of the water column were measured with digital bathythermograph (Kankyo Keishoku Kaisha, Tokyo, Japan). The samples collected at 40 m depth were approximately from the top of the thermocline and 100 m samples from the bottom of thermocline.

All bacteriological analyses were conducted immediately after collection of samples. Both total viable counts (TVC) and vibrio counts were made following membrane filtration technique with 0.45 μ m filters (Millipore). While ZoBell's 2216 e medium was used for TVC, thiosulfate citrate bilesalts sucrose agar (TCBS) medium was used for the estimation of *Vibrio* like organisms (VLO) and *V. parahaemolyticus* like organisms (VPLO). All colonies that appeared on

TCBS agar were regarded as VLO and 'typical' bluish green sucrose negative colonies on TCBS as VPLO⁴. Known aliquots of water samples were filtered and the filters were placed on the nutrient media. In the case of plankton samples, excess water was drained off and 100 ml sterile seawater was added and shaken thoroughly for 10 min. Known aliquots of suspension, free of zooplankton, were filtered and the filters were placed on nutrient media. All the plates containing filters were incubated at 28°C and colonies counted

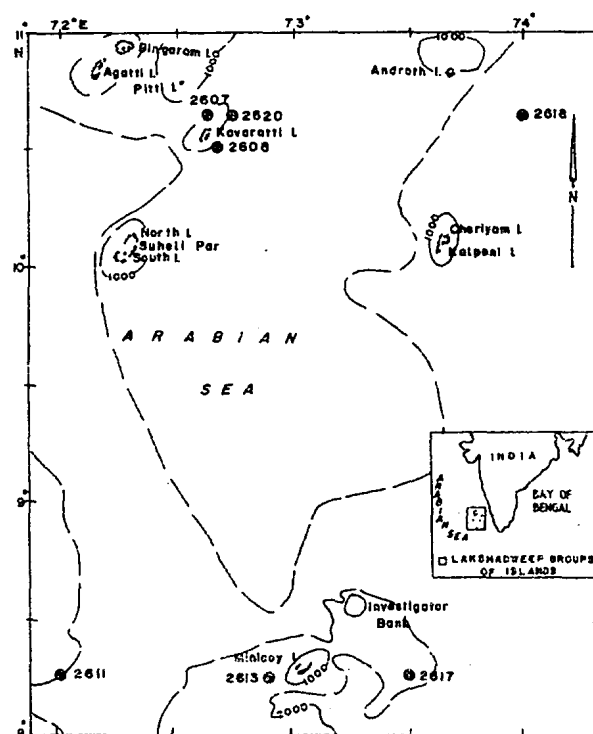


Fig. 1—Station locations

after 72 h. The microbial populations were expressed as number. (100 ml)⁻¹ or number. g⁻¹ of plankton on dry weight basis.

'Typical' green colonies (VPLO) isolated from various samples were repeatedly streaked on nutrient medium and checked for their purity. The isolates numbering 180 were subjected to 26 tests following standard methods⁵.

Results and Discussion

The results (Table 1) show that except at sts 2607 and 2608, the TVC value always increased with depth and in these stations maximum values were always recorded at 200 m [from 39.8 to 192 × 10². (100 ml)⁻¹]. However typical *V. parahaemolyticus* (VP) could not be isolated from any of the samples collected at 200 m. In general *V. parahaemolyticus* strains could be isolated only up to 40 m. Populations of VLO and VPLO fluctuated widely in the water column. Population of VPLO varied from 23.81 to 98.75 % of VLO. Compared to this, the population of *V. parahaemolyticus* varied from 0 to 9.52 % of VPLO. However the percentage of VP to TVC is mostly < 2. These results indicate the scanty distribution of typical *V. parahaemolyticus* in the water column. Similar observations¹ were made in coastal and mangrove waters, sediments and plankton but VP level was much less and it constituted only 0.001 to 0.1 % of TVC.

In the case of zooplankton which consisted of mainly copepods, chaetognaths, siphonophores, medusae and fish larvae TVC varied from 102 to 4870 × 10⁴. g⁻¹ (Table 2). All the zooplankton samples yielded typical *V. parahaemolyticus*. Variations in percentage composition of different bacterial fractions associated with zooplankton are given in Table 3. Compared to water samples, VPLO populations constituted much less percentage of VLO, i.e. 0.38 to 50.92 % except at st 2620 where it was 80.07 %. The percentage occurrence of VP in VPLO was higher on an average compared to water but much lower in TVC (0.001 to 0.12 %).

Compared to zooplankton, higher percentage of VP was noticed in water and that too in samples collected from surface layers. Their presence in surface waters and absence in deeper waters indicate the preference of VP for higher temperatures. Being a mesophile, higher populations have always been recorded in warm waters or warmer seasons of the year¹. Temperature was reported to be a major limiting factor for the distribution of *V. parahaemolyticus* in subtropical waters⁶. Eventhough the minimum temperature⁷ required for the growth of VP is only 5 - 8°C, no VP could be isolated from waters where the temperature ranged from 13.3 to 16.29°C. Since the salinity was almost uniform (34.08 - 35.60 × 10⁻³) in all stations

Table 1—Total Viable Count(TVC), *Vibrio* like Organisms (VLO), *Vibrio parahaemolyticus* like Organisms (VPLO) and *Vibrio parahaemolyticus* (VP) in Water Samples [Values × 10². (100 ml)⁻¹]

Sampling depth (m)	Temp. (°C)	TVC	VLO	VPLO	VP	VP/TVC
St 2607						
0	27.68	4.1	2.35	1.86	0.10	2.44
10	27.65	3.3	1.83	1.23	0.02	0.61
40	28.08	6.7	1.53	1.50	0.01	0.15
100	22.81	1.7	1.11	0.91	0	0
200	15.91	4.01	3.79	1.93	0	0
St 2608						
0	27.62	8.9	1.63	0.99	0.02	0.22
10	27.73	10.2	2.41	2.27	0.07	0.69
40	28.49	5.2	3.09	3.02	0.09	1.73
100	23.09	6.9	1.43	1.18	0	0
200	16.29	11.2	1.04	0.80	0	0
St 2611						
0	27.95	5.3	1.91	1.38	0.08	1.51
10	27.88	4.5	1.60	1.58	0.12	2.67
40	27.73	9.7	1.29	1.26	0.10	1.03
100	25.41	14.2	1.86	1.52	0.01	0.07
200	15.58	43.3	1.42	1.06	0	0
St 2613						
0	27.97	33.9	2.47	1.49	0.03	0.09
10	27.96	45.1	4.26	2.92	0.09	0.20
40	27.93	124.8	2.24	2.04	0	0
100	24.13	109.2	3.96	3.28	0	0
200	14.78	149.1	6.00	5.44	0	0
St 2617						
0	27.97	7.6	1.02	0.45	0.02	0.26
10	27.99	13.2	0.42	0.10	0	0
40	28.11	14.1	1.06	0.35	0	0
100	24.44	16.2	0.94	0.83	0	0
200	13.30	100.3	1.11	0.41	0	0
St 2618						
0	28.07	5.4	0.96	0.56	0.04	0.74
10	28.06	6.7	0.22	0.14	0.01	0.15
40	28.41	6.4	1.96	1.74	0.02	0.31
100	25.32	23.0	2.80	1.20	0	0
200	14.19	192.0	0.37	0.14	0	0
St 2620						
0	27.72	2.81	2.63	1.96	0.07	2.49
10	27.74	1.10	0.29	0.21	0.02	1.82
40	27.90	3.9	0.80	0.33	0.01	0.26
100	24.64	11.8	2.12	1.56	0.01	0.08
200	14.94	39.8	0.23	0.16	0	0

investigated, the observed variations in the VP population can not be related to this factor. Similar observations were also made by Kaneko and Colwell⁸. The present investigation reveals that VP occurs in open ocean which is in contrast to the earlier observation of Kaneko and Colwell⁸. These investigators could not isolate VP from seawater,

Table 2—*Vibrio parahaemolyticus* and Related Organisms Associated with Zooplankton
(Values $\times 10^4$ g⁻¹ dry wt)

St	TVC	VLO	VPLO	VP
2607	102	3.79	1.93	0.122
2608	1680	25.53	1.28	0.031
2611	4870	12.30	0.10	0.004
2613	2580	42.10	0.16	0.013
2617	261	10.23	1.50	0.068
2618	1710	44.56	14.00	1.120
2620	850	8.33	6.67	0.347

Table 3—Variations in Percentage Composition of Different Bacterial Fractions Associated with Zooplankton

St	VLO/TVC	VPLO/VLO	VP/VPLO	VP/TVC
2607	3.72	50.92	6.32	0.120
2608	1.52	5.01	2.42	0.002
2611	0.25	0.81	4.00	0.001
2613	1.63	0.38	8.13	0.001
2617	3.92	14.66	4.53	0.026
2618	2.61	31.42	8.00	0.065
2620	0.98	80.07	5.20	0.041

sediments and plankton sampled 4 to 10 miles from the shore. However organisms belonging to VPLO group were isolated from all the samples in the present study as reported by Kaneko and Colwell⁸ off South

Carolina and Georgia coasts. The only plausible explanation which can be given for the presence of VP in Laccadive sea is the influence of coral atolls. The rich flora and fauna of these atolls can supply substantial quantities of dissolved organic matter and particulates which can support a wide variety of microorganisms including *V. parahaemolyticus*.

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