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DISTRIBUTION OF LUMINESCENT BACTERIUM *VIBRIO HARVEYI* IN NETRAVATHI ESTUARY, MANGALORE*

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ABSTRACT

The seasonal distribution of bioluminescent bacteria *Vibrio harveyi* in relation to hydrographical parameters has been studied in Netravathi Estuary from April 1985 to March 1986. Differences in the distribution of bacterial density between surface and bottom waters were observed. Salinity had a major influence on the distribution of *V. harveyi* than oxygen, temperature and pH. In the sediment samples the population of *V. harveyi* fluctuated during different months.

INTRODUCTION

LUMINOUS bacteria have been isolated from the surface sea waters of tropical, temperate and polar regions and also from depths of several hundred metres (Hastings and Nealson, 1977; Ruby *et al.*, 1980). In tropical estuaries, the luminous bacteria are represented by three species *Vibrio harveyi, Photobacterium leiognathi* and *P. fischeri* (Nair *et al.*, 1979; Ramamoorthi and Jayabalan, 1982). Though the bacterial luminescence aids for easy recognition in the laboratory pertaining to isolation, identification of species requires several tests (Nealson, 1978). Information on spatio-temporal distribution of luminous microflora in relation to environmental parameters from tropical estuaries are scanty. The present study was undertaken on the distribution pattern of *V. harveyi* from the Netravathi Estuary in relation to different environmental parameters.

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MATERIAL AND METHODS

The seasonal distribution of the luminous bacterium *Vibrio harveyi* in water and sediment samples of Netravathi Estuary (12°50' N and 74°50'E) at Mangalore was studied during April

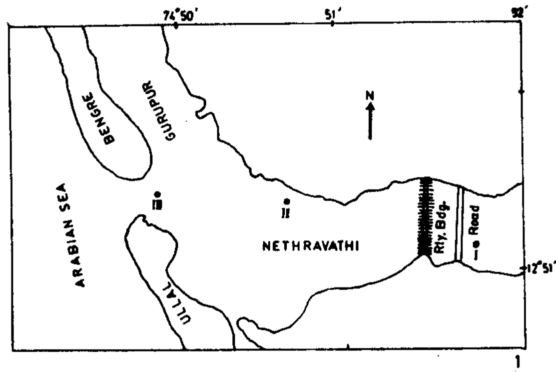

Fig. 1. Sampling stations in Netravathi Estuary.

1985 - March 1986 from 3 stations (Fig. 1). Samples were collected once a month. During Southwest monsoon season (June-August) due to heavy flood and other unfavourable weather conditions, sampling could not be made in Station I. However, from Stations II and III, surface water and sediment were collected close to the shore during monsoon months. Surface

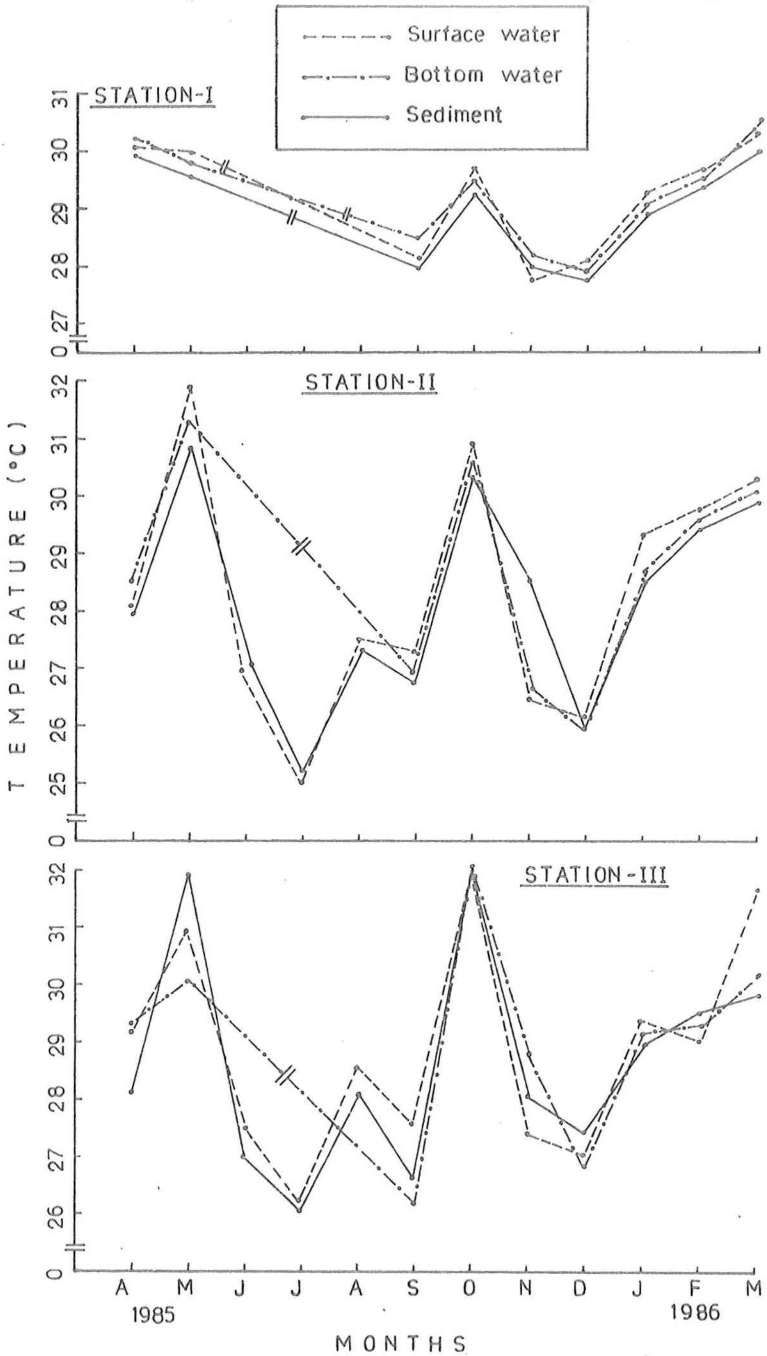

Fig. 2. Distribution of temperature in different stations.

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water samples collected by using pre-sterilized bottle and bottom water samples with Nansen water sampler were immediately transferred to sterile Mccartney bottles. The sediment samples were collected by using Peterson grab and the central portion of the mud was transferred asceptically into sterile petri-dishes.

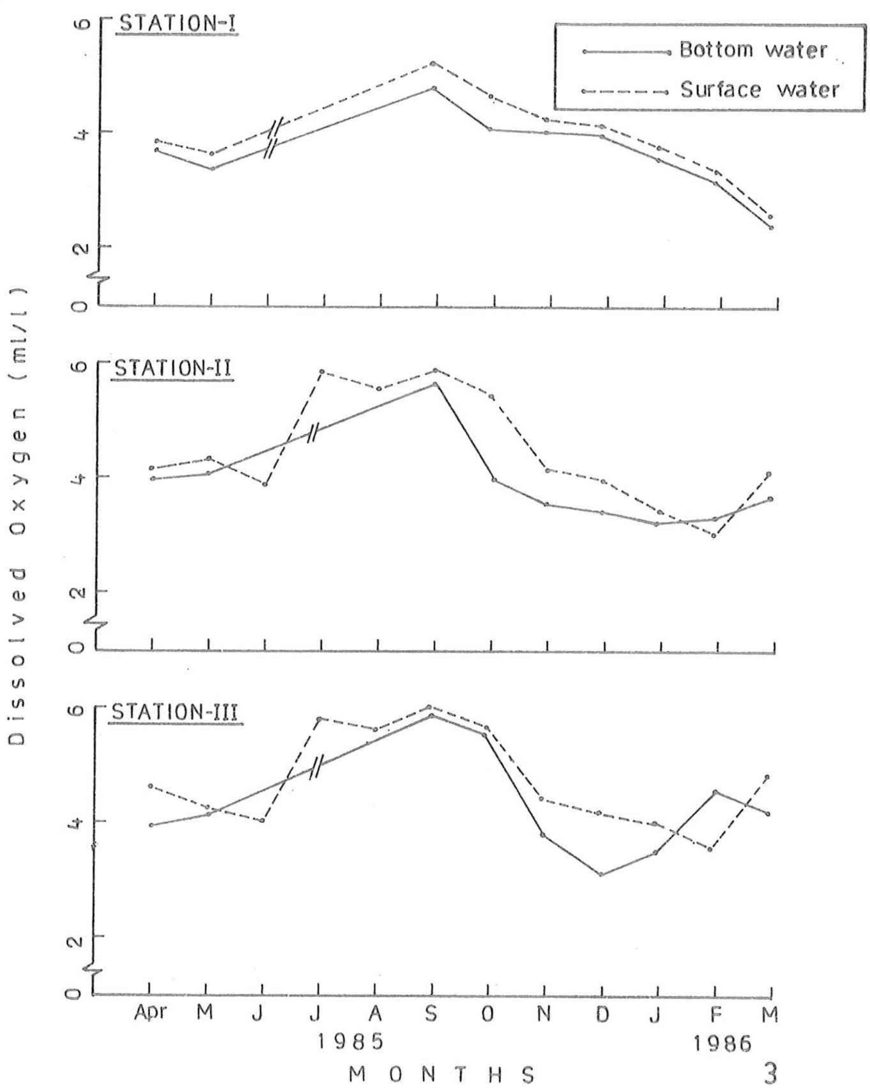

Fig. 3. Distribution of dissolved oxygen in different stations.

Temperature of surface and bottom water as well as that of sediment was recorded in the field to the nearest 0.1°C. Salinity and oxygen of water were estimated adopting the procedures given by Strickland and Parsons (1972). The pH of water and sediment was recorded by using pH meter (Elico). All the samples were processed within two hours of collection.

Sea water nutrient agar (SWC) medium (pH 7.2) with 3 ml of glycerol/litre was used to isolate luminous bacteria (Hastings and Mitchell, 1971; Ramamoorthi and Jayabalan, 1982). Cultures were grown at room temperature serially diluted and 1 ml of these were made use of for plating. For enumeration of total luminous colony forming units (LCFU),

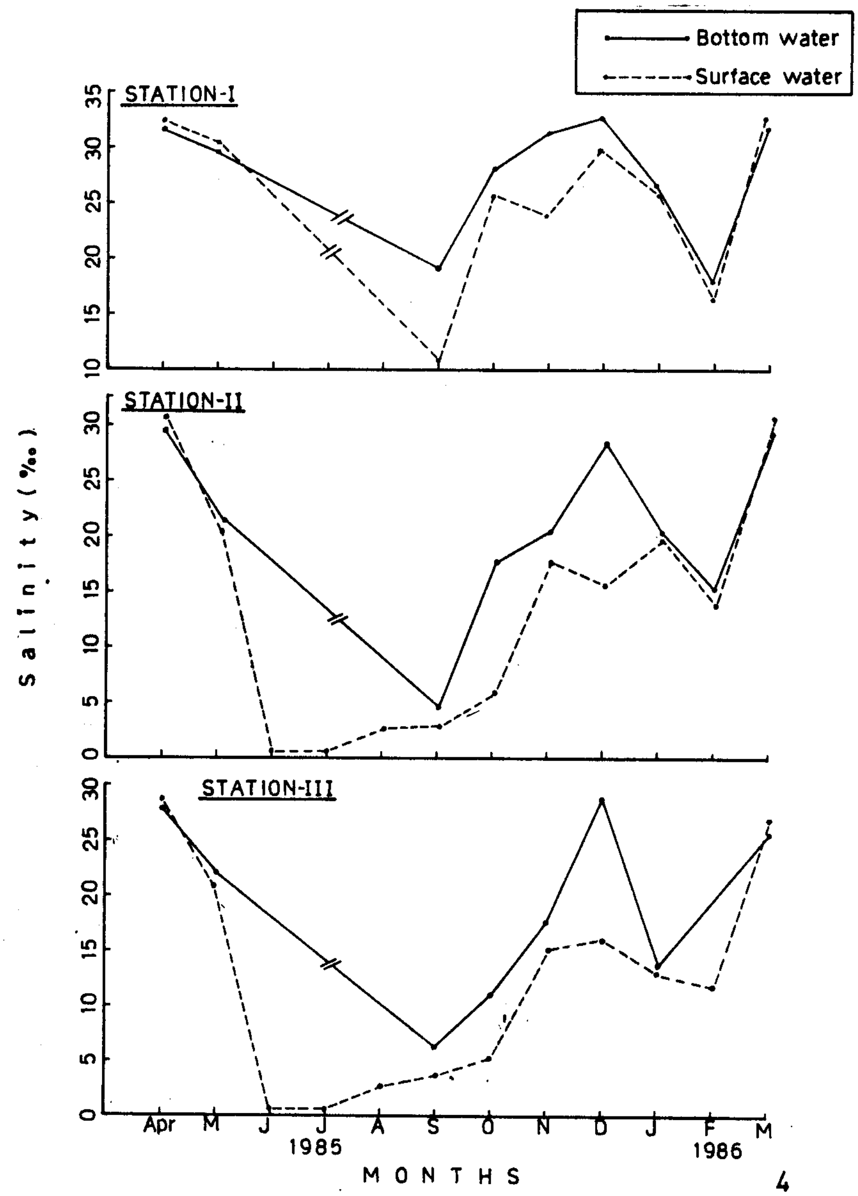

Fig. 4. Distribution of salinity in different stations.

(27 ± 2°C) for 36 hours. Pour plate technique was adopted. Water and sediment samples were petri-dishes were examined in dark and luminescent colonies were marked on outer

surface of lower petri-dishes using glass marking felt pen and then counted in light. For identification of luminous bacterium *V. harveyi*, the procedure given by Reichelt and Baumann (1973, 1975) was adopted.

RESULTS

The physico-chemical parameters of water and sediments are provided in Fig. 2, 3, 4, 5 and the percentage distribution of *V. harveyi* is given in Fig. 6, 7 and 8.

In Station I, *V. harveyi* was dominant in the surface water in almost all the sampling months with the minimum being 46.17% and the maximum being 76.92% during November and May respectively. In bottom water, *V. harveyi* increased from 60% in April to 66.67% in September. From October onwards, it ranged between 55.66% and 100%. While maximum value (100%) was recorded in Feburary, the minimum of 53.3% was observed in January. In sediment samples, *V. harveyi* recorded 58.82% in April and 64.28% in May, during September it was absent. Between October and March, the contribution of *V. harveyi* ranged from 46.66% to 68.18%.

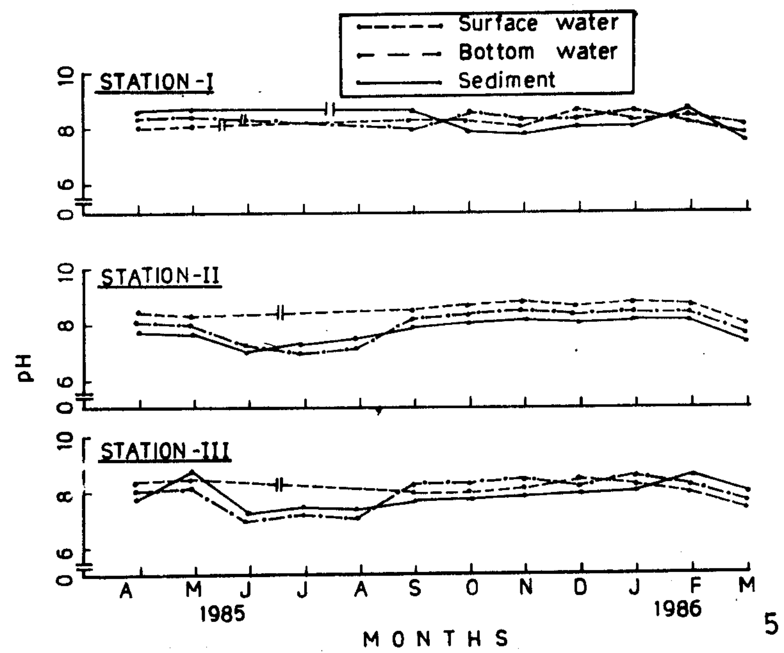

Fig. 5. Distribution of pH in different stations

In Station II, during April, the percentage of *V. harveyi* in surface water was 75% which decreased to 58% in May and remained stationary in June. *V. harveyi* was absent during July and August, but recorded 75% during september and 10% in October. From November, the percentage contribution fluctuated from 50% to 77% till March. Bottom water samples recorded 100% of *V. harveyi* during April, September and November. The minimum percentage (52.94%) was recorded in February. During the rest of the months, the values fluctuated considerably. While in sediment, *V. harveyi* recorded 100% in May and January and 50% during December, it was about during July-September.

In surface water samples of Station III, *V. harveyi* was absent in the first two sampling months. From september to March the percentage varied from 50% to 100%. The maximum percentage (100%) was observed in July and November; while the minimum

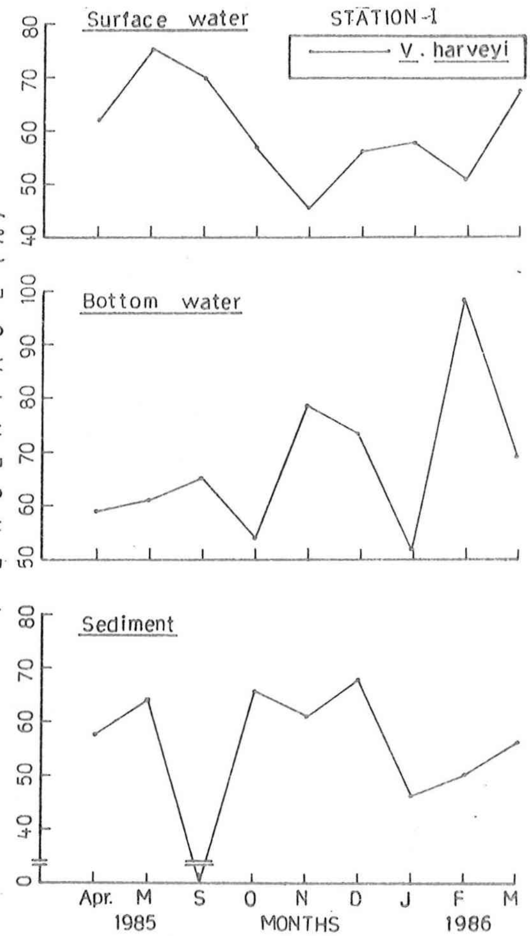

Fig. 6. Distribution (%) of *V. harveyi* in station I.

percentage (50%) was recorded in September and October. In bottom water, from an initial minimum value (50%) during April, the percentage of *V. harveyi* reached maximum (100%) in September and then reduced to 75% in October. In November, the percentage of *V. harveyi* once again reached 100%. From December onwards the occurrence ranged from 57.15% to 75.0%. Sediment samples recorded 100% of *V. harveyi* during November and February. The minimum percentage (50%) of its occurrence was noticed in June and March.

Discussion

Occurrence of three species of Luminous bacteria *viz. V. harveyi, Photobocterium leiognathi* and *P. fischeri* has been observed from Netravathi estuarine environment (Sivasankar, 1986). From the waters of Bay of Bengal, Vellar Estuary, Killai Backwater and

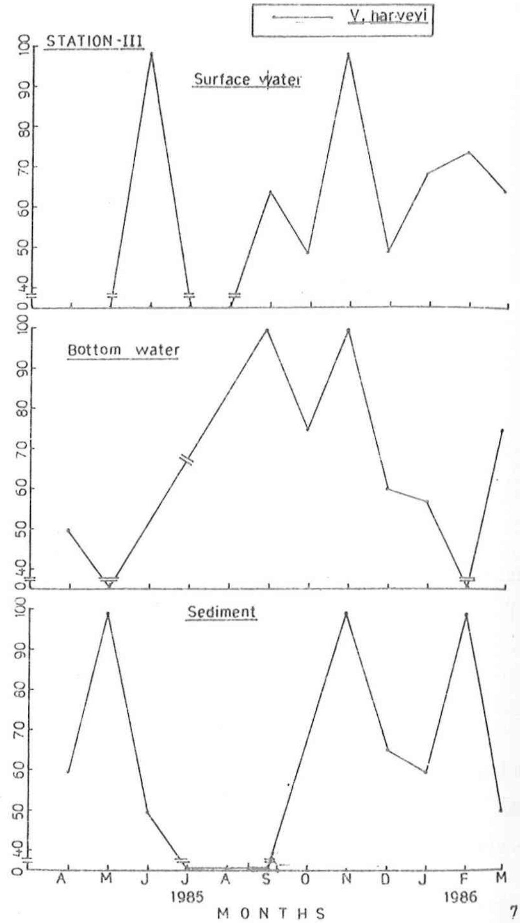

Fig. 7. Distribution (%) of *V. harveyi* in station II.

Pichavaram mangrove swamps along east coast of India, *V. harveyi* has been reported (Nair *et al.*, 1979; Ramamoorthi and Jayabalan, 1982).

In the present study, variations in the surfaee and bottom water and sediment temperature were recorded to correlate the distribution pattern of *V. harveyi.* Ruby and Nealson (1978) have noticed temperature to play a major role in the distribution of *V. harveyi* in the nearshore Californian waters. They observed the occurrence of *V. harveyi* in a very high percentage (upto 70%) during

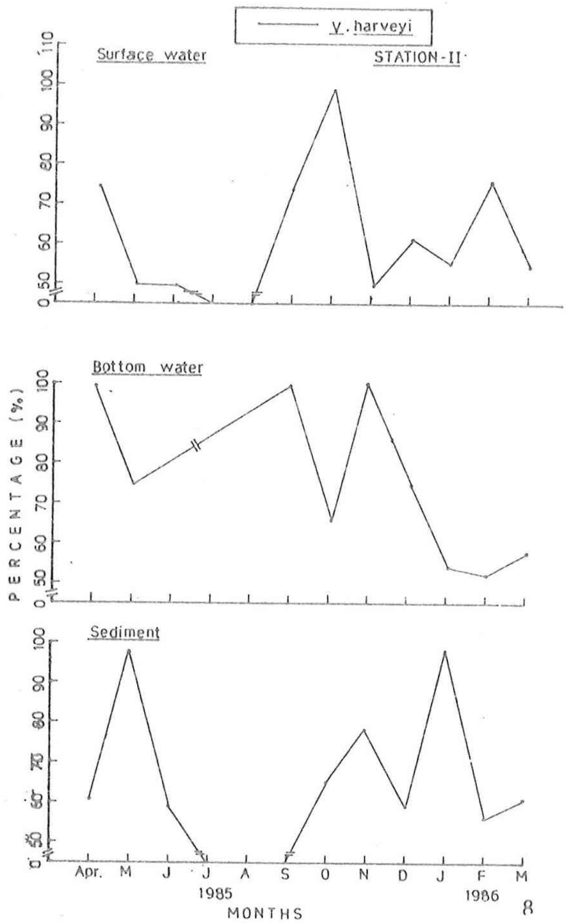

Fig. 8. Distribution (%) of *V. harveyi* in station III.

summer. It was absent during winter. Yetinson and Shilo (1978) have inferred that the temperature plays an important role in the distribution of luminescent bacteria in the Mediterranean Sea and the Gulf of Elat.

In the present study, the distribution of *V. harveyi* in relation to temperature did not show any marked variations. This might be due to marginal differences in temperature in the surface and bottom water as well as in sediment from different stations during various months. This evidently shows that in a tropical estuary like Netravathi Estuary, temperature would not play a prominent role in the seasonal fluctuations of *V. harveyi.* It has been observed that in semitropical estuarine environment, only *V. harveyi* was found in water and sediment and gastro-intestinal tract of fishes, whereas other lumuinescent bacteria were absent (O'Brien and Sizemore, 1979).

Among various species of luminescent bacteria, *V. harveyi* tolerates hypersaline conditions with a wide fluctuation in salinity ranges (Yetinson and shilo, 1978). The occurrence of *V. harveyi* in the present study in higher percentage in all the three stations both in surface and bottom waters indicates its dominance over other species. As *V. harveyi* has been reported from various biotopes like marine, estuarine, backwater and mangrove swamps (Ramamoorthi and Jayabalan, 1982), the species can be considered as a versatile form. Shilo and Yetinson (1979), have correlated the luminous bacterial populations with the local differences in salinity. The same reason may be attributed to the fluctuations of the bacterial population in different stations in the present study also. Further in support of the above, laboratory studies conducted by Eley (1982), have shown the effect of sodium chloride concentrations on the growth rate of luminous bacteria.

The influence of oxygen on the distribution pattern of luminous bacteria in an aquatic environment has so far received little attention. Nealson and Hastings (1977) observed two different patterns of growth and light emission with the stab cultures of luminous bacteria. In *V. harveyi,* the quantum of light produced was at its maximum at the surface of the agar where the oxygen level was high. Hence, they concluded that oxygen is an important parameter in controlling the synthesis of luminescent system and growth of bacteria. In the present study, it is not possible to relate the fluctuations in the population densities of *V. harveyi* with the variations in oxygen concentrations in estuarine water, as during some months *V. harveyi* was more when oxygen values were slightly less and number of bacteria was less when oxygen values were high. Similarly it is not possible to correlate the population fluctuations with pH variation.

Statistical analysis of the data shows that there was no significant difference in the distribution of *V. harveyi* between months, between stations and months. But there was a significant difference in the distribution of *V. harveyi* between stations and surface and bottom water samples at 1% level (Table 1).

In the present study, among the various environmental parameters observed, only salinity fluctuations was very wide in all the 3 stations.

In Station I where the salinity of water was higher, recorded more number of colonies followed by station II and III which recorded comparatively less salinity. Hence, it may inferred that the salinity plays a prominent role in the distribution of luminous bacteria. However, it has been observed that the surface water in a particular station recorded more colonies than the bottom water during most of the months though the salinity of bottom water was always higher than the surface water. This may be due to the combined effect of pH, oxygen, temperature and other unknown environmental parameters.

TABLE 1. *Results of analyseis of variance of the distribution of* V. harveyi *in water*

Source of variation	Degrees of Freedom	S S Q	M S S Q	F-ratio
Between Months	6	5403.1962	900.5327	1.8955
Between Stations	2	667.8190	333.9095	0.7020
Between Surface and bottom water	1	119.0819	119.0819	0.2506
Interaction between months and surface and bottom water	6	582.7395	97.1232	0.2044
Interaction between stations and surface bottom water	2	3046.4085	1523.0425	3.2058*
Interaction between months & stations	12	2295.1652	191.2637	0.4025
Error	12	5701.0343	475.0861	
Total	41	17815.4446		

* Significant at 1% level.

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