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## Note

# Density distribution of heterotrophic bacteria in the surface waters at Visakhapatnam Fishing Harbour

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**Abstract**

Heterotrophic bacteria density distribution in the surface waters at Visakhapatnam Fishing Harbour was studied from March to December 2006 at fortnightly intervals from four sampling points. Total bacteria registered a maximum density of 6.60 x $10^3$ cfu/ml. The lipolytic bacteria density (5.85 x $10^3$ cfu/ml) was relatively higher than proteolytic (2.97 x $10^3$ cfu/ml) or glycotic (3.90 x $10^3$ cfu/ml) bacterial density. Dissolved oxygen and *p*H showed all positive and all negative correlations respectively with four categories of bacteria studied. The paper discusses the impact of physico-chemical parameters on the density distribution of bacteria in the surface waters of fishing harbour.

Like all other marine organisms, heterotrophic bacteria play an important role in the marine food chain. They survive in the marine environment by obtaining nourishment from decaying organic materials and dissolved organic matter. They represent a major component of marine organisms and need nutrients like carbohydrates, proteins, lipids, etc. for growth and metabolism. Marine heterotrophic bacteria are also a potential source of many commercially important bioactive compounds. Their bioremediation capabilities are also remarkable. Earlier studies on the distribution of heterotrophic bacteria in the Indian waters indicate paucity of data on density distribution of different categories of heterotrophic bacteria in relation to ecological parameters (Velankar, 1955, 1957; Matondkar, 1981; Paliniappan and Krishnamurty, 1985; Kannan and Vasantha, 1986; Vasantha and Kannan, 1987; Alavandi, 1989; Prabhu *et al*, 1991; Ramaiah, 1994; Phillips and Permulaswamy, 1995 and Ramaiah *et al*, 1996). The present study focuses on the density distribution of different categories of heterotrophic bacteria in relation to the physico-chemical parameters in the surface waters of Visakhapatnam Fishing Harbour, in Andhra Pradesh along the east coast of India.

### Materials and methods

The investigation was carried out at Visakhapatnam Fishing Harbour (Lat 17° 42'N; Long 83° 18' E) during March to December 2006 with fortnightly sampling. The harbour waters were contaminated with fuel, fish wastes and human wastes. For the present study, investigations on the density distribution of total heterotrophic, glycolytic, proteolytic and lipolytic bacteria were carried out. Surface water samples were collected from four stations for each of these groups and were aseptically cultured in the laboratory (Colwell and Michael, 1972; Speck, 1976) at 32°C after six-fold dilution. The general bacteriological media (MM 012, HIMEDIA) was enriched with 0.2% dextrose for glycolytic bacteria; 0.4% gelatin for proteolytic bacteria culture and 0.2% of Tween 80 for lipolytic bacteria culture. The cultures, after 18 hr incubation were processed (Colwell and Michael, 1972; Speck 1976) and the colonies were counted under a binocular microscope. The densities of different categories of bacteria are expressed as .....x $10^3$cfu/ml. Simultaneously surface water samples were also collected for physico-chemical parameters. Surface water temperature was measured using a hand held (0.1°C sensitivity) thermometer. Salinity and dissolved oxygen were measured using Knudsen's method and Winkler's method respectively (Strickland and Parsons, 1972), *p*H of water sample was measured with a digital *p*H meter (Systronics). The density data were processed using 'SPSS' software 13$^{th}$ version for mean values and standard deviations. Pearson correlations were obtained for physico-chemical parameters and density distribution using the same software. The obtained correlations were tested for their significance using 't' test (Ostle, 1954).

### Results and discussion

The distribution of surface water temperature, salinity, dissolved oxygen and *p*H in the Visakhapatnam Fishing

Table 1. *Mean values and standard deviations of temperature (°C), salinity (ppt), dissolved oxygen (mg/ltr) and* pH *of Visakhapatnam Fishing Harbour surface waters. I: first fortnight; II: second fortnight)*

| Period | Temperature | | Salinity | | Dissolved oxygen | | *p*H | |
|---|---|---|---|---|---|---|---|---|
| | Mean | S.D | Mean | S.D | Mean | S.D. | Mean | S.D |
| Mar II | 31.0 | 2.13 | 32.2 | 0.17 | 6.8 | 2.23 | 7.3 | 0.39 |
| Apr I | 29.0 | 0.10 | 32.6 | 0.50 | 4.3 | 0.35 | 7.6 | 0.50 |
| Apr II | - | - | - | - | - | - | - | - |
| May I | 29.1 | 0.13 | 32.8 | 0.45 | 6.4 | 2.26 | 7.6 | 0.17 |
| May II | 28.9 | 0.26 | 29.0 | 2.04 | 4.7 | 0.26 | 8.2 | 0.35 |
| Jun I | 28.8 | 0.35 | 31.0 | 1.13 | 3.6 | 1.23 | 7.7 | 0.30 |
| Jun II | 27.0 | 0.36 | 30.7 | 0.51 | 2.6 | 0.52 | 7.3 | 0.25 |
| Jul I | 27.1 | 0.17 | 27.1 | 0.17 | 1.6 | 0.57 | 7.3 | 0.21 |
| Jul II | 27.1 | 0.43 | 34.1 | 0.17 | 2.1 | 0.30 | 8.1 | 0.55 |
| Aug I | 27.2 | 0.13 | 30.2 | 0.17 | 1.9 | 0.63 | 8.1 | 0.10 |
| Aug II | 25.9 | 0.17 | 33.0 | 0.17 | 2.5 | 1.00 | 7.3 | 0.15 |
| Sep I | 29.2 | 0.15 | 28.0 | 1.39 | 5.5 | 1.58 | 7.6 | 0.21 |
| Sep II | 27.3 | 0.17 | 29.9 | 0.56 | 6.1 | 1.32 | 7.4 | 0.14 |
| Oct I | 31.1 | 0.17 | 29.6 | 0.28 | 6.1 | 1.42 | 7.4 | 0.10 |
| Oct II | - | - | - | - | - | - | - | - |
| Nov I | 28.2 | 0.15 | 24.4 | 0.22 | 4.1 | 1.36 | 7.9 | 0.10 |
| Nov II | 28.2 | 0.05 | 28.0 | 0.13 | 4.5 | 0.53 | 7.8 | 0.32 |
| Dec I | 26.9 | 0.08 | 29.0 | 0.13 | 5.0 | 1.14 | 8.0 | 0.36 |
| Dec II | 30.0 | 0.10 | 30.0 | 0.17 | 3.7 | 0.60 | 7.7 | 0.18 |

Harbour during the study period are presented in Table 1. Table 2 provides mean density distribution (...x $10^3$ cfu/ml) of the four categories of bacteria namely total heterotrophic bacteria (TB), glycolytic bacteria (GB), proteolytic bacteria (PB) and lipolytic bacteria (LB) in the surface water during study period. Correlations between physico-chemical parameters and bacterial densities are presented in Table 3. The peak abundances of bacteria recorded in September and early December may be attributed to normal distribution of physico-chemical parameters specifically the dissolved oxygen. Consequently the dissolved oxygen showed significant positive correlation with the densities of total heterotrophic bacteria. Alavandi (1989) recorded 24.5 x $10^5$cfu/ml of heterotrophic bacteria in the coastal waters of Cochin with a peak in January and February. He attributed the peak densities to suitable salinities and temperature. But in the present study, the peak densities were recorded in September and in early December and their densities (6.60 x $10^3$ cfu/ml) were also low compared to Cochin waters. Prabhu *et al.* (1991) recorded the highest densities of 240 x $10^3$ cfu/ml) of heterotrophic bacteria in the coastal waters of Madras, which is also relatively higher than the present observations. Kannan and Vasantha (1986) and Vasantha and Kannan (1987) reported 10.9 x $10^3$ and 10.0 x $10^4$ cfu/ml of heterotrophic bacteria in the marine zones of Vellar Eastuary and Killai Backwaters of Porto Novo respectively.

Eventhough the glycolytic bacteria exhibited positive correlations with temperature, salinity and dissolved oxygen, the positive correlations are significant with respect to temperature and dissolved oxygen indicating their impact on density distribution of glycolytic bacteia. Futher the glycolytic bacterial density is also influenced by *p*H, as there exists a strong negative correlation between them. The glycolytic bacteria contributed significantly to the density of general heterotrophic bacteria during early September (1 Sep.). When compared with glycolytic and lipolytic bacteria, the proteolytic bacterial density exhibited significant positive correlations with temperature, salinity and dissolved oxygen and a significant negative correlation with *p*H. Interestingly all the four physico-chemical parameters showed significant correlations either positive or negative, with densities of proteolytic bacteria. It is further interesting to note that proteolytic bacteria did not contribute to the two peaks observed in the general heterotrophic bacteria during September and early December.

The density distribution of lipolytic bacteria revealed significant positive correlations with temperature and dissolved oxygen; and significant negative correlation with *p*H indicating the positive influence of temperature and dissolved oxygen on their densities. Lipolytic bacterial densities also contributed considerably to the peaks of general heterotrophic bacteria observed in September. But its contribution to early December (1. Dec.) peak was relatively low. Alavandi (1989) also recorded the predominance of glycolytic forms in the Cochin Backwaters. In general the peaks observed in the distribution of general heterotrophic bacteria are contributed more or less by

Table 2 . *Mean densities (... x $10^3$ cfu/ml) & standard deviations of Total Heterotrophic Bacteria (TB), Glycolytic Bacteria (GB), Proteolytic Bacteria (PB) and Lipolytic Bacteria (LB) of Visakhapatnam Fishing Harbour surface waters* (I: first fortnight; II: second fortnight)

| Period | TB | | GB | | PB | | LB | |
|---|---|---|---|---|---|---|---|---|
| | Mean | S.D | Mean | S.D | Mean | S.D | Mean | S.D |
| Mar II | 1.80 | 254.56 | 3.33 | 102.47 | 2.97 | 596.74 | 5.85 | 519.33 |
| Apr I | 3.60 | 273.86 | 3.90 | 302.77 | 2.40 | 348.81 | 2.62 | 193.11 |
| Apr II | - | - | - | - | - | - | - | - |
| May I | 1.60 | 30.00 | 1.70 | 45.27 | 1.50 | 106.81 | 1.04 | 27.28 |
| May II | 0.85 | 128.27 | 0.36 | 35.83 | 0.37 | 54.00 | 0.19 | 17.23 |
| Jun I | 0.47 | 66.27 | 0.27 | 34.81 | 1.05 | 93.77 | 0.32 | 43.92 |
| Jun II | 1.14 | 110.46 | 0.86 | 136.38 | 0.79 | 145.85 | 0.69 | 74.03 |
| Jul I | 1.10 | 59.07 | 1.49 | 108.83 | 1.09 | 75.02 | 0.81 | 40.84 |
| Jul II | 0.45 | 35.45 | 0.23 | 15.78 | 0.36 | 44.09 | 0.43 | 33.59 |
| Aug I | 0.90 | 38.26 | 0.83 | 78.23 | 0.45 | 51.93 | 0.38 | 19.90 |
| Aug II | 0.74 | 121.05 | 0.58 | 37.74 | 0.54 | 64.44 | 0.70 | 45.30 |
| Sep I | 6.60 | 698.69 | 3.30 | 561.64 | 0.45 | 150.00 | 2.18 | 249.58 |
| Sep II | 5.80 | 800.75 | 1.09 | 203.44 | 0.44 | 252.68 | 2.21 | 208.62 |
| Oct I | 1.10 | 136.38 | 1.06 | 204.44 | 1.81 | 173.85 | 1.90 | 300.16 |
| Oct II | - | - | - | - | - | - | - | - |
| Nov I | 1.90 | 123.49 | 1.70 | 425.32 | 0.34 | 85.06 | 1.88 | 123.60 |
| Nov II | 1.98 | 5.15 | 0 | 0.00 | 0.18 | 9.15 | 0.13 | 13.12 |
| Dec I | 6.03 | 626.18 | 1.08 | 18.00 | 0.45 | 45.30 | 1.49 | 182.54 |
| Dec II | 1.90 | 113.58 | 0.43 | 12.96 | 0.49 | 45.91 | 1.27 | 157.64 |

the three groups *i.e.* glycolytic, proteolytic, and lipolytic bacteria except the peak in early December (Dec I). During early December, general heterotrophic bacteria registered a density of 6.03 x $10^3$ cfu/ml, while the density of glycolytic bacteria (1.08 x $10^3$ cfu/ml), proteolytic bacteria (0.44 x $10^3$ cfu/ml) and lipolytic bacteria (1.49 x $10^3$ cfu/ml) put together was only 3.01 x $10^3$ cfu/ml. This indicates that during early December (Dec I), the general heterotrophic bacteria density may also be contributed by other groups of bacteria. (*i.e.* non-glycolytic, non-proteolytic and non-lipolytic forms).

The present study recorded the occurrence of low densities of heterotrophic bacteria in the fishing harbour waters. The dominance of lipolytic bacteria in the water samples indicates abundant availability of lipids (oils & others), compared to protein in the harbour waters. The low abundance of proteolytic bacteria in water samples indicates the fish wastes (which are more proteinaceous) did not contribute significantly to proteolytic bacteria in the surface water, which may be due to their utilization as food by other marine animals or may be contributed to harbour sediments or carried away by the coastal circulation processes to other habitats.

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Table 3. *Pearson correlations between physio-chemical parameters and bacterial densities during study period (N=17) (*indicates significant at p=0.05)*

| Bacteria | Temperature | Salinity | Dissolved oxygen | *p*H |
|---|---|---|---|---|
| General Bacteria | -0.065 | -0.216* | 0.397* | -0.039 |
| Glycolytic Bacteria | 0.384* | 0.031 | 0.412* | -0.354* |
| Proteolytic Bacteria | 0.603* | 0.391* | 0.419* | -0.497* |
| Lipolytic Bacteria | 0.549* | 0.091 | 0.609* | -0.427* |

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