

BACTERIAL (FREE LIVING AND PARTICLE BOUND) CELL-SIZE IN THE SURFACE WATERS OF RIVER CAUVERY AND ITS UPSTREAM TRIBUTARIES IN KARNATAKA STATE, INDIA

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Abstract. Cell-size here refers to mean length, as only the lengths of bacterial cells were measured in this study. Cell breadths were not measured, hence calculation of biovolume and biomass was not possible. In this study, the mean cell-lengths of heterotrophic bacterioplankton as well as their relation with environmental (water-quality) variables were analyzed for two years in the main river Cauvery and its four important upstream tributaries from February 2000 to January 2002. The initial hypothesis that all the five water courses have similar bacterial mean cell-lengths was rejected, because mean cell-lengths of free living and particle bound planktonic bacteria was more and was also significantly different in the river Lakshmanatheertha, when compared to the other four water courses studied. Season-wise grouped data revealed that, the mean cell-length of free-living bacteria was significantly less in winter season as compared to rainy and summer seasons during the second year of study only in the river Lakshmanatheertha. A correlation (*r*) analysis between the mean cell-lengths of heterotrophic bacteria and environmental (water-quality) variables revealed significant relations. Also with the help of regression analysis (*r*²) the effect of some important environmental variables on the mean cell-length of heterotrophic bacteria has been discussed in the light of recent investigations in the field of fresh water microbial ecology.

Keywords: *bacterial cell length, planktonic food web, environmental factors, bacterial production*

Introduction

Heterotrophic bacteria are accounted for the most important proportion of decomposers in the aquatic ecosystems, which are responsible for key process regulating the function and productivity of ecosystem through the microbial loop [3]. Heterotrophic bacteria in the fresh water ecosystem form a major part of the food web and mediate important processes in the carbon budget [12]. This food web plays an important role in the regulation of carbon transfer [26]. As in both fresh water and marine waters, the biomass and size distribution of bacteria are important parameters of ecosystem function. Given the important ecological role of bacterial factors, regulating the productivity (growth rate) and biomass (abundance and cell-size) of these communities are of interest. Bacterial cell-size may be related to metabolic state, as large bacterial cells are often the most active [27]. The role of large bacteria in the river is important as competitors; large bacteria limit the development of small ones, and also play a buffer role in the degradation of organic matter [14 and 26]. Further, the large allochthonous bacteria brought by the sewage are active in the river and able to grow in culture at rate twice those of smaller bacteria [14]. Use of radiolabeled thymidine and leucine incorporation techniques [4] showed lower metabolic activity for smaller-sized soil bacteria. A sharp and drastic decrease in the concentration and changes in composition of organic substrates may lead to reduction of bacterial cell-size. Reduction

or small sizes are said to be an adaptive mechanism of bacteria under starvation. Small cell-size has advantages, e.g., increased surface volume ratio, allowing a higher substrate incorporation rate per unit of biomass [47] and protection from zooplankton grazing in comparison to larger cells [21]. Furthermore, small or miniature bacteria can be obtained experimentally by starvation in culture [24], which implies morphological and physiological modifications. Thus, it is possible that cell-size diversity of the bacterioplankton corresponds to some aspects of the physiological state of the population, and may have a meaningful ecological role in the planktonic food-web [31]. The cell-size of planktonic bacteria is an important feature in the predator-prey relationship between bacteria and protistan predators, because grazing by predators is size selective and causes a profound shift in bacterial cell-size distribution [41, 36 and 6]. Larger cells are more likely to be grazed by zooplankton. After grazing of larger cells, the medium sized bacteria are then used as a food source [6]. Predation by bacterivorous protists in aquatic habitats can influence the morphological structure and physiological status of the bacterial communities [21]. However, in some habitats and seasons, metazoan grazing or lysis by phages may play an important factor in controlling bacterial size mortality [47]. Nevertheless, the effects of predation in the natural environment are difficult to demonstrate, as many other factors can affect the bacterioplankton cell-size distribution [9]. These factors include growth rate, temperature, oxic/anoxic conditions [10], resuspension [45], cell lysis initiated by abrasion, starvation or infected by viruses [27]. As far as authors know, only few studies are available in the literature on size spectra of planktonic bacteria. Examples are studies on marine plankton in the Mediterranean [38], in sediments of brackish water [11] and in sediments of Botany Bay [31]. Few studies analyzed the seasonal changes in abundance and cell size of heterotrophic bacteria in fresh water lakes, [5], on the effect of detrital addition on bacterial cell size [17], on bacterial biomass and cell size distribution [10], on role of cell size in microbial loop [9]. Further more in reservoir, specific bacterial cell size and size selective grazing [19 and 21] was also carried out. Few research studies on rivers documented that, physiological characteristic and ecological role of small and large sized bacteria in polluted rivers [14], on bacterial cell-size in three lowland water courses of North-East England [49], and on microbial food web with respect to diel fluctuation in bacterial biomass [26]. So far there are no studies on the mean cell-length of planktonic bacteria in river Cauvery and its important tributaries like Lakshmanatheertha, Harangi, Hemavathy and Lokapavani in Karnataka state, India. Hence, this investigation on the mean cell-lengths of planktonic bacteria was undertaken. The aims of this investigation were 1) to study and compare the mean cell-lengths of planktonic bacteria in the rivers Lakshmanatheertha, Harangi, Hemavathy, Lokapavani and Cauvery. 2) to test the initial hypothesis that, the mean cell-lengths of planktonic bacteria in four upstream tributaries are similar to each other, but are markedly different in the main river Cauvery. 3) to investigate the relationships between mean cell-lengths of planktonic bacteria and other microbial and water quality variables. 4) to know, the potential control of mean cell-lengths of planktonic bacteria by relevant water quality (bottom-up or nutrient) and by predation (top-down control or grazing) and/or by both.

Materials and methods

Collection and preservation of samples

The sampling sites on the main river Cauvery and its four important upstream tributaries are shown in (Fig 1.) Mid stream surface water samples were collected from the river Lakshmanatheertha at Kattemalawadi Anicut (site-1, Latitude $12^{\circ} 17''\text{N}$ and Longitude $76^{\circ} 17''\text{E}$) near Hunsur town, river Harangi at Chunchanakatte village (site-2, Latitude $12^{\circ} 50''\text{N}$ and Longitude $76^{\circ} 05''\text{E}$), river Hemavathy near Hampapura village (site-3, Latitude $12^{\circ} 40''\text{N}$ and Longitude $76^{\circ} 45''\text{E}$), river Lokapavani at Bapurayanakoppal (site-4, Latitude $12^{\circ} 25''\text{N}$ and Longitude $76^{\circ} 41''\text{E}$) and the main river Cauvery near Mahadevapura village (site-5, Latitude $12^{\circ} 25''\text{N}$ and Longitude $76^{\circ} 41''\text{E}$) between 06.30 AM and 12.30 PM, at about fortnightly intervals, from February 2000 to January 2002.

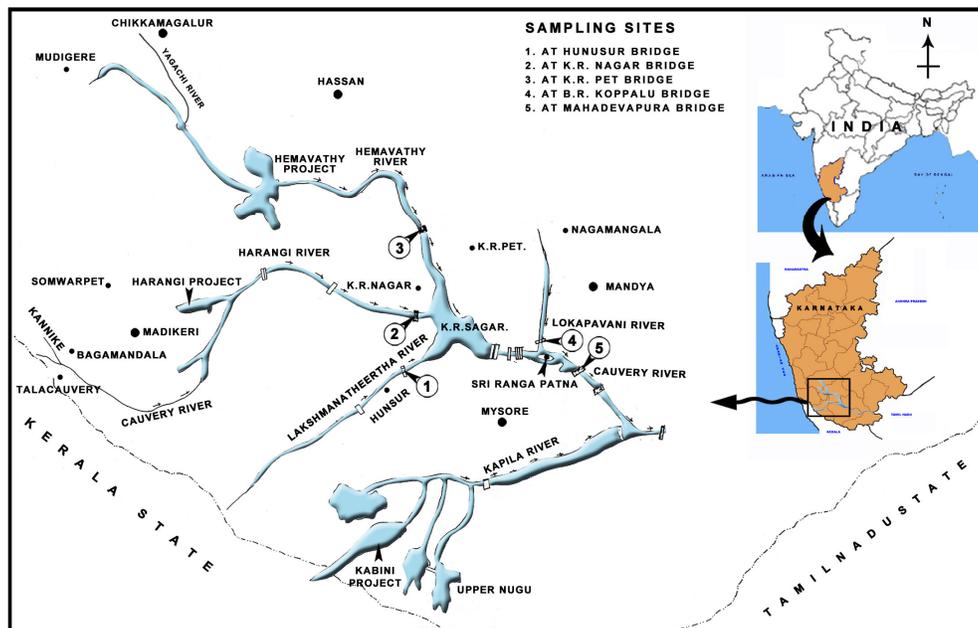


Figure 1. Map showing sampling sites on the river Cauvery and its four important upstream tributaries in Southern Karnataka State

Water samples were collected in sterile glass bottles from all the five water courses. Ten ml of water sample from each site were preserved with 2% final concentration of 0.22 μm filtered neutral formalin [23] immediately after collection. Preservation was not for more than 3-4 days before microscopical observations as sizes may change (due to cell shrinkage) on fixing for longer periods [13]. Bacteria were then stained with Acridine Orange concentrated on black 0.22 μm polycarbonate membrane filters and examined under the Epifluorescence microscope. For the analysis of other water quality variables, water samples were collected in clean polythene bucket and transferred to 5 litre capacity polythene containers. Detailed methodology followed was based on APHA [2] and as described in Yamakanamardi [49].

Measurement of mean length of planktonic bacteria

The length of 100 to 150 free living bacteria (FLB) and 100 -150 particle bound bacteria (PBB) from each water sample were measured directly from the microscopic images using G-12 eyepiece graticule at magnification 1000X [33]. The graticule contained two series of circles i.e. clear and black of increasing diameter. These circles were overlaid on each bacterium chosen without bias, so that cell length was given by the appropriate circle diameters [49]. The bacteria were placed in ten length categories that increased in a root 2 progressions from $< 0.44 \mu\text{m}$ to $>7\mu\text{m}$. The ten size categories were; 1= $< 0.44\mu\text{m}$, 2= $0.44 - 0.53\mu\text{m}$, 3= $0.53 - 0.75\mu\text{m}$, 4= $0.75 - 1.06\mu\text{m}$, 5= $1.06 - 1.495\mu\text{m}$, 6= $1.495 - 2.125\mu\text{m}$, 7= $2.125 - 3.0\mu\text{m}$, 8= $3.0 - 4.25\mu\text{m}$, 9= $4.25 - 6.0\mu\text{m}$ and 10= $>7\mu\text{m}$. Mean length of both FLB and PBB were calculated, by ascribing lengths which were the mid point of the maximum and minimum lengths in 2-9 category. Cells in category 1 were treated as being $0.44\mu\text{m}$ while cells in category 10 were taken as $7\mu\text{m}$.

Estimation of percentage of bacterial cells in each size category

The percentage of FLB and PBB cell in each size category was calculated by dividing the number of bacterial cell in each size category by the total number of cells sized and then multiplied by 100.

Statistical analysis

All the statistical analysis were carried out using SPSS for Window release 6.0 [35]. The Kolmogorov-Smirnov test was used to test for agreement with the normal distribution. Distribution of many variables were found to differ significantly ($p < 0.05$) from the normal distribution. Therefore, values for all variables were scaled, if necessary and then \log_{10} transformed. Student-Newman-Keuls one – way ANOVA post hoc test was applied for making multiple comparisons among the means. Correlations were examined using Pearson's Correlation coefficients. Values of Pearson's correlation coefficients, calculated after \log_{10} transformation, were generally used to help interpret the results. Further, multiple regression analysis was also used with bacterial variables as dependent variables and environmental variables as independent variables. Variable were entered into the equation using the stepwise entry method, with p in set at 0.05 and p out set at 0.1.

Results

Cell size here refers to mean length, as only the lengths of bacterial cells were measured in this study. These are related to cell volume and biomass, although not linearly. Cell breadths were not measured, hence calculation of biovolume and biomass was not possible. In this study bacterial cell size (mean length) and size category distribution was measured over a period of two years (Feb. 2000 – Jan. 2002). The results are as follows.

Mean length of free-living bacteria

Summary of the overall mean cell length of free-living bacteria measured was similar in the river Cauvery (Mean $2.17 \mu\text{m}$, range $1.41 - 3.04 \mu\text{m}$), river Harangi (Mean

2.07 μm , range 1.22 - 3.24 μm), river Hemavathy (Mean 2.02 μm , range 1.15 - 3.24 μm), and in river Lokapavani (Mean 2.15 μm , range 1.49 - 3.55 μm), but it was more and also significantly different in the river Lakshmanatheertha (Mean 2.56 μm , range 1.36 - 3.80 μm) (Table 1). Mean values with different superscripts are significantly different ($p < 0.05$) as shown by one-way ANOVA post hoc non-parametric Student-Newman-Keuls test (SNK test) is also shown in this table.

Since, Indian season is mainly controlled by Monsoon climate, the seasonal study year (February-January) was divided into three well marked seasons viz. Pre-monsoon or summer (February-May), Monsoon or Rainy (June- September) and Post-monsoon or winter (October-January). Thus, the statistical analysis (Mean \pm SD and F&P values obtained through ANOVA test) of the season wise grouped data of all the five water courses was carried out. In the river Lakshmanatheertha, during the second year of study the mean cell-length of free-living bacteria was significantly less in winter season than in rainy and summer seasons, both of which were similar when compared to other four water courses studied. Temporal variation of mean length of free-living bacterial cells in the river Lakshmanatheertha show more temporal fluctuations in the Pre-monsoon season of the first year and in all the five water courses during the Pre-monsoon and Monsoon seasons of the second year of study. In contrast, in all the five rivers there was greater fluctuation in the mean cell-lengths of free-living bacteria and there was a seasonal pattern, seemingly with smaller cells in winter than in summer (Fig. 2).

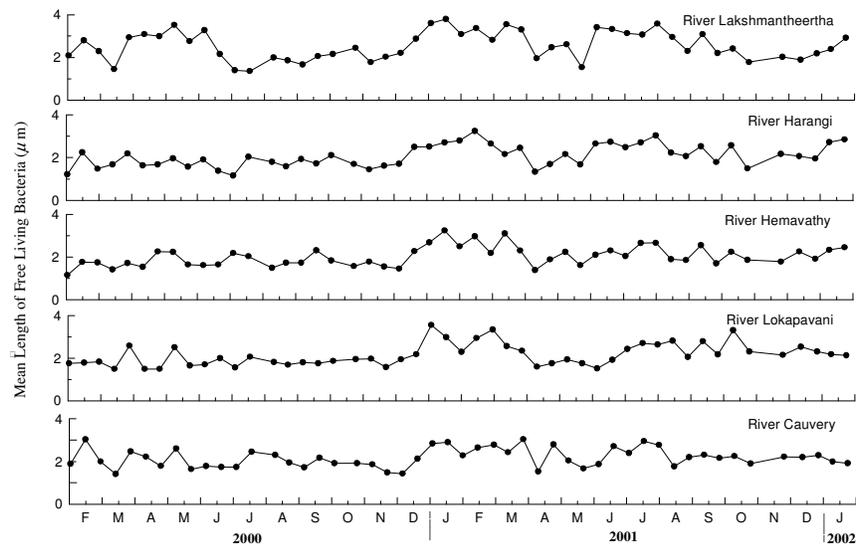


Figure 2. Seasonal changes in Free living bacterial cell size in the surface water of river Cauvery, Lokapavani, Hemavathy, Harangi and Lakshmanatheertha, February 2000 – January 2002.

The correlation between bacterial cell-size and other microbial variables is shown in (Table 2.) In the river Lakshmanatheertha the mean cell-size of free-living bacteria showed positive correlations with abundance of free living bacteria, particle bound bacteria, and total bacteria, zooplankton and mean cell-length of particle bound bacteria

and negatively correlated with Colony Forming Units as % of Acridine Orange Direct Counts (CFUs as % of AODCs). In river Harangi, the mean cell-length of free-living bacteria was correlated with mean cell-length of particle bound bacterial. In river Hemavathy, the mean cell-length of free-living bacteria was correlated with specific growth rate, zooplankton and mean length of particle bound bacteria. In river Lokapavani, the mean cell-length of free-living bacteria was correlated only with the mean length of particle bound bacteria. However, in river Cauvery, the mean cell-length of free-living bacteria was positively correlated with mean length of particle bound bacterial cells and negatively with zooplankton among bacterial variables (Table 2). The correlation between bacterial cell size and environmental variables is shown in (Table 3). The concentration of mean cell size of free-living bacteria was positively correlated with conductivity, chloride, sulphate and total anions of strong acids, and negatively correlated with the surface water velocity and dissolved oxygen in the river Lakshmanatheertha, positively correlated with dissolved oxygen in the river Harangi, and with total anions of strong acids in the river Hemavathy among the environmental variables (Table 3).

Mean length of particle bound bacteria

Summary of the overall mean cell-lengths of particle bound bacteria was similar in the river Harangi (Mean 1.70 μm , range 1.10 - 2.69 μm), river Hemavathy (Mean 1.71 μm , range 1.13 - 3.32 μm), river Lokapavani (Mean 1.84 μm , range 1.18 - 3.27 μm), and in river Cauvery (Mean 1.85 μm , range 1.23 - 2.90 μm), but it was more and also significantly different in the river Lakshmanatheertha (Mean 2.05 μm , range 1.21 - 3.01 μm) (Table 1). Temporal variation of mean length of particle bound bacterial cells did not show any obvious seasonal pattern in all the five water courses during both the year of study (Fig. 3).

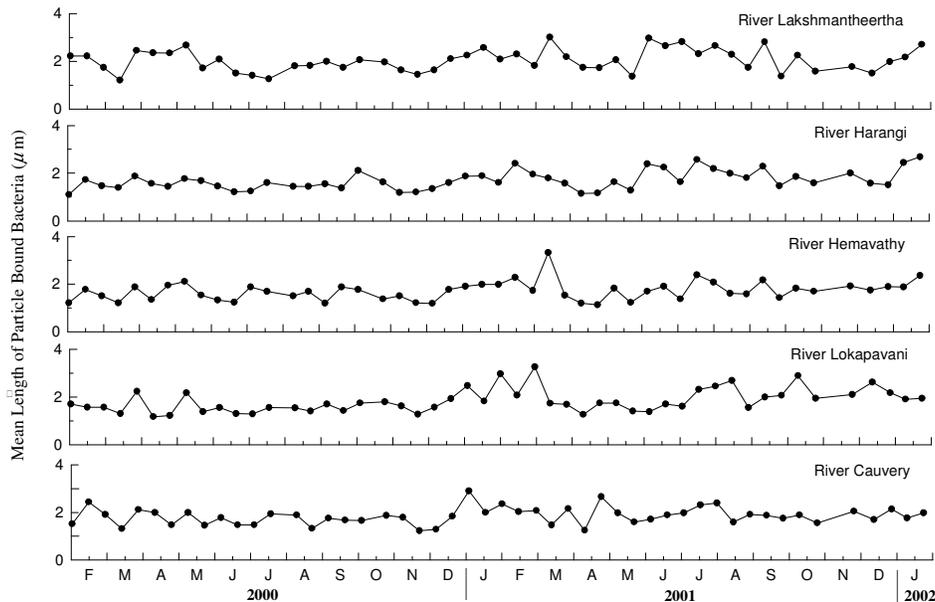


Figure 3. Seasonal changes in Particle Bound bacterial cell size in the surface water of river Cauvery, Lokapavani, Hemavathy, Harangi and Lakshmantheertha, February 2000 – January 2002.

Table 1. Summary of the overall mean cell-length of bacteria (μm) in the surface waters from Rivers Lakshmanatheertha, Harangi, Hemavathy, Lokapavani and Cauvery: Seasonal study, February 2000 to January 2002.

		MLFLB (μm)	MLPBB (μm)
River Lakshmanatheertha	Mean	02.56 ^b	02.05 ^b
	Range	01.36 - 03.80	01.21 - 03.01
	CV (%)	26	23
River Harangi	Mean	02.07 ^a	01.70 ^a
	Range	01.22 - 03.24	01.10 - 02.69
	CV (%)	25	23
River Hemavathy	Mean	02.02 ^a	01.71 ^a
	Range	01.15 - 03.24	01.13 - 03.32
	CV (%)	23	24
River Lokapavani	Mean	02.15 ^a	01.84 ^a
	Range	01.49 - 03.55	01.18 - 03.27
	CV (%)	24	26
River Cauvery	Mean	02.17 ^a	01.85 ^a
	Range	01.41 - 03.04	01.23 - 02.90
	CV (%)	20	19

Mean Values with different superscripts are significantly different ($P < 0.05$, Student-Newman-Keuls test, after \log_{10} transformation).

CV= Coefficient of Variation, MLFLB= Mean length of Free Living Bacteria, MLPBB= Mean length of Particle Bound Bacteria.

In the river Lakshmanatheertha the mean cell-size of particle bound bacteria showed correlations with abundance of free living bacteria, particle bound bacteria, and total bacteria, colony forming units, zooplankton and mean cell-length of free living bacteria. In river Hemavathy, the mean cell-size of particle bound bacteria was correlated with specific growth rate, zooplankton and mean length of free living bacteria. However, in other three water courses, the mean cell-length of particle bound bacteria correlated only with mean cell-length of free living bacteria among bacterial variables (*Table 2*). The concentration of mean cell size of particle bound bacteria was positively correlated with sulphate, phosphate and negatively with surface water velocity and dissolved oxygen in river Lakshmanatheertha, positively correlated with dissolved oxygen and negatively correlated with chlorophyll-a in river Harangi, positively correlated with chemical oxygen demand in river Hemavathy, dissolved oxygen, biological oxygen demand and chemical oxygen demand in river Lokapavani among the environmental variables (*Table 3*).

Percentage of free-living bacterial cells in each category

The overall percentage of free-living bacterial cells in each category was similar in the river Lakshmanatheertha (Mean 13.10, range 11.37 – 16.67 %), river Harangi (Mean 13.53, range 12.49 – 20.00 %), river Hemavathy (Mean 13.39, range 12.47 – 20.00 %), river Lokapavani (Mean 13.04, range 12.49 – 16.67 %), and in the river Cauvery (Mean 12.98, range 12.08 – 16.67 %). Notably, 11.37% in the river Lakshmanatheertha and 20.0% in the rivers Harangi and Hemavathy were the lowest and highest recorded percentage of free-living bacterial cells among the five water courses studied (*Table 4*).

Table 2. Relationships between mean cell-length of bacteria (μm) and other bacterial variables, February 2000 to January 2002

Sampling sites	DC-FLB	DC-PBB	DC-TB	CFUs	%CCFUs	CFUs as % of AODCs	SGR	Phytoplankton	Zooplankton	Total Plankton	ML-FLB	ML-PBB
Free Living Bacteria												
River Lakshmanatheertha	0.56***	0.51***	0.56***	NS	NS	-0.40**	NS	NS	0.43**	NS	-	0.84***
River Harangi	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	0.84***
River Hemavathy	NS	NS	NS	NS	NS	NS	0.43**	NS	0.31*	NS	-	0.82***
River Lokapavani	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	0.85***
River Cauvery	NS	NS	NS	NS	NS	NS	NS	NS	-0.29*	NS	-	0.83***
Particle Bound Bacteria												
River Lakshmanatheertha	0.40**	0.41**	0.41**	0.38*	NS	NS	NS	NS	0.36*	NS	0.84***	-
River Harangi	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.84***	-
River Hemavathy	NS	NS	NS	NS	NS	NS	0.34*	NS	0.29*	NS	0.82***	-
River Lokapavani	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.85***	-
River Cauvery	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.83***	-

DC-FLB= Directly Counted Free Living Bacteria, DC-PBB= Directly Counted Particle Bound Bacteria, DC-TB= Directly Counted Total Bacteria, CFUs = Colony Forming Units, CCFUs =Chromogenic Colony forming Units, CFUs as % AODCs= Colony Forming Units as Percentage of Acridine Orange Direct Counts, SGR=Specific Growth Rate, ML-FLB= Mean Length of Free Living Bacteria, ML-PBB = Mean Length of Particle Bound Bacteria.

Table 3. Relationships between mean cell-length of bacteria (μm) and environmental variables, February 2000 to January 2002

Sampling Sites	pH (F)	pH (L)	Temp	Cond	Turb	SWV	RF	DO	BOD	COD	CO ₂	Cl ₂	NO ₃	SO ₄	TASA	Cal	PO ₄	TSS	POM	Chl-a
Free Living Bacteria																				
River Lakshmanatheertha	NS	NS	NS	0.30*	NS	-0.43**	NS	-0.32*	NS	NS	NS	0.30*	NS	0.31*	0.34*	NS	NS	NS	NS	NS
River Harangi	NS	NS	NS	NS	NS	NS	NS	0.36*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Hemavathy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.31*	NS	NS	NS	NS	NS
River Lokapavani	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Cauvery	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Particle Bound Bacteria																				
River Lakshmanatheertha	NS	NS	NS	NS	NS	-0.33*	NS	-0.32*	NS	NS	NS	NS	NS	0.36*	NS	NS	0.29*	NS	NS	NS
River Harangi	NS	NS	NS	NS	NS	NS	NS	0.32*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.29*
River Hemavathy	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.33*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Lokapavani	NS	NS	NS	NS	NS	NS	NS	0.40**	0.32*	0.28*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Cauvery	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

pH (F) = pH measured in the field, pH (L) = pH measured in the laboratory, Temp= Temperature, Cond= Conductivity, Turb= Turbidity, SWV= Surface Water Velocity, RF= Rainfall, DO= Dissolved Oxygen measured in the Field, BOD= Biological Oxygen Demand, COD= Chemical Oxygen Demand, CO₂= Free Carbon di-Oxide, Cl₂= Chloride, NO₃=Nitrate, SO₄= Sulphate, TASA= Total Anions of Strong Acids, Cal= Calcium, PO₄= Inorganic Phosphate, TSS= Total Suspended Solids, POM= Particulate Organic Matter, Chl-a=Chlorophyll-a.

Table 4. Summary of the overall percentage of bacterial cells in each size category (%) in the surface waters from River Lakshmanatheertha, Harangi, Hemavathy, Lokapavani and Cauvery: Seasonal study, February 2000 to January 2002.

Microbial variables	River Lakshmanatheertha		River Harangi		River Hemavathy		River Lokapavani		River Cauvery	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
FLB (%)	13.10 ^a	11.37 – 16.67	13.53 ^a	12.49 – 20.00	13.39 ^a	12.47 – 20.00	13.04 ^a	12.49 – 16.67	12.98 ^a	12.08 – 16.67
PBB (%)	13.39 ^a	12.40 – 20.00	14.63 ^b	12.49 – 20.00	14.39 ^b	12.49 – 20.00	13.83 ^{ab}	12.25 – 16.67	13.33 ^a	12.34 – 20.00

Mean Values with superscripts are significantly different (P<0.05, Student-Newman-Keuls test, after log₁₀ transformation).

Season wise grouped data revealed that, the percentage of free-living bacterial cells in each size category was more and also significantly different in summer season as compared to rainy and winter seasons, both of which were similar in all the five rivers, during second year of study only (Table 5). The most frequent free-living bacterial cell-size categories in all the five water courses were 0.75 - 7.00 µm. Thus in river Lakshmanatheertha the 1.06 - 3.00 µm size categories were most frequent on 50 times out of 50 sampling days, followed by 7.00 µm (49 times), 6.00 µm (47 times), 4.25 µm (45 times) and 0.75 µm (42 times). In the river Harangi, the size category 1.06 – 3.00 µm, occurred all the 50 sampling days, followed by 0.75 µm (48 times), 4.25 µm (46 times), 7.00 µm (42 times) and 6.00 µm (38 times). In the river Hemavathy, the size category, 1.06 – 2.125 µm, occurred all the 50 sampling days, followed by 3.00 µm (49 times), 0.75 µm (47 times), 4.25 µm (45 times), 7.00 µm (43 times) and 6.00 µm (42 times). In the river Lokapavani, the most frequent category, 1.06 – 2.125 µm, occurred all the 50 times, followed by 3.00 - 4.25 µm (49 times), 0.75 µm (48 times), 7.00 µm (47 times) and 6.00 µm (43 times). Similarly, in the river Cauvery, the most frequent size category, 1.06 – 3.00 µm, occurred all the 50 sampling days, followed by 4.25 µm (49 times), 0.75 µm (48 times), 7.00 µm (47 times) and 6.00 µm (43 times). Whereas, the size category 6.00 µm occurs least in the rivers Harangi, Hemavathy, Lokapavani and Cauvery, while, the size category 0.75 µm was the least in the river Lakshmanatheertha (Table 7).

Percentage of particle bound bacterial cells in each category

The overall percentage of particle bound bacterial cells in each category was similar in the river Lakshmanatheertha (Mean 13.39, range 12.40 – 20.00 %), river Lokapavani (Mean 13.83, range 12.25 – 16.67 %), and in river Cauvery (Mean 13.33, range 12.34 – 20.00 %), but was more and also significantly different in the river Harangi (Mean 14.63, range 12.49 – 20.00 %), and in river Hemavathy (Mean 14.39, range 12.49 – 20.00 %). However, the percentage of particle bound bacterial cells was similar in the river Harangi, Hemavathy and in river Lokapavani also. The 12.25 % in the river Lokapavani and 20.0% in the rivers Lakshmanatheertha, Harangi, Hemavathy and Cauvery were the lowest and highest recorded percentage of particle bound bacterial cells among the five water courses studied (Table 4). Season wise grouped data revealed that, except in the river Lakshmanatheertha, the percentage of particle bound bacterial cells in each size category was more and also significantly different during summer

season as compared to rainy and winter seasons in other water courses studied during second year of study only (Table 6).

The distribution of particle-bound bacteria between the size categories on each sampling day is given in the Table 5. All the particle bound bacterial size categories such as 0.75 - 7.00 μm occurs more frequently in all the five water courses. Thus in river Lakshmanatheertha the 1.06 - 3.00 μm size categories was most frequent on 50 times out of 50 sampling days, followed by 0.75 μm (48 times), 4.25 μm to 7.00 μm (44 times). In river Harangi, 1.06 - 2.125 μm size category, occurred all the 50 sampling days, followed by 0.75 μm and 3.00 μm (49 times), 7.00 μm (38 times), 4.25 μm (35 times) and 6.00 μm (29 times). In river Hemavathy, 1.06 - 2.125 μm , occurred all the 50 sampling days, followed by 0.75 μm and 3.00 μm (49 times), 4.25 μm (41 times), 7.00 μm (36 times) and 6.00 μm (31 times). Similarly in river Lokapavani, 1.06 - 2.125 μm , occurred all the 50 sampling days, followed by 0.75 μm (48 times), 3.00 μm (47 times), 4.25 μm (42 times), 7.00 μm (41 times) and 6.00 μm (39 times). In river Cauvery, the most frequent size category, 0.75 - 2.125 μm , occurred all the 50 sampling days, followed by 3.00 μm (49 times), 7.00 μm (46 times), 4.25 μm (45 times), and 6.00 μm (39 times). Of particular size category 6.00 μm occurred least time in all the five water courses (Table 7).

Table 5. Seasonal variation in the % free living bacterial size category.

Sl. No.	Sampling sites	Pre-Monsoon (Summer)	Monsoon (Rainy)	Post-Monsoon (Winter)	F-value ¹	P-value ¹
February 2000 - January 2001						
1	River Lakshmanatheertha	12.84 ^a	12.41 ^a	13.72 ^a	2.529	0.1021 ^{NS}
2	River Harangi	15.21 ^a	13.34 ^a	14.19 ^a	1.734	0.1989 ^{NS}
3	River Hemavathy	13.93 ^a	13.52 ^a	14.00 ^a	0.1617	0.8524 ^{NS}
4	River Lokapavani	13.63 ^a	13.47 ^a	12.50 ^a	1.7903	0.1894 ^{NS}
5	River Cauvery	12.90 ^a	13.17 ^a	13.07 ^a	0.1858	0.8317 ^{NS}
February 2001 - January 2002						
1	River Lakshmanatheertha	14.14 ^a	12.70 ^b	12.74 ^b	6.490	0.0064 [*]
2	River Harangi	13.17 ^a	12.50 ^b	12.50 ^b	4.2233	0.0287 [*]
3	River Hemavathy	13.53 ^a	12.69 ^b	12.50 ^b	5.8298	0.0097 [*]
4	River Lokapavani	13.61 ^a	12.50 ^b	12.50 ^b	11.7183	0.0004 ^{***}
5	River Cauvery	13.76 ^a	12.50 ^b	12.50 ^b	3.5685	0.0463 [*]

Values are Mean, ¹value obtained from ANOVA post hoc nonparametric test. * = Significant, $p < 0.05$, NS = Non Significant, $p > 0.05$. Mean values with different superscripts are significantly different ($p < 0.05$, Student-Newman-Keuls test).

Table 6. Seasonal variation in the % particle bound bacterial size category.

Sl. No.	Sampling sites	Pre-Monsoon (Summer)	Monsoon (Rainy)	Post-Monsoon (Winter)	F-value ¹	P-value ¹
February 2000 – January 2001						
1	River Lakshmanatheertha	12.97 ^a	14.63 ^a	13.56 ^a	1.7769	0.2017 ^{NS}
2	River Harangi	15.42 ^a	15.66 ^a	15.05 ^a	0.1224	0.8853 ^{NS}
3	River Hemavathy	14.66 ^a	15.67 ^a	15.88 ^a	0.5884	0.5635 ^{NS}
4	River Lokapavani	14.35 ^a	14.80 ^a	13.53 ^a	0.9589	0.3981 ^{NS}
5	River Cauvery	13.82 ^a	13.47 ^a	13.69 ^a	0.0640	0.9381 ^{NS}
February 2001 - January 2002						
1	River Lakshmanatheertha	13.69 ^a	12.50 ^a	13.08 ^a	2.0629	0.1524 ^{NS}
2	River Harangi	15.96 ^a	12.50 ^b	13.10 ^b	11.5511	0.0004 ^{***}
3	River Hemavathy	14.93 ^a	12.50 ^b	12.50 ^b	6.5579	0.0061 [*]
4	River Lokapavani	15.03 ^a	12.72 ^b	12.50 ^b	11.9459	0.0003 ^{***}
5	River Cauvery	13.91 ^a	12.50 ^b	12.50 ^b	7.9071	0.0028 ^{**}

Values are Mean, ¹value obtained from ANOVA post hoc nonparametric test. * = Significant, p<0.05, NS = Non Significant, p>0.05. Mean values with different superscripts are significantly different (p<0.05, Student-Newman-Keuls test).

Table 7. Appearance of Planktonic bacterial cell-size in each size category out of 50 times.

Name of the river	0.75µm	1.06 - 2.125 µm	3.00 µm	4.25 µm	6.00 µm	7.00 µm
1 Lakshmanatheertha	42	50	50	45	47	49
2 Harangi	48	50	50	46	38	42
3 Hemavathy	47	50	49	45	42	43
4 Lokapavani	48	50	49	49	43	47
5 Cauvery	48	50	50	49	43	47
Name of the river	0.75µm	1.06 - 2.125µm	3.00 µm	4.25 µm	6.00 µm	7.00 µm
1 Lakshmanatheertha	48	50	50	44	44	44
2 Harangi	49	50	49	35	29	38
3 Hemavathy	49	50	49	41	31	36
4 Lokapavani	48	50	47	42	39	41
5 Cauvery	50	50	49	45	39	46

Discussion

This is the first comprehensive study of the bacterial cell-sizes in the river Cauvery and its four important tributaries in South Karnataka, India. Cell size here refers to mean length, as only the lengths of bacterial cells were measured in this study. These are related to cell volume and biomass, although not linearly. Cell breadths were not measured, hence calculation of biovolume and biomass was not possible. In this study

bacterial cell size (mean length) and size category distribution was measured over a period of two years (Feb. 2000 – Jan. 2002). The size of bacteria is an important trait in the predator-prey relationship of aquatic bacteria and bacterivorous protists. Grazing by bacterivorous protists is size selective [16 and 41] and thus small and large bacteria may have a refuge from protozoan grazing. Filament formation or permanent filamentous growth is one highly effective, size dependent grazing defense mechanism of aquatic bacteria [19 and 20]. The initial hypothesis that the four upstream tributaries are similar to each other in having similar mean cell-lengths, but are markedly different from that of main river Cauvery was rejected, because the mean cell-length of planktonic bacteria in the river Lakshmanatheertha was more and also significantly different than the remaining four water courses studied. The low level of water, maximum anthropogenic activities at the sampling spot, surface algal bloom, discharge of sewage, agricultural wastes and other effluents contamination and eutrophic nature of the river, might be the reason for increased mean bacterial cell-lengths in the river Lakshmanatheertha. Similarly, higher mean cell-length of planktonic bacteria was reported from an artificial lake in tropical Cameron, which actually receives considerable amount of untreated waste waters [25]. Further, the planktonic bacterial cell-size was high during low water [1]. The temporal variation in the mean cell-length of free-living bacteria showed seasonal pattern with smaller cells in winter than in summer in all the five rivers (*Fig 2*). Thus, cell-size was measured, because small size has been associated with stress and starvation [49 and 30].

Generally, season wise grouped data of all the five rivers revealed no seasonal variation in the mean length of both free-living and particle bound bacterial cells, except, in the river Lakshmanatheertha during the second year of study, where the mean cell-length of free-living bacteria was significantly less in winter than in rainy and summer seasons. This may be due to physiological stress caused by seasonal environmental changes or variations in the supply of food by phytoplankton and also in the grazing pressure from higher trophic levels [48 and 9].

The mean cell-lengths of planktonic bacteria with other microbial variables showed significant positive correlation with the abundance of bacterioplankton in the river Lakshmanatheertha. This implies that, higher the bacterial abundance higher will be the mean cell-length, which may be because of low level of water and addition of sewage, agricultural run-off and other untreated effluent caused the growth of algal bloom, responsible for eutrophic condition of water [8 and 28]. However, the negative correlation between mean cell-length of free-living bacteria and CFUs as % of AODCs in the river Lakshmanatheertha, may be because all the viable bacteria are not capable to grow on the artificial nutrient media employed under laboratory conditions to form colonies or perhaps most of these bacteria are dead [34, 40 and 37]. In the rivers Lakshmanatheertha and Hemavathy, the mean cell-length of bacteria was positively correlated with zooplankton. Similarly, a significant positive correlation was noticed between bacterial cell-size and zooflagellates in the water column of Sep reservoir, pelagic and Benthic [7, 22 and 26] ecosystems. However, in the river Cauvery, the mean cell-length of free-living bacteria was negatively correlated with zooplankton. Zooplanktons are recognized as being the main consumers of bacteria in the aquatic ecosystem. Several studies have revealed that, grazing by protists or metazoa play a dominant factor controlling the bacterial cell mortality [47]. Further, the mean cell-length of bacteria in the river Hemavathy showed positive correlation with the specific

growth rate of bacterioplankton. Similar observation was made in the Holderness Drain of North-East England [49].

The examination of correlation between mean cell-size of bacteria and environmental variables showed the presence of more correlations in river Lakshmanatheertha, when compared to other four water courses studied (*Table 6*). Hence, in the river Lakshmanatheertha, environmental variables may also have an important role for affecting the changes in the cell-size of planktonic bacteria. Parntaler, et al., [36] and Bergstein Ben-Dan, et al. [6] suggested several chemical and physical factors that might be influencing bacterial succession and changes in cell-size spectra in aquatic environments. Similarly, in the river Lakshmanatheertha, lesser DO concentration was noticed and the water in this river was slightly anoxic, due to pollution load and eutrophic condition. Hence, in the river Lakshmanatheertha the bacterial cell-size was negatively correlated with dissolved oxygen. This probably implies that, lesser the dissolved oxygen concentration more will be the bacterial cell-size [10].

The different distribution of the cell-size category in all the five water courses might be because of several chemical and physical factors [36 and 6], but the effects of predation in the natural environment are difficult to demonstrate [9]. However, based on recent intercomparison of cell-size, it would appear that much of the reported variation in bacterial cell-size is related to methodology which varies among the investigators [10]. Several studies have revealed grazing by protists as the dominant factor controlling the bacterial cell-size distribution [47]. Notable, lowest (11.37%) percentage of free-living bacterial cells in each size category in the river Lakshmanatheertha and lowest (12.25%) percentage of particle bound bacterial cells in each size category in the river Lokapavani was the lowest recorded values among five water courses studied (*Table 6*). The decrease in the percentage of bacterial cell-size may be due to grazing by protozoan or even larger predators such as rotifers [14 and 39] or due to nutrient poor environment [30]. However, the highest (20.0%) percentage of free-living bacterial cells in each size category in the rivers Harangi and Hemavathy and the highest (20.0%) percentage of particle bound bacterial cells in each size category in the rivers Lakshmanatheertha, Harangi, Hemavathy and Cauvery were the highest recorded values among the five water courses studied (*Table 6*). Large cell-size category in these water courses could have several explanations, viz. species shifts, reduced respiratory metabolism, reduced predation on large cells or greater availability of nutrients or organic substrate for growth due to anthropogenic activities [10].

In general, the more and significantly different seasonal variation in the percentage of bacterial cells in each size category during summer season in all the five water courses (*Table 6*) may be due to annual rain deficit, lack of water renewal, surface algal patches, sewage and other anthropogenic contaminations enriched nutrient level. There are only few supportive evidences, which explain that, dominance of smaller cells in nutrient poor environment to dominance of larger cells in nutrient rich environment [43 and 30]. Further, a pronounced variation in cell-size of bacteria with respect to larger cells during summer season [25] was noticed. The ups and downs in the bacterial cell-size in different season may be due to variation in the food supply and in the grazing pressure from higher trophic levels [14 and 26]. Further, bacteria are often the most stable component of planktonic communities. Refusing from grazing is one of the possible mechanisms buffering bacterioplankton against strong seasonal fluctuation in cell-size in both marine and fresh water habitats [29]. However, bacterial cell-sizes

respond to flagellate grazing with marked bidirectional shift in their size distribution [36 and 19].

The extent of the potential dependence of bacterial cell-size on environmental (water quality) variables was further investigated by step-wise multiple regression analysis. The results of all the five rivers are given in *Table 8*. The regression analysis revealed that, several key environmental variables were potentially responsible for much of the variation in bacterial cell-size, notable are SO₄, DO, SWV, PO₄, TASA, BOD, COD, Conductivity, Chl-a, and Chloride. Further, as many as 1-8 positive correlations were found to be affecting the bacterial cell-sizes in the present study. However, no environmental variables entered the regression equation in the river Lokapavani with respect to mean length of free-living bacteria and with both the (flb and pbb) bacterial cell-size in the river Cauvery. Similarly, several environmental variables such as PO₄, Chloride, COD, SO₄ and BOD were directly involved in the observed changes in the cell-size of planktonic bacteria in a flooded Sep reservoir of France [26]. The other reason for such dependence of bacterial cell-size may be due to substrate availability, nutrients, because bacterial growth in terms of size probably maintained at a maximum level by a density dependent factors such as carbon or other nutrients [11 and 15].

The 1992-1994 data from river Hull, Beverly and Barmston Drain and Holderness Drain [49], revealed that, physiological stress indicated by the presence of small cells was perhaps not the major cause of temporal variation in bacterial cell-size in river Hull and Beverly and Barmston Drain and they were less affected by the environmental factors. But it was quite opposite in the Holderness Drain, where bacterial variables were largely related to cell-length of planktonic bacteria and they might have influenced temporal variation of bacteria. The interrelationship between the cell-size of planktonic bacteria and environmental variables also showed the presence of many correlations in Holderness Drain. Particularly the environmental constraint like low pH might have stressed the bacterial population which resulted in less bacterial activity (V_{max} and V_{max} per bacterium), CFUs, CFUs as % of AODCs etc. However, in the river Hull and Beverly and Barmston Drain there was no evidence of environmental variables causing stress in sense of smaller cell-size [49]. Similarly, in the present investigation the mean cell-lengths of planktonic bacteria measured from 2000 to 2002, were more and also significantly different in the river Lakshmanatheertha when compared to rivers Cauvery, Harangi, Hemavathy and Lokapavani. Further, in the river Lakshmanatheertha, the mean cell-length of planktonic bacteria showed more correlations with environmental variables when compared to other four water courses studied (*Table 6*). Similar findings were noticed in the river Holderness Drain [49], and in the down stream tributaries of river Cauvery, such as river Arkavathy and river Shimsha (Unpublished data of Harsha from our laboratory). In contrast to this in the river Lokapavani the cell-size of free-living bacteria and the cell-size of both free-living and particle bound bacterial cells in the river Cauvery did not show any correlation with the environmental variables, which shows probably that no evidence of environmental variables, participating in the control of bacterial cell-size similar to the findings of Yamakanamardi [49] in river Hull and Beverley and Barmston Drain, and in the river Cauvery and its down steam tributaries like Suvarnavathy, Shimsha and Kapila.

Table 8. Results of stepwise multiple regression analysis between mean cell-length of bacteria (μm) and environmental variables, in the river, Lakshmanatheertha, Harangi, Hemavathy, Lokapavani and Cauvery, February 2000 to January 2002.

Bacterioplankton variables	Environmental variables
River Lakshmanatheertha	
Mean Length of Free Living Bacteria	SWV (-), SO_4 (+), ($r^2=0.35$, $F=12.86$, $P<0.001$), COND (+), DO (-), Cl_2 (+), TASA (+).
Mean Length of Particle Bound Bacteria	SO_4 (+), DO (-), ($r^2=0.23$, $F=7.17$, $P<0.005$), PO_4 (+), SWV (-).
River Harangi	
Mean Length of Free Living Bacteria	DO (+), ($r^2=0.13$, $F=7.29$, $P<0.05$).
Mean Length of Particle Bound Bacteria	DO (+), ($r^2=0.10$, $F=5.48$, $P<0.05$), Chl-a (-).
River Hemavathy	
Mean Length of Free Living Bacteria	No environmental variables entered in the regression equation.
Mean Length of Particle Bound Bacteria	DO (+), ($r^2=0.16$, $F=9.0$, $P<0.005$), COD (+), BOD (+).
River Lokapavani	
Mean Length of Free Living Bacteria	No environmental variables entered in the regression equation.
Mean Length of Particle Bound Bacteria	No environmental variables entered in the regression equation.
River Cauvery	
Mean Length of Free Living Bacteria	
Mean Length of Particle Bound Bacteria	

Environmental (independent) variables in the final regression equation ($P_{\text{in}}=0.05$, $P_{\text{out}}=0.1$) are shown: multiple coefficients of determinations (r^2) and overall F and P values for each equation are given in the parenthesis. Environmental variables which were not in the final equation but which are correlated ($P<0.05$) with the relevant Bacterioplankton variables are then listed in order of decreasing magnitude of correlation coefficient; the sign of the correlation is indicated in the parenthesis. The environmental variables were; COND=Conductivity, DO= Dissolved Oxygen, BOD= Biological Oxygen Demand, COD=Chemical Oxygen Demand, Cl_2 =Chloride, Chl-a= Chlorophyll-a, TASA= Total Anions of Strong Acids, SO_4 = Sulphate, SWV= Surface Water Velocity, PO_4 =Phosphate.

However, the mean cell-lengths of planktonic bacteria were similar in the river Cauvery and its down stream tributaries such as Kapila, Suvarnavathy, Shimsha and Arkavathy. Such variation may be due to the climatic condition, place to place geological variation, nutrient availability and grazing pressure by higher trophic level [26 and 31].

Thus, it could be concluded that the mean cell-length of planktonic bacteria in all these five watercourses studied, were controlled largely by environmental variables, which is in agreement with the other studies such as, low land water courses of north-east England [49], in a flooded Sep reservoir of France [26], in the hypertrophic Humboldt Lake and Oligotrophic Redberry lake in Saskatchewan Canada [44], and in sediments of Botany bay in Sydney, Australia which is fed by two rivers, the Cooks river and the Georges river [31]. Further, the strength of the relationship between the

overall mean cell-length of planktonic bacteria and environmental variables in the river Lakshmanatheertha with more correlations suggests that, environmental variables have retained a degree of bottom-up (nutrients) control of variation in bacterioplankton cell-size. On the other hand, the massive input of allochthonous bacteria from land, from sewage water or due to rain or from agricultural field and other anthropogenic inputs in the rivers, may also influence the magnitude and seasonal variation of the abundance of heterotrophic bacterioplankton [8 and 42]. This may be the reason for a strong positive correlation noticed between the bacterial abundance and mean cell-length of planktonic bacteria in the river Lakshmanatheertha.

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