Heterotrophic free-living and particle-bound bacterial cell size in the river Cauvery and its downstream tributaries

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This is the first comprehensive study on planktonic heterotrophic bacterial cell size in the river Cauvery and its important tributaries in Karnataka State, India. The initial hypothesis that the mean cell size of planktonic heterotrophic bacteria in the four tributaries are markedly different from each other and also from that in the main river Cauvery was rejected, because all five watercourses showed similar planktonic heterotrophic bacterial cell size. Examination of the correlation between mean heterotrophic bacterial cell size and environmental variables showed four correlations in the river Arkavathy and two in the river Shimsha. Regression analysis revealed that 18% of the variation in mean heterotrophic free-living bacterial cell size was due to biological oxygen demand (BOD) in the river Arkavathy, 11% due to surface water velocity (SWV) in the river Cauvery and 11% due to temperature in the river Kapila. Heterotrophic particle-bound bacterial cell size variation was 28% due to chloride and BOD in the river Arkavathy, 11% due to conductivity in the river Kapila and 8% due to calcium in the river Cauvery. This type of relationship between heterotrophic bacterial cell size and environmental variables suggests that,though the mean heterotrophic bacterial cell size was similar in all the five water courses, different sets of environmental variables apparently control the heterotrophic bacterial cell size in the various water bodies studied in this investigation. The possible cause for this environmental (bottom–up) control is discussed.

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1. Introduction

Heterotrophic bacteria are known to efficiently decompose organic matter and regenerate minerals in aquatic ecosystems, and their abundance represents an index of heterotrophic activity (Overbeck and Chrost 1990; Henssen and Tranvik 1998). They play an important role in the structure and functioning of the microbial food web, in relation to environmental conditions such as temperature (Felip *et al* 1996; Simon and Wunsch 1998), resource availability (Pace and Cole 1996), and predation pressure (Pace and Cole 1996; Thouvenot *et al* 1999). Given the important ecological role of heterotrophic bacteria, factors regulating the productivity (growth rate) and biomass (abundance and cell size) of these communities are of interest. The size of heterotrophic bacterial cells is an important aspect of the predator–prey relationship between bacteria and protistan predators because grazing by predators is size selective (González *et al* 1990; Šimek *et al* 1997; Hahn *et al* 2000). Moreover, larger bacterioplankton cells to be grazed more

Keywords. Heterotrophic bacterial cell size; metabolic activity; environmental variables

Abbreviations used: BOD, biological oxygen demand; SWV, surface water velocity; FLB, free-living bacteria; PBB, particle-bound bacteria; CFU, colony-forming unit; AODC, acridine orange direct count

efficiently than small cells (González *et al* 1990; Jugnia *et al* 2000). Previous studies have provided evidence that large allochthonous bacteria (≥1 μm) brought by plants are active in the river and able to grow in culture at rates twice those of smaller bacteria (<1 μm) (Servais and Garnier 1990; Garnier *et al* 1992). Predation by bacterivorous protists in aquatic habitats can influence the morphological structure, taxonomic composition and physiological status of bacterial communities. Grazing by protozoa depends on the concentration of bacteria and digestion capacity of the grazer, which is one of the main biological processes that control allochthonous bacterial density. Such grazing by protists can result in bacterial responses at the community and species level. Kjellberg *et al* (1987) suggested that heterotrophic bacterial cell size may be related to metabolic state, as large bacteria are often the most active (Rimes and Goulder 1986a, b). As competitors, large heterotrophic bacteria limit the development of small ones, but they play a buffer role in the degradation of organic matter, which explains the slow decrease in biodegradable organic matter in downstream areas. A reduction in size is said to be an adaptive mechanism of heterotrophic bacteria under starvation. Thus, the cell size of heterotrophic bacteria may have a meaningful ecological role in the planktonic food web (Letarte and Pinel-Alloul 1991). Cell size here refers to mean length, as only the lengths of heterotrophic bacterial cells were measured in this study. This is related to cell volume and biomass, although not linearly. Cell breadths were not measured, hence calculation of biovolume and biomass was not possible.

As far as we know, few studies that have taken bacteria into account are available in the literature on the size spectra of heterotrophic microorganisms. Examples are studies on seasonal changes in abundance and cell size of heterotrophic bacteria in freshwater lakes (Bennett *et al* 1990), the effect of detrital addition on bacterial cell size (Hadas *et al* 1990), bacterial biomass and cell size distribution (Cole *et al* 1993), predation of filamentous bacteria by nanoflagellates (Wu *et al* 2004). Furthermore, Hahn and Höfle (1999, 2001) hypothesized that size-selective grazing was the major force controlling both the morphological and the taxonomic structure of heterotrophic bacteria in reservoirs. A few research studies on rivers have documented bacterial and algal production (Findlay 1991), the physiological characteristics and ecological role of small- and large-sized bacteria in polluted rivers (Garnier *et al* 1992), heterotrophic bacterial cell size in three lowland water courses (unpublished results from Yamakanamardi 1995) of cold regions such as north-east England, and the microbial food web with respect to diel fluctuations in bacterial biomass (Jugnia *et al* 2000). In the studies carried out by Yamakanamardi (unpublished data, 1995) physiological stress indicated by the presence of small cells was perhaps not the major cause of temporal variation in heterotrophic bacterial cell size in the rivers Hull and Beverley, and Barmston Drain; cell size was less affected by environmental factors. It was the opposite in Holderness Drain, where bacterial variables were largely related to the cell size of planktonic bacteria and might have influenced the temporal variation in bacterial cell size.

What factors control the heterotrophic bacterial cell size distribution in freshwater habitats? This is an old, unsolved question. Knowledge of the controlling factors will greatly improve our understanding of the biology and ecology of planktonic heterotrophic bacteria present in the pelagic zone of freshwater systems. These organisms numerically and biochemically dominate the communities of freshwater systems. They are essential for the functioning of freshwater ecosystems and, in contrast to other organisms such as fish, are present in all freshwater systems. Despite the crucial role of freshwater heterotrophic bacteria in the metabolism of freshwater systems, not much is known about the factors controlling community composition, functional composition and their size distribution. The latter is an important ecological parameter, because the ecological interaction of freshwater heterotrophic bacteria with their biological and non-biological environment is usually size-dependent. The cell size of heterotrophic bacteria matters in terms of substrate uptake (surface–volume ratio), as well as in the interaction with protistan or metazoan predators. Therefore, an understanding of the mechanisms controlling the size distribution of heterotrophic bacterial communities is crucial for understanding the interaction of heterotrophic bacteria with their environment.

The present study was undertaken to know what factor(s) would cause a temporal variation in heterotrophic bacterial cell size with respect to Indian climatic conditions. To the best of our knowledge, there are no reports available on the measurement of heterotrophic bacterial cell size in lotic waters in India, and particularly on the heterotrophic bacterial cell size in the river Cauvery and its important tributaries in Karnataka State. Hence, a 24-month seasonal study of planktonic heterotrophic bacterial cell size (both free-living bacteria [FLB] and particle-bound bacteria [PBB]) was undertaken. Its main aims were (i) to study and compare the mean cell length of heterotrophic bacteria (both FLB and PBB) in five watercourses; (ii) to test the hypothesis that the mean length of heterotrophic bacteria in all the four tributaries is markedly different from each other and from that of the main river Cauvery, which has substantially different water sources from the upstream and a potentially different water quality environment; (iii) to compare the mean percentage of heterotrophic bacterial cell distribution in each size category; and (iv) to investigate the relationship between the mean cell lengths of heterotrophic bacteria with other microbial and environmental variables.

2. Materials and methods

2.1 *Collection and preservation of water samples*

Midstream surface water samples were collected from the river Cauvery near Bannur village (Site 1, 12° 25″ N and 76° 41″ E), the river Kapila at Tirumkudala Narasipura (Site 2, 12° 10″ N and 76° 55″ E), the river Suvarnavathy at Mudigundum (Site 3, 12° 09″ N and 77° 07″ E), the river Shimsha at Torekadanahalli (Site 4, 12° 50″ N and 76° 53″ E) and the river Arkavathy near Doddaladahalli (Site 5, 12° 33″ N and 77° 25″ E) between 07.30 and 12.30 IST at fortnightly intervals, from February 2000 to January 2002. Samples for bacteriological analysis were transferred to sterile glass bottles and were packed in ice for transport. For the analysis of environmental variables, water samples were collected in a clean polythene bucket and transferred to 5 litre capacity polythene containers. Ten ml of a water sample from each site was preserved with a 2% final concentration of 0.22 μm filtered neutral formalin (Hobbie *et al* 1977). The water was preserved for not more than 3–4 days before microscopical observations were carried out, as sizes may change (due to cell shrinkage) on fixing for longer periods (Fry 1990). Bacteria were then stained with acridine orange (BDH Chemicals Ltd, England), concentrated onto black 0.22 μm polycarbonate membrane filters (Millipore India Ltd) and examined under an epifluorescence microscope (Olympus, BX 40, Japan) (figure 1a). The methodology followed was based on APHA (1992) and described in Yamakanamardi (1995).

2.2 *Measurement of mean length (cell size) of heterotrophic bacteria*

The length of 100–150 heterotrophic FLB and 100–150 heterotrophic PBB from each water sample was measured directly from the microscopic images using a G-12 eyepiece graticule (Olympus Optical Co. Ltd, Japan) at magnification 1000X (May 1965). The graticule contained two series of circles, i.e. one clear and the other black of increasing diameter (figure 1b). These circles were overlaid on each bacterium chosen without bias, so that cell length was given by the appropriate circle diameters (as described in Yamakanamardi 1995). The bacteria were placed in ten length categories that increased in root 2 progressions from <0.44 μm to >7 μm. The ten size categories were; 1 = <0.44 μm, 2 = 0.44–0.53 μm, 3 = 0.53–0.75 μm, 4 = 0.75–1.06 μm, 5 = 1.06–1.49 μm, 6 = 1.49–2.12 μm, 7 = 2.12–3.0 μm, 8 = 3.0–4.25 μm, 9 = 4.25–6.0 μm and 10 = >7 μm.

The mean lengths of both heterotrophic FLB and PBB were calculated. Cells in categories 2–9 were ascribed lengths which were the mid-points of the maximum and minimum lengths for their category. Cells in category 1 were taken as being 0.44 μm while cells in category 10 were taken as 7 μm.

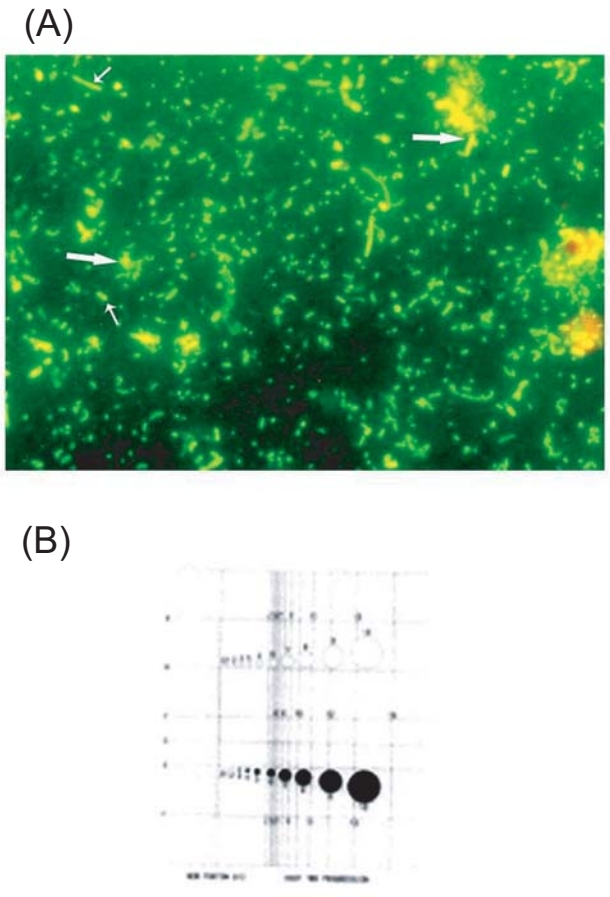

Figure 1. (A) Acridine orange-stained free-living (small arrows) and particle-bound (large arrows) heterotrophic bacteria in the surface water of the river Cauvery, 7 August 2001, 1000X. **B)** Magnified picture of G-12 eyepiece graticule; dark circles were used to measure the bacterial cell size. These circles were overlaid on each bacterium chosen without bias, so that cell length was given by the appropriate circle diameters (for details *see* the Materials and methods section).

2.3 *Estimation of percentage of heterotrophic bacterial cells in each size category*

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

2.4 *Statistical analysis*

All statistical analyses were carried out using SPSS for Windows release 6.0 (Norusis 1993). The Kolmogorov–

Smirnov test was used to test for agreement with the normal distribution. Distribution of many variables was 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 the Pearson correlation coefficient. Values of Pearson correlation coefficient 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. Variables were entered into the equation using the step-wise entry method, with P in set at 0.05 and P out set at 0.1.

3. Results

Cell size here refers to mean length, as only the lengths of heterotrophic 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. The results of heterotrophic bacterial cell size (mean length) and size category distribution are given below.

3.1 *Mean length of heterotrophic FLB*

The summary of overall mean cell size of heterotrophic FLB is given in table 1. The mean cell size of heterotrophic FLB was similar in the rivers Cauvery (mean 2.68 μm, range 1.1–3.5 μm), Kapila (mean 2.89 μm, range 1.2–4.2 μm), Suvarnavathy (mean 2.96 μm, range 1.30–4.40 μm), Shimsha (mean 2.94 μm, range 1.4–4.0 μm) and Arkavathy (mean 2.84 μm, range 1.1–3.80 μm). Temporal variations in mean cell size of heterotrophic FLB measured from February 2000 to January 2002 for all the rivers studied is shown in figure 2. Fluctuations in the mean cell size of heterotrophic FLB were greater in the rivers Cauvery (1.09–3.49 μm), Kapila (1.17–4.21 μm), Suvarnavathy (1.26–4.36 μm), Shimsha (1.35–3.83 μm) and Arkavathy (1.09–3.75 μm) during the first year of study, i.e. February 2000 to January 2001 (figure 2). In contrast, during the second year of study, there was not much fluctuation recorded. It is clear from figure 2 that during winter heterotrophic FLB cells were smaller than during the summer and rainy season in all the five watercourses studied. The largest mean cell size of heterotrophic FLB (4.36 μm) recorded in the river Suvarnavathy and smallest mean cell size (1.10 μm) recorded in the rivers Cauvery and Arkavathy were the largest and smallest mean cell sizes of heterotrophic FLB recorded among the five watercourses studied (table1).

The correlation between cell size of heterotrophic bacteria and other microbial variables is shown in table 2. In the river Suvarnavathy the mean cell size of heterotrophic FLB was positively correlated with the abundance of FLB and mean cell size of heterotrophic PBB. In the river Shimsha the mean cell size of heterotrophic FLB was correlated with the abundance of heterotrophic FLB, total bacteria and mean cell size of heterotrophic PBB (table 2). However, in the other three watercourses, the mean cell size of heterotrophic FLB correlated only with mean cell size of heterotrophic PBB among bacterial variables. The correlation between heterotrophic bacterial cell size and environmental variables is shown in table 3. Among environmental variables, the concentration of mean cell size of heterotrophic FLB was positively correlated with temperature in the river Kapila and BOD in the river Arkavathy, and negatively correlated with SWV in the river Cauvery (table 3).

3.2 *Mean length of heterotrophic PBB*

The summary of overall mean cell size of heterotrophic PBB is given in table 1. The mean cell size of heterotrophic PBB was similar in rivers Cauvery (mean 2.73 μm, range

Table 1. Summary of the overall mean cell size of heterotrophic bacteria (μm) in the surface waters of rivers Cauvery, Kapila, Suvarnavathy, Shimsha and Arkavathy: seasonal study, February 2000–January 2002.

Microbial variables	River Cauvery		River Kapila		River Suvarnavathy		River Shimsha		River Arkavathy	
	Mean (range)	CV%	Mean (range)	CV%	Mean (range)	CV%	Mean (range)	CV%	Mean (range)	CV%
Cell size of FLB (μm)	2.68 (1.1–3.5)	22	2.89 (1.2–4.2)	22	2.96 (1.3–4.4)	21	2.94 (1.4–4.0)	22	2.84 (1.1–3.8)	23
Cell size of PBB (μm)	2.73 (1.2–3.5)	21	2.93 (1.3–3.7)	15	3.02 (1.2–4.0)	17	2.95 (1.0–3.9)	20	2.84 (1.02–3.8)	22

Mean values with different superscripts are significantly different ($P<0.05$, Student–Newman–Keuls test, after $\log_{10}$ transformation). FLB, free-living bacteria; PBB, particle-bound bacteria; CV, coefficient of variation.

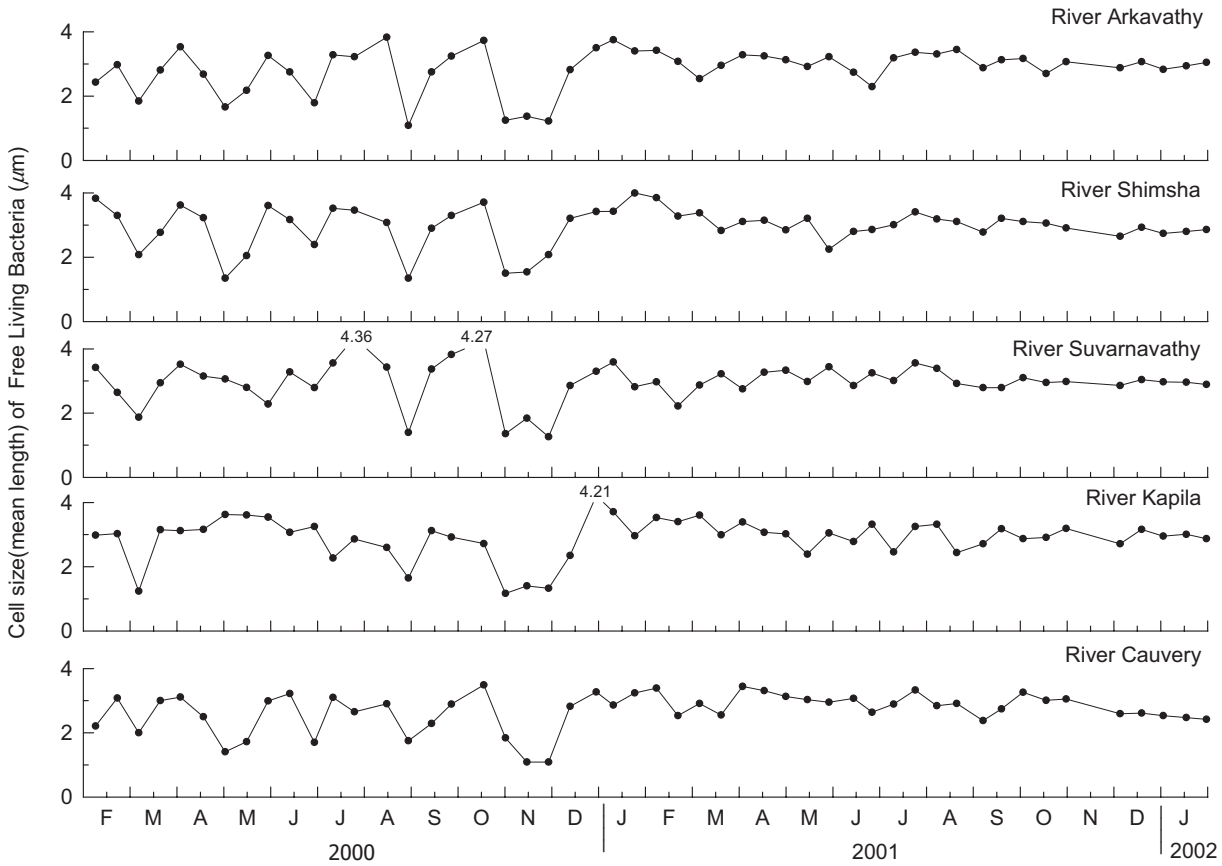

Figure 2. Seasonal changes in heterotrophic free-living bacterial cell size in the surface water of rivers Cauvery, Kapila, Suvarnavathy, Shimsha and Arkavathy, February 2000–January 2002.

1.2–3.5 μm), Kapila (mean 2.93 μm, range 1.3–.7 μm), Suvarnavathy (mean 3.02 μm, range 1.20–4.0 μm), Shimsha (mean 2.95 μm, range 1.0–3.9 μm) and Arkavathy (mean 2.84 μm, range 1.02–3.80 μm). Temporal variations in the mean cell size of heterotrophic PBB measured from February 2000 to January 2002 for all the rivers studied are shown in figure 3. In rivers Cauvery, Kapila, Suvarnavathy, Shimsha and Arkavathy the mean cell size of heterotrophic PBB did not show any marked seasonal pattern. However, during the first year of study, i.e. February 2000 to January 2001, fluctuations in the mean cell size of heterotrophic PBB were greater in rivers Cauvery (1.20–3.45 μm), Kapila (1.25–3.71 μm), Suvarnavathy (1.16–3.99 μm), Shimsha (1.0–3.86 μm) and Arkavathy (1.02–3.77 μm) (figure 3). In contrast, during the second year of study, the mean cell size of heterotrophic PBB did not fluctuate much. The largest (4.0 μm) mean cell size of heterotrophic PBB in the river Suvarnavathy and smallest (1.00 μm) in the river Shimsha were the largest and smallest cell sizes of heterotrophic PBB recorded among the five watercourses studied. Season-wise grouped data of mean cell sizes of heterotrophic PBB did not show marked differences in any of the water courses studied.

In the rivers Shimsha and Arkavathy, there were few correlations between the mean cell size of heterotrophic PBB and other bacterial variables (table 2). In the river Shimsha, the cell size of heterotrophic PBB was positively correlated with the abundance of PBB and mean cell size of heterotrophic FLB. In the river Arkavathy, the mean cell size of heterotrophic PBB was correlated with the abundance of zooplankton and mean cell size of heterotrophic FLB (table 2). However, in the other three watercourses, the mean cell size of heterotrophic PBB was correlated only with mean cell size of heterotrophic FLB. The mean cell size of heterotrophic PBB was positively correlated with calcium in the river Suvarnavathy, turbidity in the river Shimsha, and BOD, chloride and calcium in the river Arkavathy. However, among environmental variables, it was negatively correlated with conductivity in the river Shimsha (table 3).

Table 2. Correlation between mean cell size of heterotrophic bacteria (μm) and other bacterial variables, February 2000–January 2002.

Rivers	DC-FLB	DC-PBB	DC-TB	CFUs	%CCFUs	CFUs as % of AODCs	CS-PBB	SGR	Phyto-plankton	Zooplankton	Total plankton
Cell size of heterotrophic free-living bacteria											
River Cauvery	NS	NS	NS	NS	NS	NS	0.78***	NS	NS	NS	NS
River Kapila	NS	NS	NS	NS	NS	NS	0.55***	NS	NS	NS	NS
River Suvarnavathy	0.32*	NS	NS	NS	NS	NS	0.64***	NS	NS	NS	NS
River Shimsha	0.44***	NS	0.42**	NS	NS	NS	0.56***	NS	NS	NS	NS
River Arkavathy	NS	NS	NS	NS	NS	NS	0.73***	NS	NS	NS	NS
Cell size of heterotrophic particle-bound bacteria							CS- FLB				
River Cauvery	NS	NS	NS	NS	NS	NS	0.78***	NS	NS	NS	NS
River Kapila	NS	NS	NS	NS	NS	NS	0.55***	NS	NS	NS	NS
River Suvarnavathy	NS	NS	NS	NS	NS	NS	0.64***	NS	NS	NS	NS
River Shimsha	NS	0.29*	NS	NS	NS	NS	0.56***	NS	NS	NS	NS
River Arkavathy	NS	NS	NS	NS	NS	NS	0.73***	NS	NS	0.41**	NS

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; CSFLB, cell size of free-living bacteria; CSPBB, cell size of particle-bound bacteria. *, **, ***, Level of significance.

3.3 *Percentage of heterotrophic FLB cells in each size category*

The overall percentage of heterotrophic FLB cells in each size category was similar in the rivers Cauvery (mean 15.45, range 12.46–25%), Kapila (mean 14.97, range 12.40–33.33%), Suvarnavathy (mean 14.50, range 12.42–24.99%), Shimsha (mean 14.61, range 12.38–33.32%) and Arkavathy (mean 14.76, range 11.49–33.32%). The largest percentage of heterotrophic FLB cells (33.33%) in each size category was recorded in the river Kapila and the smallest (11.49%) in the river Arkavathy (table 4).

The most frequent heterotrophic FLB cell size categories in all the five watercourses were 1.49–3.00 μm and 6–7 μm. Thus, in the river Cauvery, the 1.49–3.00 μm size category was the most frequently recorded (47 times) on 50 sampling days, followed by 6–7 μm (42 times) and 0.75 μm (38 times). In the river Kapila, the most frequently recorded size category was 1.49–3.00 μm (45 times), followed by 6–7 μm (39 times) and 0.75 μm (30 times). In the river Suvarnavathy, the most frequently recorded size category was 1.49–3.00 μm (47 times), followed by 6–7 μm (37 times) and 0.75 μm (28 times). In the river Shimsha, the most frequently recorded size category was 1.49–3.00 μm (48 times), followed by 6–7 μm (35 times) and 0.75 μm (33 times). In the river Arkavathy, it was 0.73–3.00 μm (48 times), followed by 6–7 μm (45 times). The size category 4.25 μm was the most infrequently found in all the five watercourses (table 5).

Season-wise grouped data of percentages of heterotrophic FLB cells in each size category did not show significant variations in any of the rivers studied. However, in the river Cauvery there were larger numbers and significantly different percentages of heterotrophic FLB cells in each size category during the winter season in the first year of study.

3.4 *Pecentage of heterotrophic PBB cells in each size category*

The overall percentages of heterotrophic PBB cells in each size category were similar in the rivers Cauvery (mean 15.65, range 12.37–24.99%), Kapila (mean 14.54, range 11.54–24.78%), Suvarnavathy (mean 14.38, range 12.47–33.33%), Shimsha (mean 14.97, range 12.11–33.32%) and Arkavathy (mean 14.44, range 12.41–33.33%). The largest percentage of heterotrophic PBB cells (33.33%) in each size category was recorded in the rivers Suvarnavathy and Arkavathy, and

Table 3. Relationship between mean cell size of heterotrophic bacteria (μm) and environmental variables, February 2000–January 2002.

Rivers	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*
Mean cell size of heterotrophic free-living bacteria																				
River Cauvery	NS	NS	NS	NS	NS	-0.34*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Kapila	NS	NS	0.34*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Suvarnavathy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Shimsha	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Arkavathy	NS	NS	NS	NS	NS	NS	NS	NS	0.43**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mean cell size of heterotrophic particle-bound bacteria																				
River Cauvery	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Kapila	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Suvarnavathy	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.29*	NS	NS	NS	NS
River Shimsha	NS	NS	NS	-0.33*	0.30*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
River Arkavathy	NS	NS	NS	NS	NS	NS	NS	NS	0.35*	NS	NS	0.37*	NS	NS	NS	0.31*	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; CO_2, free carbon dioxide; Cl_2, chloride; NO_3, nitrate; SO_4, sulphate; TASA, total anions of strong acids; Cal, calcium; PO_4, inorganic phosphate; TSS, total suspended solids; POM, particulate organic matter; Chl-*a*, chlorophyll-*a*.
*, ** Level of significance.

the smallest (11.54%) percentage in each size category in the river Kapila among the five watercourses studied (table 4).

The most frequent heterotrophic PBB cell size categories in all the five watercourses were 0.53–3.00 μm and 6–7 μm. Thus, in the river Cauvery, the 0.75–3.00 μm size category was the most frequent (49 times) in 50 sampling days, followed by 0.53–0.75 μm (35 times) and 4.25–7.0 μm (34 times). In the river Kapila the most frequent size category was 3.0–7.0 μm (39 times), followed by 0.75–3.00 μm (27 times). In the river Suvarnavathy these were 0.75–2.12 μm (45 times), followed by 3.00–7.00 μm (39 times) and 0.53–0.75 μm (28 times). In the river Shimsha the most frequent size category was 0.75–3.00 μm (44 times), followed by 3.0–7.0 μm (35 times) and 0.53–0.75 μm (34 times). In the river Arkavathy the most frequent size category was 0.53–7.0 μm, which was found 36 times in 50 days of sampling (table 5). Season-wise grouped data of percentages of heterotrophic PBB cells in each size category did not show any significant variation in any of the rivers during both years of study.

4. Discussion

This is the first comprehensive study on the cell size of heterotrophic bacteria in the river Cauvery and its important tributaries in Karnataka State, 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 the biovolume and biomass was not possible. In this study, cell size (mean length) of heterotrophic bacteria and heterotrophic bacterial cell size distribution were also calculated over a period of two years (February 2000–January 2002). Bacterial cell sizes vary either inversely or independently of trophic conditions (Van Es and Meyer-Reil 1982; Bird and Kalff 1984). However, during the present investigation, surprisingly, the mean cell size of both heterotrophic FLB and PBB were similar in all the five watercourses studied (table1). Hence, the initial hypothesis that the mean cell size of heterotrophic bacteria in all the four tributaries are markedly different from each other and from that of the main river Cauvery, which has substantially different water sources from the upstream and a potentially different water quality environment, was rejected, because all five watercourses showed similar planktonic heterotrophic bacterial cell size. The size of bacteria is an important factor in the predator–prey relationship of aquatic bacteria and bacterivorous protists. Grazing by bacterivorous protists (flagellates and ciliates) is size selective (González *et al* 1990; Šimek and Chrzanowski 1992) and thus small and large bacteria may take refuge from protistan grazing. Filament formation or permanent filamentous growth is a highly effective, size-dependent

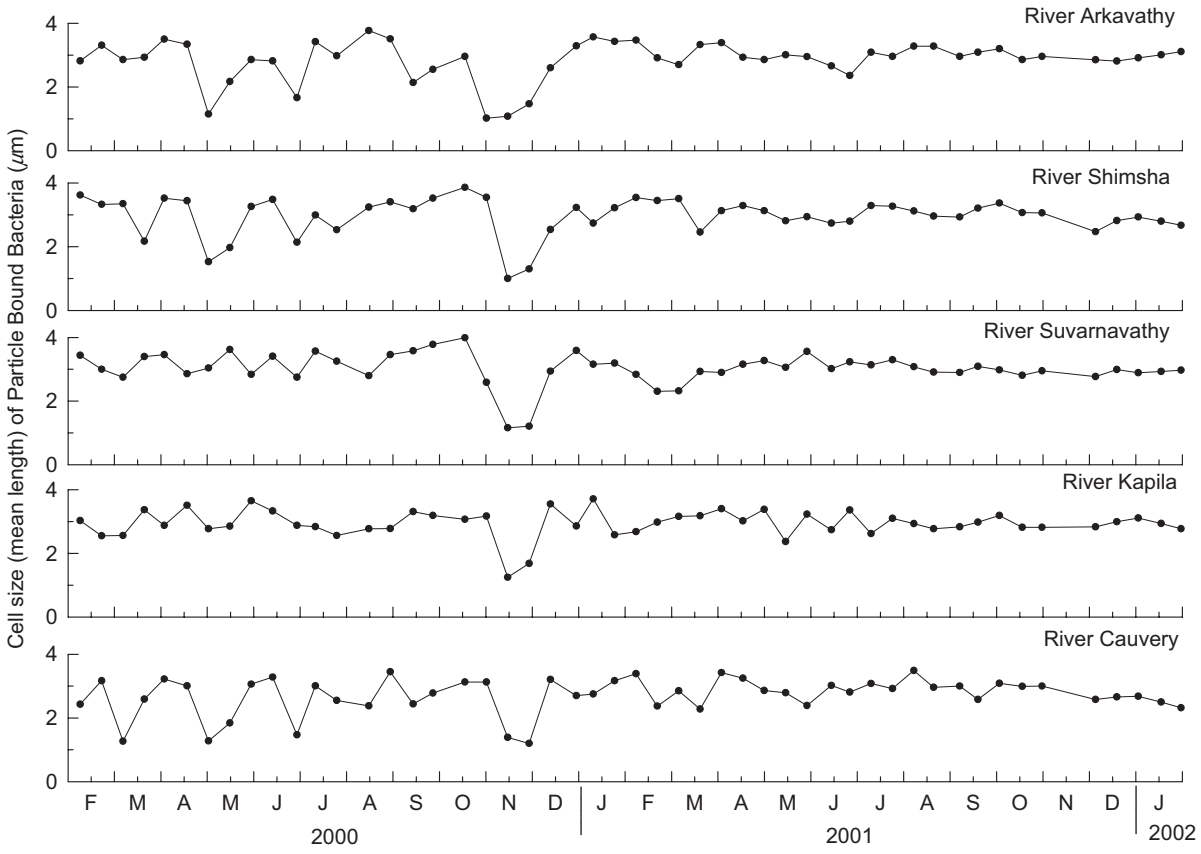

Figure 3. Seasonal changes in heterotrophic particle-bound bacterial cell size in the surface water of rivers Cauvery, Kapila, Suvarnavathy, Shimsha and Arkavathy, February 2000–January 2002.

grazing defence mechanism of aquatic bacteria (Hahn *et al* 1999, 2000).

Experimental field studies have demonstrated that the natural bacterial community with respect to cell size is mainly dependent on physical and chemical factors, such as temperature, substrate availability and nutrient supply, as well as protistan grazing pressure (Hahn and Höfle 1999, 2001; Jugnia *et al* 2000).

Temporal variations in the mean cell size of both heterotrophic FLB and PBB fluctuated only during the first year of study (figures 2 and 3) The increase and decrease noticed in bacterial cell size during the first year of study through the various seasons were probably due to variations in the supply of food by phytoplankton and the grazing pressure from higher trophic levels (Wright 1988; Callieri and Heinimaa 1997). Application of Pearson correlation between mean cell size of heterotrophic bacteria and other microbial variables revealed that the mean cell size of heterotrophic FLB was positively correlated with the abundance of heterotrophic FLB in the rivers Suvarnavathy and Shimsha, as well as with total bacteria in the river Shimsha. Further, the cell size of heterotrophic FLB was positively correlated with the mean length of heterotrophic PBB in all the watercourses studied (table 2). The mean cell size of heterotrophic PBB was positively correlated with the mean size of heterotrophic FLB in all the five watercourses studied. However, it was positively correlated with only abundance of heterotrophic PBB in the river Shimsha and with zooplankton in the river Arkavathy (table 2). The different correlations between cell size and heterotrophic bacterial abundance suggest that the extent of their participation in terms of growth rate and biomass (Cole *et al* 1993).

The mean cell size of heterotrophic FLB was positively correlated with temperature in the river Kapila, BOD in the river Arkavathy and negatively correlated with SWV in the river Cauvery. The mean cell size of heterotrophic PBB was positively correlated with calcium in the river Suvarnavathy; turbidity in the river Shimsha; and BOD, chloride and calcium in the river Arkavathy. This suggests that the greater availability of nutrients and organic substrates such as chloride and calcium contributed by anthropogenic inputs such as discharge of untreated effluents and domestic sewage might have supported heterotrophic bacterial growth in terms of cell size (Cole *et al* 1993; Callieri and Heinimaa 1997).

Table 4. Summary of the overall percentage of heterotrophic bacterial cells in each size category (%) in the surface waters of rivers Cauvery, Kapila, Suvarnavathy, Shimsha and Arkavathy: seasonal study, February 2000–January 2002.

Microbial variables	River Cauvery	River Kapila	River Suvarnavathy	River Shimsha	River Arkavathy
	Mean (range)	Mean (range)	Mean (range)	Mean (range)	Mean (range)
FLB (%)	15.45 (12.46–25.00)	14.97 (12.40–33.33)	14.50 (12.42–24.99)	14.61 (12.38–33.32)	14.76 (11.49–33.32)
PBB (%)	15.65 (12.37–24.99)	14.54 (11.54–24.78)	14.38 (12.47–33.33)	14.97 (12.11–33.32)	14.44 (12.41–33.33)

Mean values with different superscripts are significantly different ($P<0.05$, Student–Newman–Keuls test, after $\log_{10}$ transformation). FLB, free-living bacteria; PBB, particle-bound bacteria.

Table 5. Heterotrophic bacterial cell size in each size category (out of 50 samplings).

% of heterotrophic free-living bacterial size category				
	Name of the river	1.49–3.00 μm	6.00–7.00 μm	0.75 μm
1	Cauvery	47	42	38
2	Kapila	45	39	30
3	Suvarnavathy	47	37	28
4	Shimsha	48	35	33
		0.73–3.00 μm	6.00–7.00 μm	
5	Arkavathy	48	45	
% of heterotrophic particle-bound bacterial size category				
	Name of the river	0.75–3.00 μm	0.53–0.75 μm	3.00–7.00 μm
1	Cauvery	49	35	34
2	Kapila	27	28	39
3	Suvarnavathy	45	28	39
4	Shimsha	44	34	35
5	Arkavathy	32	36	36

Bacterial cell size was negatively correlated with SWV in the river Cauvery and with conductivity in the river Shimsha (table 3). Schauer *et al* (2005) also reported a different response in a cosmopolitan group of planktonic freshwater bacteria with respect to their tolerated ranges of pH and conductivity. The mean cell size of heterotrophic FLB in the rivers Suvarnavathy and Shimsha, and mean cell size of heterotrophic PBB in the rivers Cauvery and Kapila did not show correlation with any of the environmental variables, which suggests that no environmental variables participated in the control of bacterial cell size in these watercourses. Our results agree with the findings of Yamakanamardi (unpublished data, 1995) in the rivers Hull and Beverley, and Barmston Drain. In contrast, Schauer *et al* (2005, 2006) reported that the composition of SOL communities in natural habitats was largely controlled by abiotic factors (water chemistry) such as pH, conductivity, altitude and trophy. Hence, it is suggested that other variables such as grazing may be more important in controlling bacterioplankton in these watercourses (Cole *et al* 1993; Yamakanamardi [unpublished data, 1995]; Callieri and Heinimaa 1997).

The mean percentage of heterotrophic bacterial cells in each size category of both FLB and PBB was also similar in all the five watercourses studied (table 4). The highest percentage (33.33%) of heterotrophic FLB cells in each size category in the river Kapila and the highest percentage (33.33%) of heterotrophic PBB cells in each size category recorded in the river Suvarnavathy were the highest compared with the other watercourses studied (table 4). Several explanations could be offered for the large cell size in each size category in these two watercourses. These include species shifts, reduced respiratory

Table 6. Results of step-wise multiple regression analysis between mean cell size of bacteria and environmental variables in the rivers Cauvery, Kapila, Suvarnavathy, Shimsha and Arkavathy, February 2000–January 2002.

Bacterioplankton variables	Environmental variables
River Cauvery	
Mean cell size of free-living bacteria	SWV (–), (R^2= 0.11, F= 6.20, P<0.05)
Mean cell size of particle-bound bacteria	No environmental variables entered in the regression equation
River Kapila	
Mean cell size of free-living bacteria	Temp (+), (R^2= 0.11, F= 6.14, P<0.05)
Mean cell size of particle-bound bacteria	No environmental variables entered in the regression equation
River Suvarnavathy	
Mean cell size of free-living bacteria	No environmental variables entered in the regression equation
Mean cell size of particle-bound bacteria	Ca (+), (R^2= 0.08, F= 4.35, P<0.05)
River Shimsha	
Mean cell size of free-living bacteria	No environmental variables entered in the regression equation
Mean cell size of particle-bound bacteria	Cond (–),(R^2= 0.11, F= 5.93, P<0.05), Tur (+)
River Arkavathy	
Mean cell size of free-living bacteria	BOD (+),(R^2= 0.18, F= 10.95, P<0.05)
Mean cell size of particle-bound bacteria	Cl_2 (+), BOD (+), (R^2= 0.28, F= 9.28, P<0.001), Cal (+) TASA (+)

Environmental (independent) variables in the final regression equation (P in=0.05, P out=0.1) are shown: multiple coefficients of determinations (R^2) and overall F and P values for each equation are given in parentheses. 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 parentheses. The environmental variables were Temp, temperature; Cond, conductivity; Turb, turbidity; BOD, biological oxygen demand; Cl_2, chloride; Cal, calcium; TASA, total anions of strong acids; SWV, surface water velocity

metabolism, reduced predation on large cells or greater availability of nutrients and organic substrate conditions for growth contributed by anthropogenic inputs such as discharge of untreated effluents and domestic sewage (Cole *et al* 1993). Furthermore, the lowest percentage (11.49%) of heterotrophic FLB cells in each size category in the river Arkavathy and the lowest percentage (11.54%) of heterotrophic PBB cells in each size category recorded in the river Kapila were the lowest compared with the other three watercourses studied (table 4). The decrease in percentage of bacterial cells in these watercourses may be due to grazing by protozoa or larger predators such as rotifers (Sanders *et al* 1989; Garnier *et al* 1992).

The extent of potential dependence of planktonic heterotrophic bacterial cell size on environmental variables was further investigated by step-wise multiple regression analysis (table 6). The regression analysis revealed that 18% variation in bacterial cell size of heterotrophic FLB was due to BOD (+) in the river Arkavathy and 11% due to SWV (-) in the river Cauvery. The 11% variation in bacterial cell size of heterotrophic FLB was due to temperature (+) in the river Kapila. However, none of the environmental variables in the rivers Suvarnavathy and Shimsha entered in the equation with respect to mean cell size of FLB (table 6) whereas 28% variation in mean cell size of heterotrophic PBB was due to chloride (+) and BOD (+) in the river Arkavathy, and 11% due to conductivity (–) in the river Shimsha. Furthermore, 8% variation in the mean cell size of heterotrophic PBB was due to calcium in the river Suvarnavathy. However, none of the environmental variables entered the regression equation in the rivers Cauvery and Kapila with respect to mean cell length of heterotrophic PBB (table 6). This kind of relationship between cell size and environmental variables suggests that temperature, calcium and chloride content, and BOD were directly responsible for the observed changes. This was in agreement with Jugnia *et al* (2000) in the flooded Sep reservoir of France. Another reason for such dependence of bacterial cell size may be due to substrate availability and nutrients, because bacterial growth in terms of size is probably maintained at a maximum level by density-dependent factors such as carbon or other nutrients as proposed by Gasol and Vaque (1993), and Ekebom (1999).

The 1992–1994 data from the rivers Hull and Beverly, and Barmston and Holderness Drains (unpublished data, Yamakanamardi 1995) revealed that physiological stress indicated by the presence of small cells was perhaps not the major cause of temporal variation in heterotrophic bacterial cell size in the rivers Hull and Beverley, and Barmston Drain

and they were less affected by environmental factors. It was the opposite in Holderness Drain, where bacterial variables were largely related to cell length of planktonic bacteria and they might have influenced temporal variation of bacterial cell size. The interrelationship between planktonic heterotrophic cell size and environmental variables also highlighted the presence of many correlations in Holderness Drain. In particular, environmental constraints such as a low pH might have stressed the bacterial population which resulted in low bacterial activity (V_{max} and V_{max} per bacterium), colony-forming units (CFUs), CFUs as a percentage of acridine orange direct counts (AODCs), etc. However, in rivers Hull and Beverley and Barmston Drain there was no evidence of environmental variables causing stress resulting in smaller cell size (unpublished data, Yamakanamardi 1995). In the present investigation, mean cell length of heterotrophic bacteria and environmental variables showed four correlations in the river Arkavathy and two correlations in the river Shimsha, thus supporting the findings of Yamakanamardi (unpublished data, 1995) in the Holderness Drain. In contrast to this, in the rivers Suvarnavathy and Shimsha, the mean length of FLB did not show any correlation with any of the environmental variables. The mean cell size of PBB in the rivers Cauvery and Kapila also did not show any correlation with any of the environmental variables, which suggests that environmental variables probably do not have a role to play in controlling the bacterial cell size, as found by Yamakanamardi (unpublished data, 1995) in the rivers Hull and Beverley, and Barmston Drain. Bacterial cell size upstream of the Krishna Raja Sagara reservoir was the opposite of the downstream findings. The mean planktonic bacterial cell size in the river Laxmantheertha was significantly high and different from that of the rivers Harangi, Hemavathy, Lokapavani and the main river Cauvery (unpublished data of Mahadeveswamy from our laboratory). However, during the present study, there was no difference in bacterial cell size in downstream tributaries. Such variations between upstream and downstream water may be due to climatic conditions, local geological variations, nutrient availability and grazing pressure by higher trophic levels (Jugnia *et al* 2000; Lee and Patterson 2002).

The obvious conclusion is that, though the heterotrophic bacterial mean cell length was similar in all the five watercourses, different sets of environmental variables apparently controlled cell size in the different water bodies studied. Similar observations were made for the hypertrophic Humboldt lake and oligotrophic Redberry lake in Saskatchewan, Canada (Tumber *et al* 1993), in a recently flooded oligomesotrophic reservoir in France (Jugnia *et al* 2000) and in sediments of Botany Bay in Sydney, Australia, which is fed by two rivers—the Cooks and the Georges rivers (Lee and Patterson 2002).

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