Hydrobiologia (2014) 726:65–80
DOI 10.1007/s10750-013-1752-x

PRIMARY RESEARCH PAPER

Are prokaryotic cell shape and size suitable to ecosystem characterization?

R. La Ferla · G. Maimone · G. Caruso · F. Azzaro · M. Azzaro · F. Decembrini · A. Cosenza · M. Leonardi · R. Paranhos

Received: 5 April 2013 / Revised: 11 November 2013 / Accepted: 13 November 2013 / Published online: 24 November 2013

Abstract Estimation of microbial biomass depends on cell shape and size determinations, and thus, there is a wide biovolume variability among morphotypes. Nevertheless, data on morphology and morphometry of prokaryotic cells under different trophic status are seldom published, due to the methodological difficulties of cell measurements. The main question addressed in this paper concerns the suitability of prokaryotic size and shape for environmental characterization. Microbial biovolumes were compared among different ecosystems, located in temperate and tropical regions. Samples were taken from fresh, brackish, mixohaline, and estuarine waters that were classified as oligo-, meso-, eu-, and hypertrophic by comparing synoptically different trophic indices. Prokaryotic cell abundance and volume were quantified by Image Analysis, used to calculate biomass, and correlated to environmental variables. Some samples were analyzed by flow cytometry also, and data from sub-populations with a different apparent DNA content were available. Prokaryotic abundances generally increased from oligo- to hypertrophic waters while cell volumes increased from oligotrophic to eutrophic waters. Although significant correlations between cell volumes and environmental variables were detected (positive with salinity and negative with Chlorophyll-*a*), different morphotypes dominated each studied regions. Our results sustain the hypothesis that prokaryotic cell size and shape could be useful to ecosystem characterization.

Keywords Prokaryotic cell size · Abundance · Morphology · Image analysis · Tropical and temperate aquatic systems · Trophic status

Handling editor: Stefano Amalfitano

Electronic supplementary material The online version of this article (doi:10.1007/s10750-013-1752-x) contains supplementary material, which is available to authorized users.

R. La Ferla (✉) · G. Maimone · G. Caruso · F. Azzaro · M. Azzaro · F. Decembrini · A. Cosenza · M. Leonardi
Istituto per l'Ambiente Marino Costiero (IAMC-CNR), Spianata San Raineri, 98122 Messina, Italy
e-mail: rosabruna.laferla@iamc.cnr.it

R. Paranhos
Institute of Biology, Federal University of Rio de Janeiro (UFRJ), Av. Prof. Rodolpho Rocco 211, Ilha do Fundão, Rio de Janeiro CEP 21941-617, Brazil

Introduction

The inclusion of prokaryotes (bacteria and archaea) in environmental or biodiversity monitoring programs is extremely rare. In the frame of the European Marine Strategy Framework Directive (MSFD) for the assessment of the "good environmental status (GES)" of the EU's marine waters, the Directive Guidance on the interpretation and application of the "Biological diversity" descriptor advocates that microbes (viruses

and bacteria) are included in monitoring programs (Cochrane et al., 2010). In fact, they could be considered biological indicators suitable for water quality assessment owing to their small size, short generation time, high sensitivity, and comprehensive responses to environmental conditions (Chuan et al., 2009). It is believed that the morphology of microbial cells may be a sensitive marker of changes in aquatic and terrestrial ecosystems; understanding the mechanisms that control the flexibility of cell morphology of prokaryotes could be crucial for revealing their interaction with environment (Young, 2006; Straza et al., 2009) and, to some extent, the distribution patterns of different genotypes and community composition (Pernthaler & Amann, 2005; Sjöstedt et al., 2012). Moreover, specific morphotypes may perform specific physiological activities (Cottrel & Kirchman, 2004; Posch et al., 2009) and, in turn, trophic changes might produce morphometric and morphological alterations in organisms of the aquatic food web (Racy et al., 2005). However, studies on prokaryotic specific cell volume show interesting link with biomass in aquatic environments (La Ferla et al., 2010) suggesting the direct use of cell size to better quantify the prokaryotic standing stock.

To date, the mechanisms controlling size and shape distribution are not fully known. The top-down control mechanism based on grazing activity by predators surely constrains the prokaryotic cell size and the community composition (Chrzanowski & Šimek, 1990; Šimek et al., 2001). Among the bottom-up control mechanisms, temperature, nutrient conditions, and primary producers are often used as explanatory variables (Gasol & Duarte, 2000).

A discrete variability of prokaryotic cell size has already been observed in aquatic ecosystems (Chrzanowski et al., 1988; Šestanović et al., 2005; Mahadevaswamy et al., 2008). Relatively few studies have so far dealt with prokaryotic size and shape in relation with environmental parameters (La Ferla et al., 2012; Sjöstedt et al., 2012 and references therein), and even a lower number has considered whether these parameters vary in relationship with the trophic status in different water bodies (Nakano & Kawabata, 2000; Gurung et al., 2002; Lind & Barcena, 2003). Comparative studies between tropical and temperate aquatic environments have rarely been done on this specific topic (Furtado et al., 2001; Hernández-Avilés et al., 2012).

In this study, we explored the variations of prokaryotic cell size and shape in selected freshwater, brackish, mixoeuhaline and estuarine water bodies of temperate (Italy), and tropical (Brazil) environments, in relation to physical, chemical, and trophic parameters.

The prokaryotic cell abundances and volumes were quantified by image analysis, and biomass was determined multiplying the abundance measurements by the conversion factors derived from cell volume estimates. At the same time, auxiliary physical, chemical, and trophic parameters were determined. In addition, in a few stations, flow cytometry analysis was performed to distinguish sub-populations having a different apparent DNA content.

The specific goals of this study were: (i) to evaluate the relation between prokaryotic organisms and environmental parameters in order to look for the main factor driving cell size distribution, (ii) to investigate the prokaryotic size distribution in different trophic states, and (iii) to assess whether the cell volume can be used as a satisfactory descriptor of trophic status of different water bodies.

Materials and methods

Study sites and sampling

The research was carried out in different freshwater, brackish, mixoeuhaline, and estuarine environments located at different latitudes in Sicily (Italy) and in the State of Rio de Janeiro (Brazil) (supplementary Table 1).

The freshwater environment examined in this study, the artificial Pozzillo Lake, is a large reservoir located in a continental area of the Sicily that receives the waters of the Salso River and of the streams descending from the surrounding hills. The brackish Oliveri-Tyndari lagoon system, in the North coast of Sicily, is constituted of four brackish-mixoeuhaline ponds, each one showing peculiar hydrobiological features, in relation to the different inputs of marine and continental waters. The Cape Peloro lagoon system, located in the N-E coast of Sicily, is constituted by two neighboring ponds, Ganzirri (max depth 7 m) and Faro (max depth 28 m), both characterized by high biodiversity and productivity which makes them suitable for exploitation of biological resources.

Finally, Guanabara Bay is a tropical estuarine system located in Rio de Janeiro which harbors the second largest city in Brazil. The bay is considered one of the most eutrophized areas in the world. Since the human impacts are not uniformly distributed, the bay water quality differs spatially depending on the pollution focuses, tidal influence, and circulation water patterns (Mayr et al., 1989).

Pozzillo Lake as well as Cape Peloro and Oliveri-Tyndari lagoon systems were sampled in December 2008 and in March 2009 with a Niskin bottle aboard a rubber dinghy. In Guanabara Bay, sampling was performed in November 2009 with Van Dorn bottles aboard a motor boat. In Pozzillo Lake, water samples were collected at one station in 2008 (P) and at five stations in 2009 (P.1, P.A, P.B, P.C, and P.D). In Oliveri-Tyndari system, water samples were collected from the central part of four ponds i.e., Porto (POR), Mergolo (M), Marinello (MAR), and Verde (V). In Cape Peloro system, samples were drawn from three stations in Ganzirri pond (G-1, G-6, and G-9) and one station in Faro pond (F). In Guanabara Bay, six stations were sampled: GB.1, GB.7, GB.34, and GB.PQ at two depth layers, GB.Urca, and GB.Cajù at surface layer only.

Physical, chemical and trophic parameters

Temperature (*T*), salinity (*S*), and dissolved oxygen (DO) measurements were taken using a handheld multiparametric probe sensor (SBE 19 Plus). For DO, water samples were analyzed using Winkler's method (Carpenter, 1965). Nutrient determinations of nitrite (NO_2^-), nitrate (NO_3^-), and dissolved reactive phosphorus (DRP) were performed according to Strickland & Parsons (1972), while ammoniacal nitrogen (NH_4^+) according to Aminot & Chaussepied (1983). Total suspended matter (TSM) was evaluated by a gravimetric method and Chlorophyll-*a* (Chl-*a*) according to Lazzara et al. (1990).

Guanabara Bay water samples underwent physical and chemical measurements by standard oceanographic methods (Grasshoff et al., 1999).

The waters samples were classified as oligo-, meso-, eu-, and hypertrophic by comparing synoptically different trophic indices determined applying the criteria indicated by the Organization for Economic Cooperation and Development (OECD; Giovanardi & Tromellini, 1992) together with the classifications derived from the trophic status index TRIX, according to the Italian D.Lgs. 152/99, and the Trophic State Index (TSI), by Carlson (1977). Obviously, the definition of the trophic state is only related with the temporary condition of the considered water bodies at the sampling moment. In this study, the seasonal variability of the trophic conditions was not considered.

The trophic state classification in the OECD is performed for studying eutrophic marine coastal environments and simply consists in a geometric mean (based on log 10 transformation) of the total phosphorous, total nitrogen, Chlorophyll-*a* concentrations.

The TRIX index is the only index recognized by the Italian law for classifying the trophic status (quality) of marine coastal environments. It is suitable for systems strongly influenced by terrigenous inputs (TRIX index = (Log(Chl-*a*|OD%|NP) − (−1.5))/1.2).

The TSI of Carlson (1977) allows the classification of lakes according to their algal biomass, as derived from measurements of chlorophyll pigments, or Secchi depth, or total phosphorus. In this study, to estimate TSI, Chl-*a* was chosen in accordance to Carlson (1983) formula TSI (Chl-*a*) = 9.81 ln(Chl-*a*) + 30.6.

Prokaryotic abundance, size, biomass, and shape

All the samples for the prokaryotic abundance (PA), size (VOL), and shape determinations were directly collected in sterile condition in falcon tubes (polyethylene), immediately fixed with prefiltered formaldehyde (0.22 μm porosity; final conc. 2%) and stored in the dark at 4°C to prevent contamination till the laboratory treatment (within 10 days). Two replicates of water samples were filtered through polycarbonate black membranes, porosity 0.22 μm (GE Water & Process Technologies), and stained for 10 min with 4′,6-diamidino-2-phenylindole (DAPI, Sigma, final concentration 10 μg ml^{-1}) according to Porter and Feig (1980). A Zeiss AXIOPLAN 2 Imaging (magnification: Plan-Neofluar 100× objective and 10× ocular; HBO 100 W lamp; filter sets: G365 exciter filter, FT395 chromatic beam splitter, LP420 barrier filter) equipped with the digital camera AXIOCAMHR (Zeiss) was used. The images were captured and digitized on a personal computer using the AXIOVISION 3.1 software for the subsequent morphometric analysis. The standard resolution of 1,300 × 1,030 pixels was used for the image

acquisition. The pixel size in the resulting image was 0.106 μm by automatic calibration. Further calibration was performed by measuring a FITC-stained suspension of monosized latex beads (diameter, 2.13 μm). According to Lee & Fuhrman (1987), the pixels that constituted the fluorescent "halo" around the bacterial cells were not measured. To prevent the DAPI fading, the cell counts were done on pictures and at least 10 fields of view were evaluated for each sample. The recognition of the cells was done by one only experienced operator who discharged the misclassified objects from count and measurement. The volume (VOL, expressed in μm^3) of individual cell was derived from two linear dimensions (width, *W*, and length, *L*) manually obtained. Curved objects were drawn by curve spline. VOL of a single cell was calculated according to the geometrical formula adopted by Krambeck et al. (1981):

$$\begin{aligned} &\mathrm{VOL} = (\pi/4)W^2(L - W/3) \quad \text{for coccal forms,} \\ &W = L \end{aligned}$$

assuming that the cells are cylindrical straight rods with hemispherical or, in the case of coccoid forms, spherical caps (Massana et al., 1997).

To convert VOL in cell carbon content (CCC, fg C $cell^{-1}$), the allometric relation proposed by Loferer-Krößbacher et al. (1998) and routinely adopted for DAPI-stained cells in marine and limnetic environments was used:

$$\mathrm{CCC} = 218 \times \mathrm{VOL}^{0.86}$$

assuming that 80% of the biovolume consisted of water, while the other part of the dry weight (20%) is considered to be constituted by 50% carbon (Bölter et al., 2006).

Prokaryotic biomass (PB, μg C l^{-1}) was calculated by multiplying the PA of each sample by the corresponding CCC derived from VOL:

$$\mathrm{PB} = \mathrm{PA} \times \mathrm{CCC}.$$

Errors during biomass calculation by PA and VOL accounted for >5 and ~3%, respectively, as already estimated by Bölter et al. (2002).

The shape classification was performed by the experience of the same operator. Cells were operationally defined as cocci if their length and width differed by less than 0.10 μm, coccobacilli if their length and width differed by more than 0.10 μm, and rods if their length was at least double their width; C-shaped and S-shaped cells were defined vibrios and spirillae, respectively; cells exceeding 4 μm in length were defined as filamentous bacteria.

Flow cytometry

Water samples for flow cytometry were preserved by fixation with filtered (0.22 μm) paraformaldeyde 2% (final concentration) for 15 min and stored in liquid nitrogen. The samples were stained with Syto13 at a 2.5 μM final concentration (Gasol & del Giorgio, 2000; Andrade et al., 2003). Prokaryotic abundance cytometric counts (PA^C) were obtained with a CyAn ADP flow cytometer (Dako, USA) equipped with a solid state laser (488 nm, 25 mW) and filter modifications (green FL1 to 515 ± 30 nm, red FL4 to 660 ± 30 nm). For calibration of side scatter and green fluorescence signals, and as an internal standard for cytometric counts and measures, fluorescent latex beads (1.58 μm diameter) were systematically added. Based on optics and fluorescence signals, HNA and LNA cells abundances were also determined (Gasol & del Giorgio, 2000).

Statistical analyses

By using the beanplot package (Kampstra, 2008) from R software (v. 2.14.2), beanplots of the PA, VOL, and PB in the aquatic systems with different trophic status (oligo-, meso-, eu-, and hypertrophic) were generated. This kind of plots is an extended version of the well-known boxplots; in this case, the empirical distribution of the data is also shown. Descriptive statistical analysis and Spearman Rank correlations were performed with the SigmaStat software V3.0.

For each trophic state, the Shannon index of diversity (H') was calculated from the relative frequencies of volumes (Size Diversity Index, SDI) and of each morphotypes (Morphological Diversity Index, MDI) according to Racy et al. (2005).

Multivariate analysis among PA, VOL, PB, and environmental parameters was performed using the Primer 6 package (Clarke & Gorley, 2006). Hierarchical cluster analysis (HCA) using the Euclidean distance was applied to test the resemblance level of the different water bodies as well as principal component analysis (PCA) to reduce the environmental variables down to a few components (Jolliffe, 2005).

PCA generated new variables, called principal components (PC), which explained the dispersion of the samples. Variables with the highest loadings had the greatest influence on the samples separation.

Results

Physical and chemical parameters, and trophic status

Physical (*T*), chemical (*S*, DO, NH_4^+, NO_2^-, NO_3^-, and DRP), and trophic parameters (Chl-*a* and TSM) of each sample in relation with the different trophic status were reported in supplementary Table 2, together with prokaryotic parameters. Obvious differences in *T* between the temperate and tropical systems (*T* range 10–29°C) and in *S* between freshwater and the other environments (*S* range 0.51–37.89) were found. Low DO values, together with high NO_2^- and Chl-*a* concentrations, were recorded at some GB stations, where anoxia was detected. Pozzillo Lake showed low TSM values. Low *S* values were observed in Marinello pond and at st. GB.34-0.5m in 2009, when compared to neighboring stations.

By using together the three above referred indices to describe the trophic state, similar classifications of the water samples were obtained. Some stations kept the same trophic status in the different periods, and namely those collected from Pozzillo Lake and Verde (classified as eutrophic), Ganzirri (mesotrophic), and Porto (oligotrophic) ponds. Guanabara Bay was in hypertrophic condition, except for stn.s. GB.PQ-9m and GB.1-20m which were oligotrophic and mesotrophic, respectively. Marinello and Mergolo waters were both classified as mesotrophic in December 2008 and oligotrophic in March 2009. Conversely, Faro pond waters were classified as oligotrophic and mesotrophic in December 2008 and March 2009, respectively.

Prokaryotic abundance, size, biomass and shape

Mean values of PA, VOL, and PB, together with HNA and LNA percentages, detected in the different sampling stations in relation with trophic states, are reported in supplementary Table 2. PA ranged from 0.33 to 5.91 cell $\times$ 10^7 ml^{-1} in oligo- and hypertrophic waters, respectively. VOL values ranged from 0.053 to 0.466 μm^3 and CCC from 16.98 to 101.69 fg C $cell^{-1}$, detected in oligo- and eutrophic waters, respectively. PB accordingly to PA varied from 88.37 to 2411.28 μg C l^{-1} in oligo- and hypertrophic waters, respectively.

Four beanplots show the distribution of the mean values of PA, VOL, and PB in each trophic status (Fig. 1). On the whole, PA increased from the oligo- to hypertrophic systems. Nevertheless, PA was distributed in homogeneous cores in the oligo-, meso-, and eutrophic waters, respectively, and the respective mean values were lower than or close to the total mean value. In the hypertrophic water, the mean PA value peaked and the widest distribution of values were observed around the average. Taking into account all the measured cells (about 4,500), in oligotrophic waters, the majority of VOL were distributed in a core which fell close to the mean value (0.123 ± 0.060 μm^3). In mesotrophic waters VOL distribution was quite similar to the oligotrophic ones, but a secondary core was well-defined and the mean value increased (0.156 ± 0.096 μm^3). In eutrophic waters, the largest cells were detected and the mean VOL raised considerably (0.258 ± 0.127 μm^3) also with respect to the global average (0.172 ± 0.099 μm^3). In hypertrophic waters, few variability of VOL was observed, and the cell sizes were entirely distributed in a single core near the overall average (0.154 ± 0.031 μm^3) (Fig. 1). The mean CCC values ($\pm$SD) calculated in the oligo-, meso-, eu-, and hypertrophic waters were 34 ± 14, 41 ± 20, 62 ± 25, and 41 ± 7 fg C $cell^{-1}$, respectively, depicting a distribution which was similar to that of cell volumes.

Also PB increased with the increasing trophic levels. Its distribution appeared to be mainly modulated by VOL in eutrophic waters and, in a lesser extent, in the mesotrophic ones while by PA in oligo- and hypertrophic waters (Fig. 1).

The class frequency of the dimensional sizes (as percentage) is reported in Fig. 2. Considering all the studied water bodies, the most frequent size-class fell in the range from 0.02 to 0.049 μm^3 accounting for a mean percentage of 21% of the total. Just after, the size-class 0.05–0.079 μm^3 accounted for the 19%. Within this size-class, a weak different cell distribution among the different trophic states was noticed. The size-class >0.6 μm^3 contributed as a mean to only the 6% of the total, but in the eutrophic waters, it reached the 12% of the total.

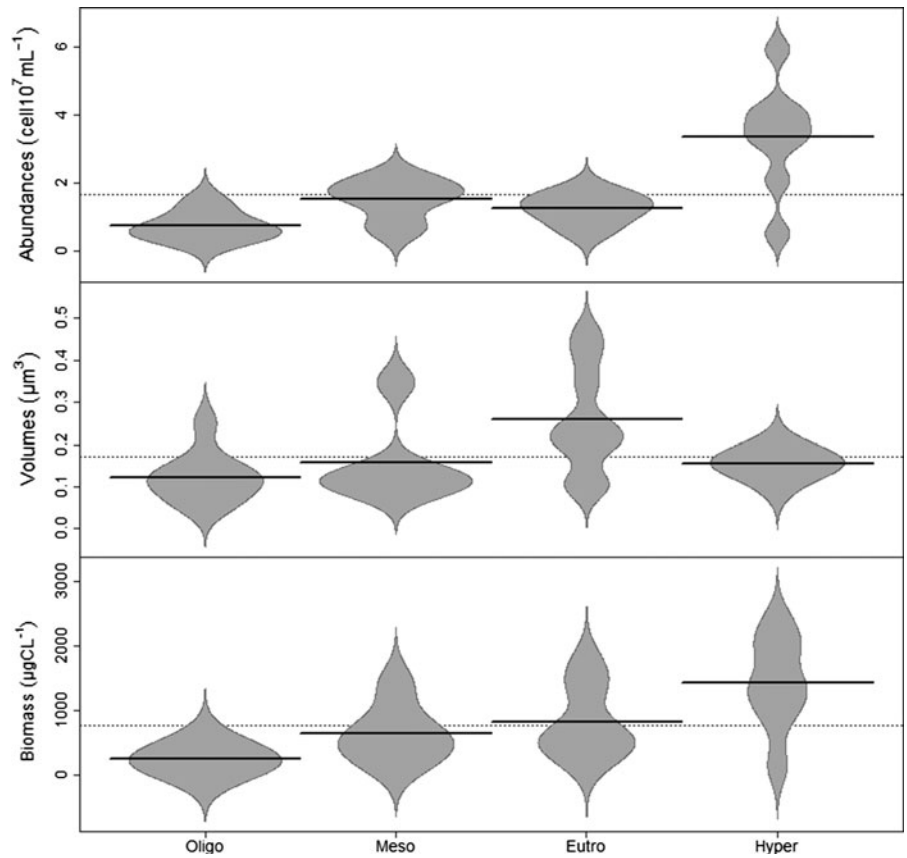

Fig. 1 Beanplots of the entire dataset of the volumes, abundances, and biomass of prokaryotic cells distributed in each trophic status. *Dashed lines* overall mean values; *black lines* mean values within each trophic status; *gray areas* empirical distribution of each parameter

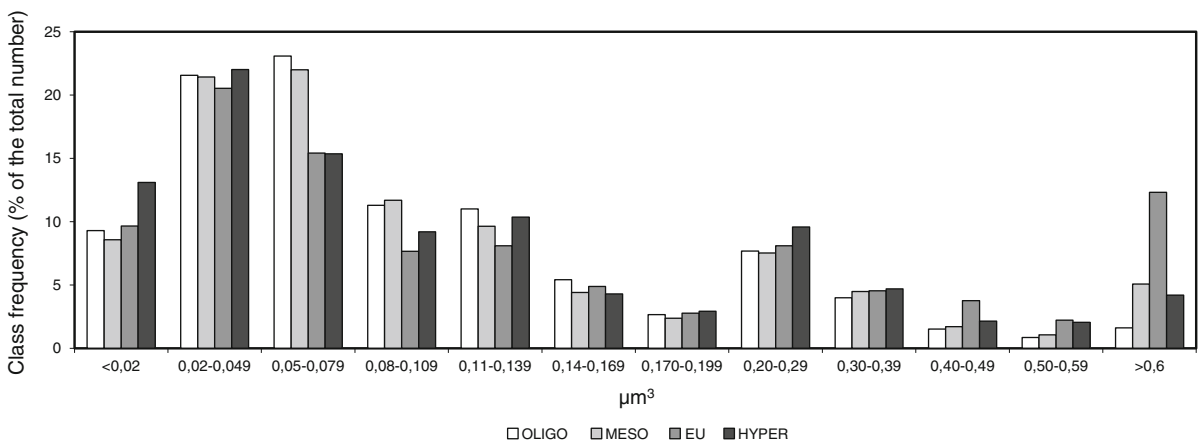

Fig. 2 Class frequency of the dimensional sizes (as percentage of the total) in the different trophic states

PA^C obtained only in Guanabara Bay (samples $n = 10$) fell in a range of 0.29–4.99 cells $\times$ 10^7 ml^{-1}, recorded at GB.7-20m and GB.34-0.5m, respectively (data not shown). PA^C were similar to the image analysis counts (PA) resulting in a highly significant correlation ($R^2 = 0.9065$; linear regression: $y = 0.8246x - 0.4016$). However, although SYTO is considered better to stain cell content than DAPI, PA^C

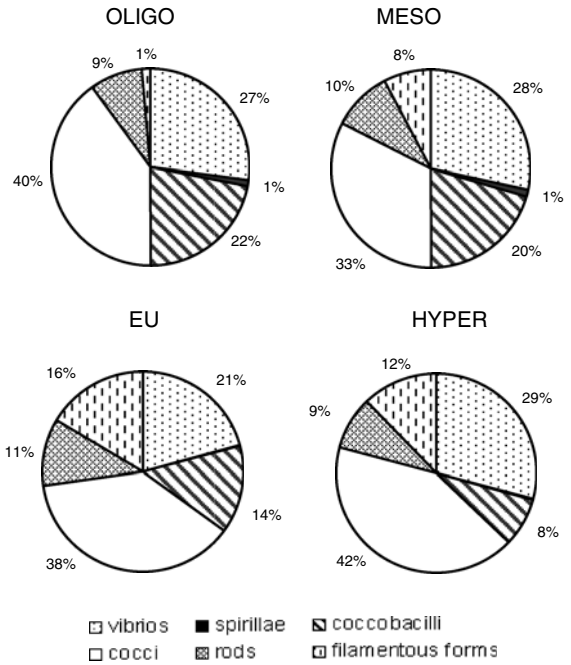

Fig. 3 Incidence of the morphotype abundances (as percentage of the total) in the different trophic states

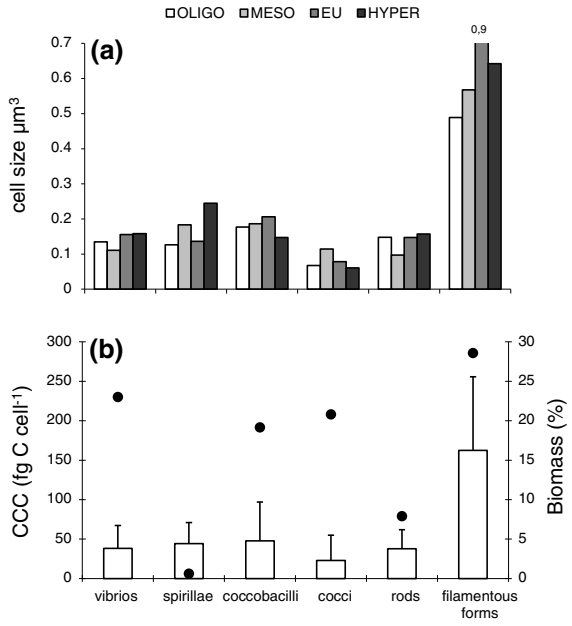

Fig. 4 Cell size of the morphotypes in the different trophic states (**a**) and cell C content together with the relative biomass (as percentage of the total, *filled circle*) of each morphotype (**b**)

were always slightly lower than PA. This result could be a consequence of the weak fluorescence signal by smaller cells, whereas the weakening over time must be excluded because of the prompt analyses, performed within a few days after the sample collection.

HNA cells prevailed over the LNA cells with the only exception of GB.1-20m and GB.7-20m samples (supplementary Table 2).

The different shapes of the cells were ascribable to the six following morphotypes: vibrios, cocci, coccobacilli, spirillae, rods, and filamentous forms. Cocci, including cyanobacteria, were the most frequent morphotype in all the trophic water bodies, accounting on average for the 38% of the total (Fig. 3); slightly lower counts were obtained in mesotrophic waters. Vibrios represented the second frequent morphotype (on average the 26% of the total) with a lower percentage in eutrophic waters than in the others. With the increase of the trophic status, coccobacilli incidence decreased, while from oligo- to eutrophic waters, the filamentous forms increased. In this latter category, the straight filaments without visible septae and bacterial chains were included. Few rods (9%) and negligible spirillae were observed, both showing a distribution scarcely affected by trophic conditions.

With respect to cell-size (Fig. 4a), cocci showed the smallest volumes (mean value: 0.08 μm^3); rods and vibrios (both 0.14 μm^3) were particularly small in mesotrophic waters; coccobacilli and spirillae had similar mean VOL (0.17 and 0.18 μm^3, respectively), but the last ones showed a greater size in hypertrophic waters than in others. On the whole, spirillae were thinner than vibrios (mean width 0.22 and 0.36 µm, respectively). Finally filamentous forms showed the greatest size (mean VOL 0.66 μm^3), particularly in eutrophic waters.

CCC and related biomass (as percentage of the total biomass) of each morphotype are shown in Fig. 4b. Filamentous forms accounted for the highest CCC value (>150 fg C $cell^{-1}$), while the other forms did not reach 50 fg C $cell^{-1}$. The greatest weight in terms of biomass was due to the filamentous forms—accounting for the 29% of the total biomass—albeit they were less numerous, followed by vibrios (23%) that were relatively abundant. Cocci, small but numerically predominant, contributed to the 20% and coccobacilli to the 19% of the total biomass. Notwithstanding their discrete size, rods and spirillae accounted barely for the 8 and <1% of the total biomass.

Table 1 Spearman-rank significant correlations among prokaryotic cell volumes, abundance, and biomass versus the environmental parameters in the studied waters—all together and in the different trophic states

	n	PA		VOL		PB	
		r	<*p*	*r*	<*p*	*r*	<*p*
All together							
T (°C)	37	0.483	0.002	n.s.	–	n.s.	–
S	37	n.s.	–	−0.599	0.000	−0.468	0.003
DO	37	−0.499	0.001	n.s.	–	−0.479	0.002
Chl-*a*	37	0.521	0.001	0.327	0.048	0.629	0.000
PA	37	–	–	0.350	0.033	0.889	0.000
PB	37	0.889	0.000	0.711	0.000	–	–
HNA (%)	10	0.933	0.000	0.713	0.019	0.957	0.000
LNA (%)	10	−0.894	0.000	−0.693	0.022	−0.936	0.000
TSM	37	0.422	0.009	n.s.	–	n.s.	–
Oligo-							
DO	9	n.s.	–	−0.661	0.042	−0.729	0.020
DRP	9	−0.661	0.042	n.s.	–	−0.717	0.024
PB	9	n.s.	–	0.683	0.036	–	–
Meso-							
PA	11	n.s.	–	n.s.	–	0.791	0.002
PB	11	0.791	0.002	0.800	0.001	n.s.	–
Eu-							
DO	9	n.s.	–	−0.717	0.024	n.s.	–
PA	9	n.s.	–	n.s.	n.s.	0.800	0.006
PB	9	0.800	0.006	0.867	0.000	–	–
Hyper-							
T (°C)	8	0.761	0.021	n.s.	–	0.822	0.005
S	8	−0.857	0.001	n.s.	–	−0.905	0.000
NO_2^-	8	0.714	0.037	n.s.	–	n.s.	–
PA	8	n.s.	–	n.s.	–	0.952	0.000
PB	8	0.952	0.000	n.s.	–	–	–

For parameter abbreviations see the text

n.s. not significant

Statistical analyses

The seasonal variability of the studied systems as well as its ecological implications was deliberately neglected by the given statistics.

Analyzing all the samples together, without distinguishing among the trophic status, the Spearman-rank analysis showed few but highly significant correlations among PA, VOL, and PB versus the other environmental parameters (Table 1). PA correlated positively with *T*, Chl-*a*, HNA%, and TSM and negatively with DO and LNA%. VOL correlated negatively with *S* and positively with Chl-*a*. Moreover VOL was more significantly related to PB than to PA, and positive correlations of VOL with HNA% and negative with LNA% were also computed. PB showed negative correlations with *S*, DO, and LNA%, and positive with Chl-*a* and HNA%.

Grouping the data within the different trophic status (again Table 1), PA did not correlate with PB in oligotrophic waters only, and it showed negative relations with DRP and *S* in oligo- and hypertrophic waters, respectively. Significant inverse relationship between VOL and DO in eutrophic and hypertrophic waters only was found; VOL did not correlate with PA while it did with PB in oligo-, meso- and eutrophic

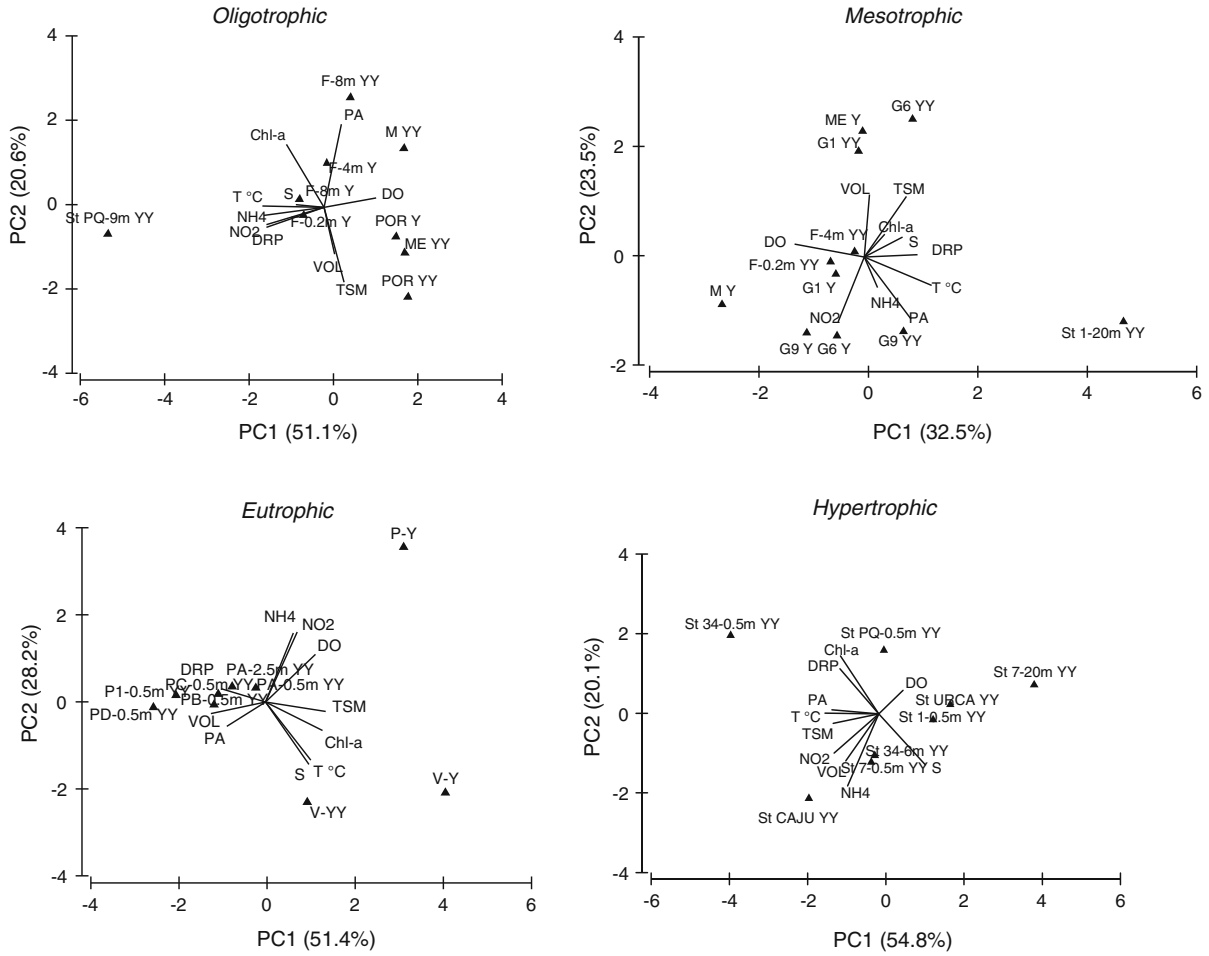

Fig. 5 Principal components analysis (PCA) among the physical, chemical, and biological parameters considering the trophic states separately. PCA with eigenvalues >1 were considered only. $Y = 2008$, $YY = 2009$

waters. In hypertrophic waters, PB was positively correlated to *T* and negatively to *S*.

Grouping the samples according to their trophic status, the reciprocal interactions among the physical, chemical, and biological parameters were analyzed by PCA, and the projection of the factor loadings on the plane described by the 1st and 2nd principal factors was reported (Fig. 5). Only the PCs with eigenvalues >1 were considered. In oligotrophic waters, PC1 (explaining up to the 51.1% of total variability) consisted of physical and chemical parameters (*T* and nutrients), while PC2 (representing the 20.6% of total variability) was composed by biological and trophic indicators (PA, TSM, and Chl-*a*). In mesotrophic waters, PC1 (accounting for the 32.5% of total variability) was dominated by *T* and DO, while PC2 (explaining the 23.5% of total variability) by nutrients, VOL and TSM. In eutrophic waters, PC1 explained the 51.4% of total variability and was constituted by physical, trophic, and biological parameters (TSM, Chl-*a*, VOL, DO, and *T*); PC2 (accounting for the 28.2% of variability) was constituted by chemical and hydrological parameters (nutrients, *S*, *T* and DO). Finally, in hypertrophic waters, PC1 (explaining the 54.8% of variability) was represented by PA, *T*, *S*, and TSM, while PC2 (accounting for the 20.1% of variability) was related to NH_4^+, Chl-*a*, *S*, VOL, and DRP. Overall, the PCA biplots highlighted the close relationships existing between VOL and TSM.

HCA, performed on 37 complete data from the four trophic systems, yielded the dendrogram reported in Fig. 6, where one cluster, grouping together some

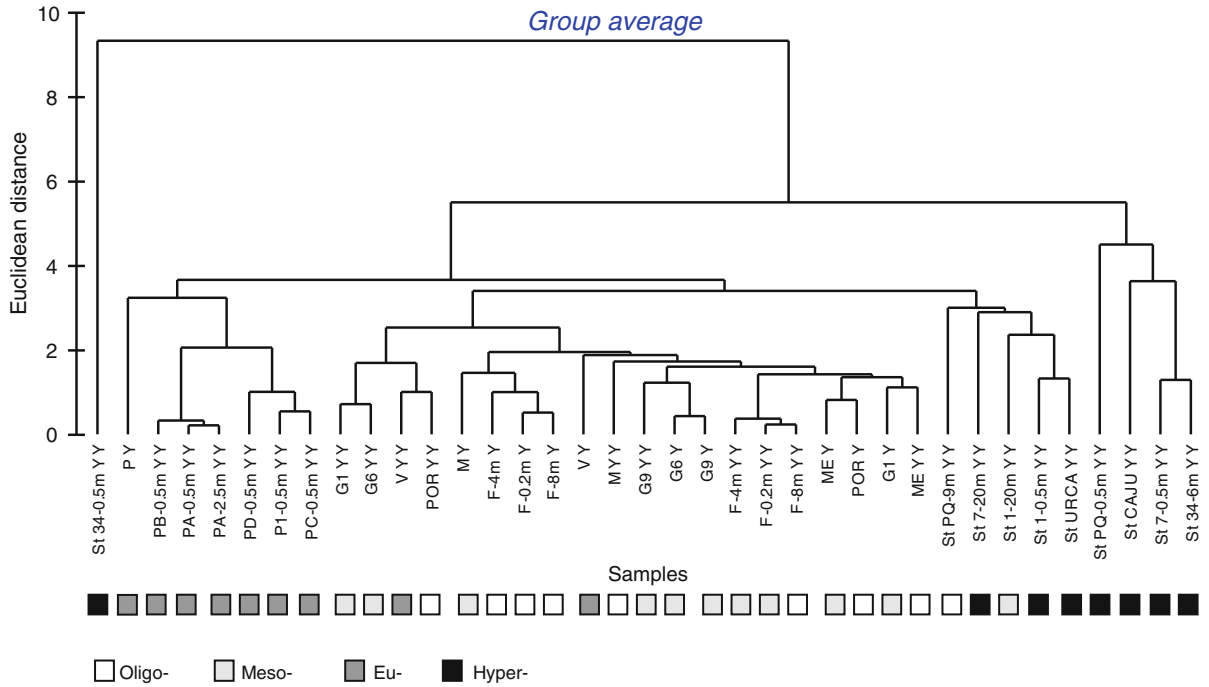

Fig. 6 Dendrogram obtained by hierarchical cluster analysis using the Euclidean distance to test the resemblance level of different trophic water bodies. $Y = 2008$, $YY = 2009$

hypertrophic samples (Euclidean distance 4.49), was clearly separated from the other water bodies (3.4). Within the last, a group which enclosed most of the eutrophic samples (3.23) and two mixed clusters were further differentiated. The St.GB.34-0.5m appeared to be incongruent with the other samples (9.35) forming a cluster by itself.

As regards the SDI and the MDI, both the indices showed small variation (data not shown). However, SDI increased from oligo- to eutrophic waters (from 0.85 to 0.93, respectively). MDI displayed the opposite trend and decreased from 0.69 to 0.60, respectively. Nevertheless, in hypertrophic waters, an inversion was found with a weak deflection of SDI (0.91) in concomitance with an increase of MDI (0.66).

Discussion

Few papers investigate the phenotypic characteristics of planktonic cells notwithstanding they provide an approach for analyzing the ecosystems structure allowing to better quantify biomass as well as cell heterogeneity in mixed assemblages (Quinones et al., 2003). Changes in size and morphology of unicellular organisms, included prokaryotic cells, have been proposed as sensitive indicators of trophic and climatic changes in aquatic ecosystems (Pernthaler & Amann, 2005; Young, 2006). As a consequence, several automated or semiautomated procedures by image analysis (Bloem et al., 1995; Bölter et al., 2006) and different algorithms for the different dyes (Loferer-Krößbacher et al., 1998) have been applied for the cell volume calculation. Recently, Zeder et al. (2011) showed the impact of different algorithms on the accuracy for different morphologies, and provided a novel algorithm that is accurate for all cells, independent of their shape.

Relationships of prokaryotic cell size and shape versus environmental parameters

Convergences and divergences between our dataset and results reported in the literature are examined. On the whole, prokaryotic cell size results to be independent from the water temperature, as shown by the lack of significant correlations, corroborating evidences in several marine waters and sediments in the

Mediterranean Sea (Šestanović et al., 2005; La Ferla et al., 2012). Similar findings were also found in temperate and tropical aquatic environments (Gocke et al., 2004; Mahadevaswamy et al., 2008; Hernández-Avilés et al., 2012). Conversely, in a study on cell size seasonal variation in Lake Aarlington, the cell volume inversely correlated with water temperature but not appreciably above 17°C (Chrzanowski et al., 1988). In our study case, excluding the samples warmer than 20°C, the relationship between VOL and T is described by a polynomial regression ($y = 0.0099x^2 - 0.2865x + 2.1929$; $r = 0.477$; $P > 5\%$; $n = 27$). It is also conceivable that water temperature acts simultaneously with other factors (organic nutrient quality and quantity, phytoplankton activity, flagellate grazing) in controlling bacterial dynamics as it has already been observed in Canadian Fjord (Albright & McCrae, 1987). An on-field study on cell volume in an epilimnetic lake suggested that temperature versus VOL negative correlation could imply that cells may be less subject to predation pressure during warm condition, merely reflecting the cropping of larger cells by predators (Chrzanowski et al., 1988). Anyhow, temperature exerts a direct significant influence on the cell abundance as it has already been shown by Araújo & Godinho (2008), Ducklow et al. (2012). Stepwise multiple regression analysis revealed that temperature influenced also the relative bacterial richness in the North Sea (Winter et al., 2005). However, in our study case, lower bacterial numbers are found in temperate systems than in tropical one as also referred by Hoppe et al. (1998).

On the whole, water salinity influences negatively both VOL and PB, but not PA unlike what detected by Gocke et al. (2004) in a hypertrophic tropical lagoon in Colombia where only small cocci and short rods were considered, excluding larger rods and filamentous bacteria. Since salinity is an index of dilution by freshwater input, it is reasonable to assume that volume variability is an indirect response to riverine or terrestrial inputs. The prokaryotic cell volume was positively affected by drainage of allochthonous matter and the degree of humification (Teixeira et al., 2011).

In our study areas significant inverse relationships between VOL and DO in eu- and hypertrophic waters are found, in agreement with previous results in temperate and tropical lakes (Hernández-Avilés et al., 2012). Nevertheless, in that study case, the direct competition between small cell bacteria and larger primary producers or the availability of labile substrates—freshly produced by phytoplankton in well-oxygenated water—and the availability of refractory sources—below the hypolimnion—were invoked as reasonable causes. However, differently from that paper, no stratification occurs in our samples due to the shallowness of the examined waters. In GB samples only, in two samples taken at 20 m, seawater intrudes at this deep layer. Instead, no correlations between nutrient concentrations and VOL, as well as PA and PB are detected on the whole dataset, conversely to other studies where the stimulation of photosynthesis by nutrients determined a cascade effect on secondary production and PB (Wu et al., 2007). In oligo- and hypertrophic waters only, negative and positive relationships between PA versus DRP and NO_2^-, respectively, are detected. Conversely, a negative impact by NO_3^- on prokaryotic cell size was shown by Kalcheva et al. (2010) who stated that the quantities of nutrients determined only the potential productivity of a lake, while the actual productivity depended on the structures of communities.

Significant positive correlations among Chl-*a* versus PA, VOL, and PB indicate a direct link of phytoplankton dynamics with prokaryotic variables, suggesting the prokaryotic utilization of autochthonous and labile organic substrates, produced by photosynthesis or released by phytoplankton. Since under C and P limitation, the width–length ratio (*W*/*L*) of bacterial cell increases, while under N limitation, it decreases (Vrede et al., 2002), in temperate lakes, the bacterial growth was limited by P (mean *W*/*L* ratio: 0.68), while in tropical lakes by N (mean *W*/*L* ratio: 0.21) (Hernández-Avilés et al., 2012). In our samples, the ratio is lower (mean *W*/*L* ratio: 1.44, with a peak of 2.2 in the eutrophic water), suggesting C and P as limiting factors for cell size.

As regards the HNA cells, the general conclusion from the literature is that this kind of cells is more responsive to Chl-*a* variability and more active on a cell basis than LNA cells. In our study, HNA% always prevails over LNA% except for two samples where seawater intrudes at depth. However, the contribution of LNA cells to total bacterial production is still a subject of much debate (van Wambeke et al., 2011).

As concerns the cell morphology and environmental parameters, a shift of the cell shapes has already been assessed from rods to coccal forms in relation

with water temperature increases (Sjöstedt et al., 2012). Interestingly, this finding might be due to an adaptive strategy of the cells to the increasing temperature or, differently, to the dominance of a certain phylotype on others; it was not clear whether the changes in volume were at the species level or at the community level.

Prokaryotic size and shape versus trophic status

Besides PA, which has been found to vary from oligo- to hypertrophic systems in the present and other studies (Ducklow & Shiah, 1993; Cotner & Biddanda, 2002), VOL increases with increasing trophic states but it undergoes a reduction in hypertrophic waters. A similar dichotomy between cell abundances and cell sizes has already been observed in hyper-eutrophic lakes (Sommaruga & Robarts, 1997), and it could be due to several factors. In dystrophic conditions, heterotrophic production could stimulate cell duplication at the expenses of cell enlargement (Racy et al., 2005) as well as the drainage from continental waters could sustain bacterial growth by furnishing allochthonous organic carbon of non-algal origin (Araújo & Godinho, 2008). Moreover, the drainage of allochthonous sources in the hypertrophic waters seemed to negatively affect the cell size owing to more recalcitrant organic carbon (Tranvik, 1992). In our study, in the hypertrophic waters, at all constituted by Guanabara Bay samples, high inputs of industrial and municipal waste enter the bay probably acting as an important forcing in controlling the prokaryotic assemblage, by stimulating cell reproduction but not cell growth. Also heavy metal contamination was recognized to affect the shape, structure, and taxonomic distribution of prokaryotic community (Ellis et al., 2003).

Shape and size of cells can be related to nutrients availability because the variation in surface-to-volume ratio implies changes in the capacity of nutrients absorption. However, in oligotrophic environments, prokaryotes are favoured by their small size and assimilate more efficiently nutrients at lower concentrations than phytoplankton. Moreover, nutrient and substrate resources did not always work simultaneously in mesocosm experiments (Øvreås et al., 2003). These findings agree with our results where the cell-size variability is not related to the availability of inorganic nutrients. Nevertheless, the correlation between prokaryotic parameters and Chl-*a* hints a positive relationship with phytoplankton biomass and autochthonous production. However, conflicting results, reporting no relation between Chl-*a* and prokaryotic parameters, were gathered in floodplain environments (Teixeira et al., 2011).

Since the cell C content is the direct result of cell-size variability, our data corroborate the suggestion that volume-to-biomass conversion factors are highly variable in relationship with trophic dynamics, as well as temporally and geographically (Kroer, 1994; La Ferla et al., 2010). In fact in the oligo-, meso-, eu-, and hypertrophic waters, wide contents of carbon per cell are obtained, 1.7, 2.0, 3.1, 2.0 times higher than the most currently adopted conversion factor in marine ecology (20 fg C $cell^{-1}$, according to Lee & Fuhrman, 1987). In natural assemblages, the use of a single coefficient applied for all cells implies that the C content per cell is rather constant, assessing that small cells have a higher dry weight than larger ones. Instead the allometric model—adopted in this paper—assumes that the dry weight-to-volume ratio is linearly size-dependent, and smaller organisms have a higher dry weight-to-volume ratio than larger ones (Norland, 1993). Consequently the measurement of the individual cell size should be applied in as much as the a priori determined C conversion factor could underestimate or overestimate the actual biomass.

Morphotype distribution is an aspect of a prokaryotic population that allows the comparison among several communities with ecological significance (Racy et al., 2005). Despite their small variations, SDI and MDI show opposite trends varying with the trophic status of our sampled waters. SDI is low in the oligo- and mesotrophic environments and high in the eu- and hypertrophic ones. Conversely MDI—which includes both the richness and evenness of morphotypes—is lower in the eutrophic water than in the others indicating high variability in cell size and low variability in cell morphotypes. In fact, in eutrophic environments, cocci dominate presumably as a consequence to their efficient reproductive strategy (Racy et al., 2005), besides being more resistant to predatory pressure than rods (Chrzanowski & Šimek, 1990). Such a predominance could indicate also no nutrient limitation for this prokaryotic shape (Jochem, 2001; Teixeira et al., 2011). Differently from cocci, spirillae show relatively large cell size but low abundance and negligible biomass. Conversely, filamentous forms

Table 2 A synthesis of the ranges of prokaryotic cell size (as μm^3) determined in waters within different environments and with different trophic status

	Min	Max	References
Oligo-			
NW-MED (Catalan-Balearic Basin)	0.05	0.08	van Wambeke et al. (2002)
Tyrrhenian Sea: July	0.026	0.102	La Ferla et al. (2010)
Tyrrhenian Sea: December	0.033	0.12	La Ferla et al. (2010)
Ionian Sea	0.003	0.927	La Ferla et al. (2004)
NW-Atlantic (Long Island)	0.036	0.073	Lee & Fuhrman (1987)
This study	0.053	0.252	This study
Mean value	*0.034*	*0.259*	
Meso-			
Pitumbu-Jiqui system Brazil	0.120	0.540	Araújo & Godinho (2008)
Lake Biwa Japan	0.080	0.140	Gurung et al. (2002)
Riverine and transition zone Europe	0.084	0.359	Lind & Barcena (2003)
This study	0.120	0.210	This study
Mean value	*0.101*	*0.312*	
Eu-			
Municipal Lake Yaoundé Cameroon	0.050	0.200	Jugnia et al. (1998)
Riverine and transition zone Europe	0.100	0.466	Lind & Barcena (2003)
North Adriatic Sea	0.015	0.303	La Ferla & Leonardi (2005)
This study	0.100	0.466	This study
Mean value	*0.066*	*0.359*	
Hyper-			
Riverine and transition zone Europe	0.095	0.199	Lind & Barcena (2003)
Furuike Pond Japan	0.082	0.194	Nakano & Kawabata (2000)
This study	0.095	0.199	This study
Mean value	*0.091*	*0.197*	

greatly contribute to the total biomass as a result of their great size, although their abundance is low. In hyper-eutrophic pond, filamentous forms greatly contributed to total PB, also if they accounted for only a minor percentage of the total abundance, thus, contributing disproportionally to changes in total biomass (Nakano & Kawabata, 2000; Pernthaler & Amann, 2005; Posch et al., 2009). Although the role of filamentous bacteria in the carbon flux through aquatic systems is still not well known, the presence of elongated forms could be indicator of adverse stressing conditions other than being a strategy against predation by protozoa (Kalcheva et al., 2010).

Prokaryotic cell volume as descriptor of trophic status

The comparison between the prokaryotic cell sizes measured in several aquatic systems—in Table 2 and references therein—corroborates our hypothesis that this topic needs to be tested over a larger number of samples. Nevertheless, statistics adopted in this study do not give a robust evidence about the utilization of prokaryotic cell VOL in the panel of conventionally used trophic variables to better characterize an aquatic ecosystem (nutrients, TSM, Chl-*a*).

Shifts in morphology and species have already been proved by image analysis, FISH, CARD-FISH, DGGE analysis, and flow cytometer (Šimek et al., 2001; Pernthaler & Amann, 2005; Sjöstedt et al., 2012). Some consistency concerning phylotypes and morphotypes has been highlighted in some freshwater systems (Posch et al., 2009). In fact, phylogenetic lineage analysis showed that in an alpine lake at least two bacterial groups, namely Actinobacteria were constituted by small-morphotypes, while Cytophaga by large-rods, cocci, and filaments. At the same time, in brackish areas affected by intrusion of marine water, small horseshoe cells SAR11 constituted about 30% of all prokaryotes (Piccini et al., 2006) notwithstanding this group is considered to be rare or absent in fresh- or brackish waters.

Conclusions

The prokaryotic size and shape distributions differed in the different trophic states, and a dichotomy between cell abundances and cell sizes has been observed. Volumes differently modulated the PB in the oligo-, meso-, eu-, and hypertrophic waters, by wide contents of carbon per cell.

Comparison between the prokaryotic cell sizes measured in several aquatic systems corroborates our hypothesis that investigating the cell-size distribution

of prokaryotes besides their abundance is an important topic which could be used as a satisfactory descriptor of trophic status of different water bodies.

Acknowledgments The authors wish to thank the editor and two anonymous reviewers for their useful comments. They also thank two colleagues of IAMC—Mr. Francesco Soraci, for his help in the laboratory, and Mr. Enrico Minicante Armeli, for data processing—as well as the Municipality of Regalbuto (Enna, Italy) for its endorsement. This study was supported by funds by: CNPq and FAPERJ in the frame of project "Microbial Observatory of Rio de Janeiro"; CNR in the frame of the program of Short Term Mobility 2009 (AMMCNT—CNR n. 0051228) "Confronto tra popolamenti procariotici dell'Oceano Atlantico e del Mediterraneo attraverso l'uso congiunto di tecniche citofluorimetriche e di analisi d'immagine. Analisi di campioni epi-, meso- e bati-pelagici ed interpretazione di dati pregressi per la progettazione di studi futuri sui cambiamenti globali"; Joint project CNR-CAS (AMMCNT—CNR n. 0038575) "Understanding expression and regulation of microbial enzymes involved in organic matter decomposition at different trophic levels and the interrelationship between alkaline phosphatase and eutrophication in Italian and Chinese water bodies."; Marine Strategy Framework Directive (MFSD) of European Commission.

References

Albright, L. J. & S. K. McCrae, 1987. Annual cycle of bacterial specific biovolumes in Howe Sound, a Canadian West Coast Fjord Sound. Applied and Environmental Microbiology 53: 2739–2744.

Aminot, A. & M. Chaussepied, 1983. Manuel des analyses chimiques en milieu marin. CNEXO (Centre National pour l'Exploration des Océans), Brest: 395.

Andrade, L., A. M. Gonzalez, F. V. Araujo & R. Paranhos, 2003. Flow cytometry assessment of bacterioplankton in tropical marine environments. Journal of Microbiological Methods 19: 89–94.

Araújo, M. F. F. & M. J. L. Godinho, 2008. Seasonal and spatial distribution of bacterioplankton in a fluvial–lagunar system of a tropical region: density, biomass, cellular volume and morphologic variation. Brazilian Archives of Biology and Technology 51: 205–214.

Bloem, J., M. Veninga & J. Shepard, 1995. Fully automatic determination of soil bacterium numbers, cell volumes, and frequencies of dividing cells by confocal laser scanning microscopy and image analysis. Applied Environmental Microbiology 61: 926–936.

Bölter, M., J. Bloem, K. Meiners & R. Möller, 2002. Enumeration and biovolume determination of microbial cells—a methodological review and recommendations for applications in ecological research. Biology and Fertility of Soils 36: 249–259.

Bölter, M., J. Bloem, K. Meiners & R. Möller, 2006. Enumeration and biovolume determination of microbial cells. In Bloem, J., D. W. Hopkins & M. Benedetti (eds), Microbiological Methods for Assessing Soil Quality. CABI Publishing, Wallingford, UK: 93–113.

Carlson, R. E., 1977. A trophic state index for lakes. Limnology and Oceanography 22: 361–369.

Carlson, R. E., 1983. Discussion on "Using differences among Carlson's trophic state index values in regional water quality assessment", by Richard A. Osgood. Water Resources Bulletin 19: 307–309.

Carpenter, J. H., 1965. The accuracy of the Winkler method for the dissolved oxygen analysis. Limnology and Oceanography 10: 135–140.

Chrzanowski, T. H. & K. Šimek, 1990. Prey-size selection by freshwater flagellated protozoa. Limnology and Oceanography 35: 1429–1436.

Chrzanowski, T. H., R. D. Crotty & G. J. Hubbard, 1988. Seasonal variation in cell volume of epilimnetic bacteria. Microbial Ecology 16: 155–163.

Chuan, T., T. Jing, W. Xin, Y. Wenjin, L. Xianglong, L. Daotang & Y. Hong, 2009. Spatiotemporal transition of bacterioplankton diversity in a large shallow hypertrophic freshwater lake, as determined by denaturing gradient gel electrophoresis. Journal of Plankton Research 31: 885–897.

Clarke, K. R. & R. N. Gorley, 2006. PRIMER v6: User Manual/ Tutorial. PRIMER-E, Plymouth.

Cochrane, S. K. J., D. W. Connor, P. Nilsson, I. Mitchell, J. Reker, J. Franco, V. Valavanis, S. Moncheva, J. Ekebom, K. Nygaard, R. Serrão Santos, I. Naberhaus, T. Packeiser, W. van de Bund & A. C. Cardoso, 2010. Marine Strategy Framework Directive. Guidance on the Interpretation and Application of Descriptor 1: Biological Diversity. Report by Task Group 1 on Biological diversity for the European Commission's Joint Research Centre, Ispra, Italy.

Cotner, J. B. & B. A. Biddanda, 2002. Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5: 105–112.

Cottrel, M. T. & D. L. Kirchman, 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. Aquatic Microbial Ecology 34: 139–149.

Ducklow, H. W. & F. Shiah, 1993. Bacterial production in estuaries. In Ford, T. (ed.), Aquatic Microbiology: An Ecological Approach. Blackwell, New York: 261–288.

Ducklow, H. W., X. A. G. Moràn & A. E. Murray, 2012. Bacteria in the greenhouse: marine microbes and climate change. In Mitchell, R. & J. D. Gu (eds), Environmental Microbiology. John Wiley & Sons Inc., Hoboken: 1–31.

Ellis, R. J., P. Morgan, A. J. Weightman & J. C. Fry, 2003. Cultivation-dependent and -independent approaches for determining bacterial diversity in heavy-metal-contaminated soil. Applied and Environmental Microbiology 69: 3223–3230.

Furtado, A. L. S., P. Casper & F. A. Esteves, 2001. Bacterioplankton abundance, biomass and production in a Brazilian coastal lagoon and in two German lakes. Anais da Academia Brasileira de Ciências 73: 39–49.

Gasol, J. M. & P. A. del Giorgio, 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina 64: 197–224.

Gasol, J. M. & C. M. Duarte, 2000. Comparative analyses in aquatic microbial ecology: how far do they go. FEMS Microbiology Ecology 31: 99–106.

Giovanardi, F. & E. Tromellini, 1992. Statistical assessment of trophic conditions: application of the OECD methodology to the marine environment. In Vollenweider, R. A., R. Marchetti & R. Viviani (eds), Marine Coastal Eutrophication. Elsevier, Amsterdam: 211–233.

Gocke, K., C. Hernández, H. Giesenhagen & H. G. Hoppe, 2004. Seasonal variations of bacterial abundance and biomass and their relation to phytoplankton in the hypertrophic tropical lagoon Ciénaga Grande de Santa Marta, Colombia. Journal of Plankton Research 26: 1429–1439.

Grasshoff, K., K. Kremling & M. Erhardt, 1999. Methods of Seawater Analysis, 3rd edn. Wiley-VCH Verlag, Germany: 600 pp.

Gurung, T. B., J. Urabe, K. Nozaki, C. Yoshimizu & M. Nakanishi, 2002. Bacterioplankton production in a water column of Lake Biwa. Lakes Reservoirs 7: 317–323.

Hernández-Avilés, J. S., R. Bertoni, M. Macek & C. Callieri, 2012. Why bacteria are smaller in the epilimnion than in the hypolimnion? A hypothesis comparing temperate and tropical lakes. Journal of Limnology 71: 104–111.

Hoppe, H. G., H. C. Giesenhagen & K. Gocke, 1998. Changing patterns of bacterial substrate decomposition in a eutrophical gradient. Aquatic Microbial Ecology 15: 1–13.

Jochem, F. J., 2001. Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Aquatic Microbial Ecology 25: 179–194.

Jolliffe, I., 2005. Principal component analysis. In Everitt, B. S. & D. C. Howell (eds), Encyclopedia of Statistics in Behavioral Science. Wiley, New York.

Jugnia, L. B., R. D. Tadonléké, T. Sime-Ngando, S. M. Foto & N. Kemka, 1998. Short-term variations in the abundance and cell volume of bacterioplankton in an artificial tropical lake. Hydrobiologia 385: 113–119.

Kalcheva, H., D. Terziyski, R. Kalchev, K. Dochin & A. Ivanova, 2010. Control of zoo plankton and nutrients on bacterioplankton in fish ponds with carp larvae. Bulgarian Journal of Agricultural Science 16: 284–297.

Kampstra, P., 2008. A boxplot alternative for visual comparison of distributions. Journal of Statistical Software, Code Snippets 28: 1–9. http://www.jstatsoft.org/v28/c01/.

Krambeck, C., H. J. Krambeck & J. Overbeck, 1981. Microcomputer assisted biomass determination of plankton bacteria on scanning electron micrographs. Applied Environmental Microbiology 42: 142–149.

Kroer, N., 1994. Relationships between biovolume and carbon and nitrogen content of bacterioplankton. FEMS Microbial Ecology 13: 217–223.

La Ferla, R. & M. Leonardi, 2005. Ecological implications of biomass and morphotype variations of bacterioplankton: an example in a coastal zone of the Northern Adriatic Sea (Mediterranean). Marine Ecology 26: 82–88.

La Ferla, R., A. Lo Giudice & G. Maimone, 2004. Morphology and LPS content for the estimation of marine bacterioplankton biomass in the Ionian Sea. Scientia Marina 68: 23–31.

La Ferla, R., M. Azzaro, G. Budillon, C. Caroppo, F. Decembrini & G. Maimone, 2010. Distribution of the prokaryotic biomass and community respiration in the main water masses of the Southern Tyrrhenian Sea (June and December 2005). Advances in Oceanography and Limnology 2: 235–257.

La Ferla, R., G. Maimone, M. Azzaro, F. Conversano, C. Brunet, A. S. Cabral & R. Paranhos, 2012. Vertical distribution of the prokaryotic cell size in the Mediterranean Sea. Helgoland Marine Research 66: 635–650.

Lazzara, L., F. Bianchi, M. Falcucci, V. Hull, M. Modigh & M. Ribera D'Alcalà, 1990. Pigmenti clorofilliani. Nova Thalassia 11: 207–223.

Lee, S. & A. Fuhrman, 1987. Relationship between biovolume and biomass of naturally derived bacterioplankton. Applied and Environmental Microbiology 53: 1298–1303.

Lind, O. T. & E. Barcena, 2003. Response of riverine and transition zone bacterioplankton communities to a pulsed river inflow. Hydrobiologia 504: 79–85.

Loferer-Krößbacher, M., J. Klima & R. Psenner, 1998. Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis. Applied and Environmental Microbiology 64: 688–694.

Mahadevaswamy, M., S. M. Yamakanamardi & T. S. Harsha, 2008. Bacterial (free-living and particle bound) cell-size in the surface waters of River Cauvery and its upstream tributaries in Karnataka State, India. Applied Ecology and Environmental Research 6: 29–47.

Massana, R., J. P. Gasol, P. K. Bjørnsen, N. Blackburn, A. Hanström, S. Hietanen, B. H. Hygum, J. Kuparinen & C. Pedrós-Alió, 1997. Measurement of bacterial size via image analysis of epifluorescence preparations: description of an inexpensive system and solutions to some of the most common problems. Science Marine 61: 397–407.

Mayr, L. M., D. R. Tenenbaum, M. C. Villac, R. Paranhos, C. R. Nogueira, S. L. C. Bonecker & A. C. Bonecker, 1989. Hydrobiological characterization of Guanabara Bay. In Maggon, O. T. & C. Neves (eds), Coastlines of Brazil. American Society of Civil Engineering, New York: 124–139.

Nakano, S. & Z. Kawabata, 2000. Changes in cell volume of bacteria and heterotrophic nanoflagellates in a hypereutrophic pond. Hydrobiologia 428: 197–203.

Norland, S., 1993. The relationship between biomass and volume of bacteria. In Kemp, P. F., B. F. Sherr, E. B. Sherr & J. J. Cole (eds), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton: 303–307.

Øvreås, L., D. Bourne, R. A. Sandaa, E. O. Casamayor, S. Benlloch, V. Goddard, G. Smerdon, M. Heldal & T. F. Thingstad, 2003. Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. Aquatic Microbial Ecology 31: 109–121.

Pernthaler, J. & R. Amann, 2005. Fate of heterotrophic microbes in pelagic habitats: focus on populations. Microbiology and Molecular Biology Reviews 6: 440–461.

Piccini, C., D. Conde, C. Alonso, R. Sommaruga & J. Pernthaler, 2006. Blooms of single bacterial species in a coastal lagoon of the Southwestern Atlantic Ocean. Applied and Environmental Microbiology 72: 6560–6568.

Porter, K. G. & Y. S. Feig, 1980. The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography 25: 943–948.

Posch, T., J. Franzoi, M. Prader & M. M. Salcher, 2009. New image analysis tool to study biomass and morphotypes of

three major bacterioplankton groups in an alpine lake. Aquatic Microbial Ecology 54: 113–126.

Quinones, R. A., T. Platt & J. Rodríguez, 2003. Patterns of biomass-size spectra from oligotrophic waters of the Northwest Atlantic. Progress in Oceanography 57: 405–427.

Racy, F., M. J. L. Godinho, M. H. Regali-Seleghim, N. R. S. Bossolan, A. C. Ferrari & J. V. Lucca, 2005. Assessment of the applicability of morphological and size diversity indices to bacterial populations of reservoir in different trophic states. Acta Limnologica Brasiliensia 17: 395–408.

Šestanović, S., M. Šolić, N. Krstulović & D. Bogner, 2005. Volume, abundance and biomass of sediment bacteria in the eastern mid Adriatic Sea. Acta Adriatica 46: 177–191.

Šimek, K., J. Penrhtaler, M. G. Weinbauer, K. Hornák, J. R. Dolan, J. Nedoma, M. Mašin & R. Amann, 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. Applied and Environmental Microbiology 67: 2723–2733.

Sjöstedt, J., A. Hagström & U. L. Zweifel, 2012. Variation in cell volume and community composition of bacteria in response to temperature. Aquatic Microbial Ecology 66: 237–246.

Sommaruga, R. & R. Robarts, 1997. The significance of autotrophic and heterotrophic picoplankton in hypertrophic lakes. FEMS Microbiology Ecology 24: 187–200.

Straza, T. R. A., M. T. Cottrell, H. W. Ducklow & D. L. Kirchman, 2009. Geographic and phylogenetic variation in bacterial biovolume as revealed by protein and nucleic acid staining. Applied and Environmental Microbiology 75: 4028–4034.

Strickland, J. D. & T. R. Parsons, 1972. A practical handbook of seawater analysis. Bulletin Fisheries Research Board of Canada 167: 1–310.

Teixeira, M. C., N. F. Santana, J. C. Rodriguez de Azevedo & T. A. Pagioro, 2011. Bacterioplankton features and its relations with DOC characteristics and other limnological variables in Paraná river floodplain environments (PR/MS – Brazil). Brazilian Journal of Microbiology 42: 897–908.

Tranvik, L. J., 1992. Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia 229: 107–114.

van Wambeke, F., S. Heussner, F. Diaz, P. Raimbault & P. Conan, 2002. Small-scale variability in the coupling/uncoupling of bacteria, phytoplankton and organic carbon fluxes along the continental margin of the Gulf of Lions, Northwestern Mediterranean Sea. Journal of Marine Systems 33–34: 411–429.

van Wambeke, F., P. Catala, M. Pujo-Pay & P. Lebaron, 2011. Vertical and longitudinal gradients in HNA–LNA cell abundances and cytometric characteristics in the Mediterranean Sea. Biogeosciences 8: 1853–1863.

Vrede, K., M. Heldal, S. Norland & G. Bratbak, 2002. Elemental composition (C, N, P) and cell volume of exponentially growing and nutrient-limited bacterioplankton. Applied and Environmental Microbiology 68: 2965–2971.

Winter, C., A. Smit, G. J. Herndl & M. G. Weinbauer, 2005. Linking bacterial richness with viral abundance and prokaryotic activity. Limnology and Oceanography 50: 968–977.

Wu, Y., T. Platt, C. C. L. Tang & S. Sathyendranath, 2007. Short-term changes in chlorophyll distribution in response to a moving storm: a modelling study. Marine Ecology Progress Series 335: 57–68.

Young, K. D., 2006. The selective value of bacterial shape. Microbiology and Molecular Biology Reviews 70: 660–703.

Zeder, M., E. Kohler, L. Zeder & J. Pernthaler, 2011. A novel algorithm for the determination of bacterial cell volumes that is unbiased by cell morphology. Microscopy and Microanalysis 17: 799–809.