Assessment of the applicability of morphological and size diversity indices to bacterial populations of reservoirs in different trophic states.

RACY[1], F. ; GODINHO[1], M. J. L.; REGALI-SELEGHIM[1], M. H. ; BOSSOLAN[2], N. R. S.; FERRARI[1], A. C. & LUCCA[1], J. V.

[1] Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos - Via Washington Luiz, km 235, São Carlos (SP) 13565-905, e-mail fracy@iris.ufscar.br, pmhrs@iris.ufscar.br

[2] Instituto de Física de São Carlos, Universidade de São Paulo, C.P. 369, 13560-970 São Carlos, SP, Brazil, e-mail Nelma@ifsc.usp.br

ABSTRACT: Assessment of the applicability of morphological and size diversity indices of bacterial populations of reservoirs in different trophic states. The morphology and size of bacterial cells, in samples taken at monthly intervals over half a year from the surfaces of three Brazilian reservoirs in different trophic states (Barra Bonita – eutrophic, Broa – mesotrophic and Lagoa Dourada - oligotrophic), were analysed with the aid of a computerized system, from digitalized images obtained by epifluorescence microscopy (with DAPI fluorescent stain). For each reservoir, densities (D) were estimated, and the relative frequencies of biovolumes and of each morphotype identified (coccus, coccobacillus, bacillus, vibrio, filament and spirillum) were converted into indices of size diversity (SDI) and morphological diversity (MDI), using the Shannon Index for calculations. These values were compared with the mean trophic state index (mTSI) calculated for each environment. Among the biological variables, bacterial population density was the most significant indicator of the trophic state of the environments. The MDI proved to be significantly different between the eutrophic and oligotrophic environments, as well as between the mesotrophic and oligotrophic reservoirs, but not consistently so between the eutrophic and mesotrophic, possibly because of the similarity of the trophic states in the last two. The SDI was found to differentiate significantly between the eutrophic and oligotrophic environments, but not between either of these and the mesotrophic. In the oligotrophic environment there was an extremely significant negative linear correlation ($r = -0.9735$, $P = 0.001$) between the monthly values of mTSI and MDI; this correlation was specially significant with chlorophyll a concentration (Chla), but not significant with the other components of mTSI.

Keywords: bacteria, morphology, size, diversity indices, trophic level.

RESUMO: Avaliação da aplicabilidade de índices de diversidade morfológica e de tamanho em populações bacterianas de reservatórios em diferentes estados tróficos. A morfologia e o tamanho de células bacterianas, coletadas em intervalos mensais durante meio ano na superfície de três reservatórios em diferentes estados tróficos (Barra Bonita – eutrófico; Broa – mesotrófico e Lagoa Dourada – oligotrófico), foram analisados com o auxílio de um sistema computadorizado, a partir de imagens digitalizadas obtidas por microscopia de epifluorescência (coloração com DAPI). As densidades populacionais (D) foram calculadas em cada ambiente e as freqüências relativas dos biovolumes e de cada morfotipo identificado (coco, cocobacilo, bacilo, vibrio, filamento e espirilo) foram convertidas em índices de diversidade de tamanho (IDT) e de diversidade morfológica (IDM), calculados pelo índice de Shannon. Estes valores foram comparados com o índice de estado trófico médio (IETm) calculado para cada ambiente. Entre as variáveis biológicas, a densidade populacional bacteriana foi o indicador mais significativo do estado de trofia dos ambientes. O IDM revelou-se um indicador significativo entre os ambientes eutrófico e oligotrófico, assim

como entre os mesotrófico e oligotrófico, mas pouco consistente entre eutrófico e mesotrófico, possivelmente devido à proximidade do estado trófico entre estes últimos. O IDT revelou-se um indicador significativo entre os ambientes eutrófico e oligotrófico, mas não entre estes e o mesotrófico. No ambiente oligotrófico constatou-se uma correlação linear negativa, extremamente significante (r = - 0,9735 e P = 0,001), entre os valores mensais de IET e IDM; esta correlação mostrou-se especialmente significativa com a concentração de clorofila a (Cla), mas não significativa com os demais componentes do IETm.
Palavras-chave: bactérias, morfologia, tamanho, índices de diversidade, nível trófico

Introduction

In the last decades, eutrophication (specially cultural or antropic) of freshwater bodies has become one of the greatest environmental concerns, with particular reference to lakes and reservoirs (e.g. Rocha et al., 1997). Disquiet over this phenomenon has led to intense research into its ecological consequences and a search for biological indicators that can be used to quantify water degradation. However, Esteves (1998) warned that despite the constant preoccupation of limnologists with finding organisms and environmental variables that could identify the trophic state of aquatic ecosystems, the most used indicators are adequate for temperate lakes and cannot be used in the tropics.

The idea of using microorganisms (particularly bacteria) as biological indicators is based on features (biological relevance, ease of measurement by standard methods with low error, relatively low cost and little effort required to obtain a large amount of information, among others) that make them ideal for this purpose (Cairns Jr. et al., 1993).

Concerning their biological importance in aquatic habitats, the bacterial component has been recognised, ever since the classic ecological research carried out by Lindemann (1942), as essential to decomposition processes. This focus was widened to include the concept of the microbial loop in aquatic food webs (Pomeroy, 1974; Azam et al., 1983), which conferred on microorganisms (specially bacteria) an even greater significance, given that they were now regarded also as an alternative pathway for matter and energy in the aquatic food web (Sherr & Sherr, 1988).

Structure of a community and the flux of matter and energy are dependent on the number and kind of organisms (Gasol & Vaqué, 1993). Structural and functional features are also explained by the three main criteria used in Ecology: size, genetics and trophic function (Gasol et al., 1997). But, size should be the principal criterion when grouping living organisms at the community level and the "size spectrum" generated allows easy comparison between communities.

Individual size of organisms is also related to important community properties, such as growth-rate, abundance, diversity and sinking rate in pelagic ecosystems. As a direct relation exists between the size and mass of macro or microorganisms (Peters, 1987), the individual size should be applicable in the ecological study of aquatic microbes.

The mean size in a community summarises all the individual measurements in a single number (or two, if a measure of variability, such as standard deviation, is added), but sometimes the size distribution may change, even though the mean remains the same. Therefore, important information might be lost if the mean cell-size alone were recorded. So, "size distribution" is an aspect of a population that allows a quantitative comparison among several communities with ecological significance. However, this kind of analysis has rarely been applied to bacteria or to any of the microbial loop components (Gasol et al., 1997).

In addition, trophic state or food availability in the environment seems to influence, direct or indirectly, changes in the morphology of assemblages, which are

governed by genetic control (e.g. rodA and mre genes) (Hahn & Höfle, 1999; Steinberger et al., 2002; Lindström, 2000; Simek et al., 1999).

On the other hand, the number of organisms living in a particular area is determined by speciation, extinction, immigration and local losses, and the relative importance of each process depends on the scale of the investigation (global, regional or local). No less important are the effects of the temporal and spatial scales of these processes on the number of individuals in the populations under study and, consequently, on all the related properties (size, shape, density, biomass etc.) (Godfray & Lawton, 2001).

Trophic changes will produce morphometric and morphological alterations in organisms of the aquatic food web, as well as in their abundance. Thus, the aim of this study is to investigate size diversity (measured here by a size diversity index) and morphological diversity (measured by a morphological diversity index), to find possible correlations between these indices and the trophic states of three different reservoirs, in order to obtain information about the structure and role of the bacterial communities in these environments.

Material and methods

Sampling sites

The three studied reservoirs, in the State of São Paulo, SE Brazil, (Fig.1 and Tab. I), have quite different trophic states: Barra Bonita – eutrophic, Broa – mesotrophic and Lagoa Dourada – oligotrophic, according to Rocha et al. (1997). For this study, one sampling station was selected in the pelagic zone of the reservoirs.

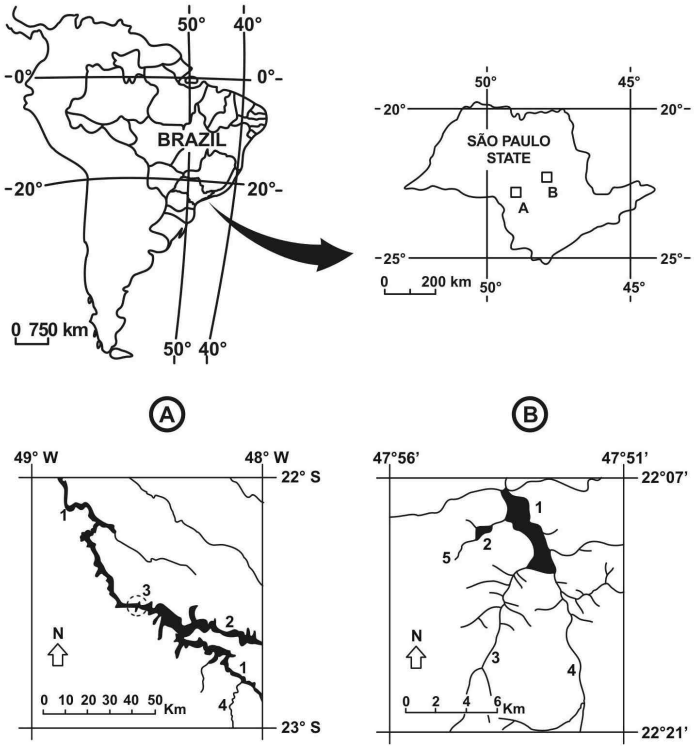

Figure 1: Localization of the sampling sites in São Paulo State (Brazil). **(A) Barra Bonita** Reservoir: **A1** – dammed portion of Tietê River, **A2** – dammed portion of Piracicaba River, **A3** – Barra Bonita dam, **A4** – Peixe River. **(B) Broa** and **Lagoa Dourada** Reservoirs: **B1** – Broa Reservoir, **B2** – Lagoa Dourada Reservoir, **B3** – Lobo River, **B4** – Itaqueri River, **B5** – Perdizes Stream.

Table I: Geographic and morphometric data **(GMD)** of the sampling sites and sampling points in Barra Bonita **(BB)**, Broa **(BR)** and Lagoa Dourada **(LD)** and their respective references **(Ref)**.

	BB		BR		LD	
GMD	**data**	**Ref**	**data**	**Ref**	**data**	**Ref**
altitude	466 m	ps	715 m	ps	724 m	ps
surface area	324.84 km^2	1	6.8 km^2	3	0.076815 km^2	4
perimeter	525 km	1	21 km	3	1.56 km	5
average depth	10.2 m	2	3 m (maximum 12)	3	2.6 m	4
estimated volume	2,600 x 10^6 m^3	2	22 x 10^6 m^3	3	0.20274 x 10^6 m^3	4
AMRT	100 days	2	20 days	2	-	-
SPLt	22°31'60.8" S	ps	22°10'62.9" S	ps	22°11'43.1" S	ps
SPLg	48°31'58.4" W	ps	47°53'69.3" W	ps	47°54'76.3" W	ps
SPMD	9.6 m	ps	7.0 m	ps	4.5 m	ps

AMRT = annual mean residence time SPLt = sampling point latitude SPLg = sampling point longitude SPMD = sampling point mean depth References: ps = present study 1 = (Tundisi & Matsumura-Tundisi, 1990) 2 = (Calijuri & Tundisi, 1990) 3 = (Tundisi & Matsumura-Tundisi, 1995) 4 = (Melão & Rocha, 2004) 5 = (Pompêo & Moschini-Carlos, 1995)

Barra Bonita Reservoir (BB) is a reservoir whose water comes largely from the rivers Tietê and Piracicaba (Calijuri & Tundisi, 1990), both heavily polluted with domestic and industrial waste (Tundisi & Matsumura-Tundisi, 1990). The catchment area is predominantly farmland, and there is little urban development around the sampling site.

Broa Reservoir (BR) is a turbulent but not very deep reservoir in the middle of the São Paulo State, built in 1936 (for power supply) and currently used for recreation and research. The original vegetation in the basin is predominantly cerrado (neotropical scrub forest), while in more fertile and humid areas is found gallery forest. There is also some agriculture in the catchment area and some parts are reforested with Pinus sp. and Eucalyptus sp. (Tundisi & Matsumura-Tundisi, 1995).

Lagoa Dourada Reservoir (LD) is a small reservoir located in the basin of the Lobo stream. The dam was built in the mid-1980s and is used mainly for leisure activities. The main vegetation in the catchment area is cerrado, with little urban development on the left bank and a small area of pasture on the right. A large part of the bottom is covered with macrophytes (e.g. Utricularia gibba) (Pompêo & Moschini-Carlos, 1995).

Sampling procedure

Samples were taken monthly from January to June 2004, except at Barra Bonita, where sampling began in February. Sampling was done on the same day at Lagoa Dourada and Broa Reservoirs (January 23, February 17, March 16, April 18, May 16 and June 20) and one day earlier at Barra Bonita Reservoir.

At each site, water samples were taken at the surface (S), 0.25 cm from the air-water interface. Water samples were taken in van Dorn bottles of 2.5 L, from which 1.5 L was stored in plastic flasks, coated to keep out the light, and 200 mL in a brown glass bottle, for bacterial analyses. The samples were transferred to the laboratory under refrigeration. Those for bacterial analyses were fixed in the field, by adding aqueous formaldehyde solution (pH 7.0 neutralized with NaOH 1N) to a final concentration of 2%, and kept refrigerated (»8° C) until analysed (Sherr & Sherr, 1993).

For pigment analyses, triplicate sub-samples of 60 to 100 mL were withdrawn from the main sample of 1.5 L, within 8 h of collection, and filtered in a Manifold filter set (Millipore®) with GF/F glass microfibre filters (Whatman®). The filters were kept in the freezer for subsequent pigment extraction. The filtrate was stored in plastic bottles and the remainder of the sample in a 1 L bottle, both being frozen for up to 3 months, for mineral nutrient analyses (total phosphorus – P_T).

Physicochemical and microbial analyses

Chlorophyll a (Chla) was extracted in hot 90% ethanol (Rai, 1973), following the recommendations made by Marker et al. (1980), and calculated by the equation proposed by Lorenzen (1967). Total phosphorus (P_T) was analysed by the method given by Valderrama (1981). Water transparency was measured at each sampling station with a 30 cm diameter Secchi disk (Sd).

Values of the Carlson (1977) Trophic State Indices (TSI) were then calculated from the data for Chla – TSI(Chla), P_T – TSI(P_T) and Sd – TSI(Sd) at each sampling site. The TSI equations are:

$$TSI(Chla) = 10\left(6 - \frac{2.04 - 0.68 \ln Chla}{\ln 2}\right)$$

$$TSI(P_T) = 10\left(6 - \frac{\ln 48 / P_T}{\ln 2}\right)$$

$$TSI(Sd) = 10\left(6 - \frac{\ln Sd}{\ln 2}\right)$$

The average of these three TSI values was used to determine the mean trophic state (mTSI) of each water-body, following the classification proposed by Kratzer & Brezonik (1981).

Bacteria were prepared for analysis by staining fractions (Lagoa Dourada = 2.0 mL, Broa = 1.0 mL and Barra Bonita = 0.5 mL) of the samples for 30 min. with 4',6-diamidino-2-phenylindole – DAPI (Sigma®), following Porter & Feig (1980), at a final concentration of 1.0 to 5.0 mg/mL (Velji & Albright, 1993), and filtered on to Sudan black-stained membranes (pore 0.2 mm; diameter 25 mm) of polycarbonate (Nuclepore®) (adapted from Hoff, 1993). Bacteria were analysed in an Olympus BHS2 epifluorescence microscope, with mercury steam light (HBO 100 W - OSRAM®) and Olympus® (DM-400, L435 and U-UG-1) UV light filter sets, at a magnification of 1,250X (neofluar UVPL 100 1.30 oil objective - Olympus®). Images (4 to 6 fields per slide, making a total of 200 to 250 cells) were captured by a CCD camera (Optronics®). The counts and measurements (cells lengths and widths) were recorded by the computer program Image-Pro Plus® ver. 4.0 - Media Cybernetics – (adapted from Massana et al., 1997).

Bacterial densities (D) were calculated as described by Jones (1979). Bacteria identified in this study were classified into the following morphotypes: cocci, coccobacilli, bacilli, vibrios, spirilla and filaments (Tortora et al., 1998). The classification was made on the basis of their length/width (L/W) ratio, according to the following criterion: cocci, from 1.0 to 1.25; coccobacilli, from 1.26 to 1.75; bacilli, 1.75 to 5.0; organisms whose L/W ratio exceeded 5 were defined as filaments (Racy, 2004). Vibrios were identified by their characteristic sickle-shape and spirilla were recognised by their S-shape and treated as bacilli for biovolume estimation. The biovolumes were calculated from the measures by applying suitable formulae for each geometrical shape, as described by Sun & Liu (2003).

Diversity Indices

In each sample the absolute frequency (number of individuals) of each morphological category (coccus, coccobacillus, bacillus, filament, vibrio and

spirillum) was determined and transformed into the corresponding relative frequency (p_i) of each morphological category (by dividing each absolute frequency by the total number of organisms in the sample) and a Shannon index of diversity was derived from these relative frequencies (Zar, 1999) of the morphotypical categories in each sample, defined here as the Morphological Diversity Index (MDI) (Racy, 2004), according to the following equation:

$$MDI = -\sum_{i=1}^{k} p_i \log p_i$$

Similarly, biovolume values found in each sample were grouped into size categories and the absolute frequency (number of individuals) of each category was determined and transformed into the relative frequency of each size category by dividing each absolute frequency by the total number of organisms in the sample. For each sample, a Shannon index of diversity was calculated from these relative frequencies (Zar, 1999) and designated the Size Diversity Index (SDI) (modified from Gasol et al., 1997).

The variation in SDI, MDI and D values of the samples was compared with that of the mean Trophic State Index (mTSI) of each site, as well as with the individual variables used to calculate that index (Chla, P_T and Sd).

Statistical analysis

The statistical analysis of the data (Pearson test for linear correlation (r), analysis of variance (ANOVA) and Tukey-Kramer multiple comparison of the means (Zar, 1999) was carried out with the program GraphPad InStat® 3.0 (1997), which automatically performs tests (e.g. Bartlett, Kolmogorov-Smirnov) to ensure the reliability of the ANOVA results. The limiting significance levels applied in multiple comparison tests were q = 3.702 and P = 0.05.

Results and discussion

Trophic state

The monthly variations in Chla and P_T concentrations and in transparency (Sd) in the three reservoirs are presented in Tab. II. During the study Chla varied widely in Barra Bonita and Lagoa Dourada; P_T was more variable in Barra Bonita than elsewhere and the water transparency in Lagoa Dourada and Broa varied very little, while in Barra Bonita the variation was considerable.

Despite the decreasing of the mean values of Chla and P_T from Barra Bonita to Lagoa Dourada, the considerable variability from site to site of the coefficient of variation (CV) for Chla, but not for P_T, reveals that only P_T clearly reflects the difference between the three environments, in all months. If the sites are compared in terms of the mean values for Sd, the decreasing gradient is different: Lagoa Dourada ® Barra Bonita ® Broa. Average depth and area of the reservoir at Barra Bonita are greater than Broa, a turbulent and shallow reservoir (Tundisi & Matsumura-Tundisi, 1995). This fact may imply a proportionately greater dispersion of dissolved organic matter (DOM) and/or particulate organic matter (POM) in the water column of Barra Bonita than Broa, since the latter reservoir is submitted to sediment inputs into a smaller area and depth, leading to a lower transparency (Sd) than Barra Bonita. Similarly, when the monthly Chla values in Barra Bonita and Broa are compared, it can be seen that in certain months (April and May) the individual absolute value of Chla in Broa is higher than that in Barra Bonita.

Table II: Monthly surface concentrations (mg/L) of chlorophyll a (Chla) and total phosphorus (P_T) and Secchi disk (Sd) transparency (m) in Barra Bonita (BB), Broa (BR) and Lagoa Dourada (LD).

	BB			BR			LD		
Month	**Chla**	**P_T**	**Sd**	**Chla**	**P_T**	**Sd**	**Chla**	**P_T**	**Sd**
January	-	-	-	6.76	15.09	1.90	0.00*	9.87	3.80
February	12.66	96.65	2.50	4.21	12.16	1.80	0.87	7.26	3.70
March	31.22	65.33	1.20	5.09	17.38	1.90	1.40	9.22	3.70
April	5.33	45.43	3.40	5.85	26.19	2.20	3.97	13.46	4.30
May	4.59	76.75	3.40	4.68	19.66	1.60	0.00*	11.83	4.40
June	7.26	31.73	2.90	4.71	16.40	1.90	2.79	8.89	4.50
$\bar{x}$	12.21	63.18	2.68	5.22	17.81	1.88	2.26	10.09	4.07
SD	11.1	25.6	0.9	0.9	4.8	0.2	1.4	2.2	0.4
CV (%)	90.8	40.5	33.9	17.8	26.9	10.3	62.1	22.0	9.2

* value possibly too low to be detected with the method and equipment used $\bar{x}$ = mean **SD** = Standard Deviation **CV** = Coefficient of Variation.

Such observations, which are characteristic of tropical and subtropical freshwater systems, have led to discussions on the applicability of Carlson's index to determine trophic state of reservoirs (Mercante & Tucci-Moura, 1999; Salas & Martino, 1991). So, total nitrogen (N_T) concentration for determination of the TSI, at least in subtropical Florida lakes, has being used for trophic classification (Kratzer & Brezonik, 1981).

In temperate lakes, the values of TSI(Cla), TSI(P_T) and TSI(Sd) for a same environment are, usually, similar (Carlson, 1977). The same do not occur in tropical and subtropical environments (e.g. Mercante & Tucci-Moura, 1999). In accordance with these authors, in the present work, for each studied reservoir, the calculated TSI values for the three variables were not similar and this could raise doubts about the classification of the reservoirs. When the mean value (mTSI) was calculated, the doubts disappear and the values found (Tab. III) confirm the previously published trophic states of the three water-bodies (Rocha et al., 1997). So, it was decided to establish the trophic state of the reservoirs from the mTSI that seems to be a reliable indicator of their trophic state.

Table III: Monthly values of the trophic state indices: based on chlorophyll a (Chla), on total phosphorus (P_T), on Secchi disk (Sd), the mean trophic state index (mTSI) and the trophic state (TS) of the reservoirs at Barra Bonita (BB), Broa (BR) and Lagoa Dourada (LD).

	BB					BR					LD				
Month	**Chla**	**P_T**	**Sd**	**mTSI**	**TS**	**Chla**	**P_T**	**Sd**	**mTSI**	**TS**	**Chla**	**P_T**	**Sd**	**mTSI**	**TS**
January	-	-	-	-	-	49	43	51	48	M	0	37	41	26	O
February	55	70	47	57	E	45	40	52	45	M	29	33	41	34	O
March	64	64	57	62	HE	47	45	51	48	M	34	36	41	37	O
April	47	59	42	50	M	48	51	49	49	M	44	42	39	42	M
May	46	67	42	52	E	46	47	53	49	M	0	40	39	26	O
June	50	54	45	50	M	46	45	51	47	M	41	36	38	38	O
$\bar{x}$	52.5	62.9	46.7	54.0	E	46.7	45.3	50.9	47.6	M	24.6	37.2	39.8	33.9	O
SD	7.6	6.4	6.2	5.5		1.7	3.7	1.5	1.3		19.8	3.2	1.3	6.5	
CV (%)	14.6	10.1	13.4	10.2		3.6	8.2	2.9	2.8		80.3	8.5	3.3	19.2	

TS from Kratzer & Brezonick (1981) - O = oligotrophic M = mesotrophicE = eutrophic HE= hypereutrophic - $\bar{x}$ = mean SD = Standard Deviation CV = Coefficient of Variation

Nevertheless, in Barra Bonita and Lagoa Dourada, the observed trophic states varied during the period of sampling. In Barra Bonita, for example, the March sample was taken during an algal bloom, which produced the highest mTSI value, due to the Chla (see Tab. II). For Lagoa Dourada, the high April values of Chla and P_T resulted in a TSI, which on this sampling date would define this site as mesotrophic, according

to the classification used. Broa was the reservoir that exhibited the most uniform mTSI, with CV = 2.8, but its mean trophic state, in most months, were nearer to eutrophy than oligotrophy.

The highly consistent means from the three mTSIs, evidenced by the low values of CV, especially in Barra Bonita and Broa, allowed the reservoirs to be classified as follows: Barra Bonita, eutrophic; Broa, mesotrophic, and Lagoa Dourada, oligotrophic.

The multiple comparison test of the means (Tukey-Kramer) showed that there was no significant difference between the mean mTSI at Barra Bonita and Broa ($q = 3.110$ and $P > 0.05$), but the difference was extremely significant between those at Broa and Lagoa Dourada ($q = 6.907$ and $P < 0.001$) and between those at Barra Bonita and Lagoa Dourada ($q = 9.695$ and $P < 0.001$).

Bacterial analyses

The morphological analyses of the three sites revealed that throughout the study the cocci and coccobacilli, taken together, showed the highest relative frequencies among the morphotypes, sometimes attaining 80% of the bacterial population (in Barra Bonita), with their frequencies increasing with the environments trophic level (Fig.2). The relative frequencies of bacilli, vibrios, filaments and spirilla together represented, on average, 19.20% of the total bacteria in Barra Bonita, 26.70% in Broa and 43.36% in Lagoa Dourada (Racy, 2004).

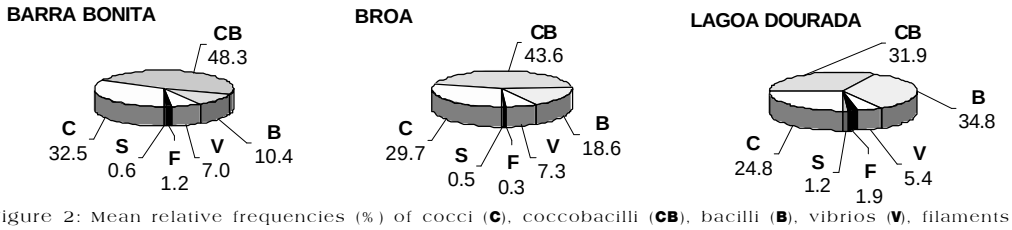

Figure 2: Mean relative frequencies (%) of cocci (**C**), coccobacilli (**CB**), bacilli (**B**), vibrios (**V**), filaments (**F**) and spirilla (**S**) in the studied water-bodies.

The values obtained for bacterial SDI, MDI and D are presented in Tab. IV. The monthly variations in SDI at each site were small, as can be seen by the low standard deviations (SD) and CVs, but the differences among the means for each site, taken over the whole period, were significant ($P = 0.0172$ by ANOVA). The mean SDI was highest in oligotrophic and lowest in eutrophic and mesotrophic environments. The Tukey-Kramer multiple comparison test identified the difference between the SDI mean values at Barra Bonita and Lagoa Dourada as the most significant ($q = 4.528$ and $P < 0.05$), whereas those between Barra Bonita and Broa ($q = 1.433$ and $P > 0.05$) and between Broa and Lagoa Dourada ($q = 3.246$ and $P > 0.05$) as not significant.

Table IV: Monthly values of the size diversity index (**SDI**), morphological diversity index (**MDI**) and bacterial density (**D** 10^6 cells x mL^{-1}) in Barra Bonita (**BB**), Broa (**BR**) and Lagoa Dourada (**LD**).

	BB			BR			LD		
Month	**SDI**	**MDI**	**D**	**SDI**	**MDI**	**D**	**SDI**	**MDI**	**D**
January	-	-	-	2.206	0.545	8.10	2.059	0.618	2.48
February	2.077	0.495	9.40	2.193	0.571	5.98	2.191	0.575	2.31
March	2.086	0.535	9.60	2.080	0.536	4.07	2.209	0.570	2.41
April	2.076	0.497	8.27	2.151	0.530	4.90	2.304	0.546	2.45
May	2.100	0.553	9.60	2.111	0.545	5.83	2.288	0.604	1.86
June	2.134	0.509	8.17	2.072	0.486	5.63	2.292	0.571	2.10
$\bar{x}$	2.09	0.52	9.01	2.14	0.54	5.75	2.22	0.58	2.27
SD	0.02	0.03	0.72	0.06	0.03	1.35	0.09	0.03	0.24
CV (%)	1.2	4.9	8.0	2.7	5.2	23.5	4.2	4.5	10.7

$\bar{x}$ = mean **SD** = Standard Deviation **CV** = Coefficient of Variation

Bacterial SDI (richness and evenness of biovolumes) can be thus a possible indicator of trophic level. However, it is more suitable to distinguish eutrophic from oligotrophic environments (those with very different TSI).

At each site, the monthly variation of MDI (Tab. IV) was also small (with small values of SD and CV), but the statistical analysis showed that the differences between the means at the three sites were not casual (P = 0.0040 by ANOVA).

The much lower P-value (4 times less, approximately) for the MDI in comparison with the SDI reveals that it is a more reliable indicator of the trophic level of an environment than SDI. For the MDI, the multiple comparison test showed again that there was no significant difference between the mean MDIs at Barra Bonita and Broa (q = 1.557 and P › 0.05), but the difference was significant between those at Broa and Lagoa Dourada (q = 4.168 and P ‹ 0.05) and very significant between those at Barra Bonita and Lagoa Dourada (q = 5.531 and P ‹ 0.01), the same pattern obtained for the TSI in these environments.

The statistical analyses showed significant variation among the mean bacterial densities (D) of the reservoirs (P ‹ 0.0001 by ANOVA). The multiple comparison test showed that there was an extremely significant difference between the mean values of D at Barra Bonita and Broa (q = 8.378 and P ‹ 0.001), Broa and Lagoa Dourada (q = 9.399 and P ‹ 0.001) and at Barra Bonita and Lagoa Dourada (q = 17.339 and P = ‹ 0.001). Average bacterial density in Broa was around 153% higher than in Lagoa Dourada; in Barra Bonita, it was about 56% higher than in Broa and 297% higher than in Lagoa Dourada (Tab. III). This positive relationship of bacterial densities with environmental trophic state was also found for other authors in freshwater (e.g. Crisman et al., 1984).

Among the bacterial variables, the population density was the most visible indicator of the trophic level of the environments. In these environments and periods, a rise in bacterial population density is the clearest response to eutrophication. This behavior of the populational bacterial density resembles that of eukaryote populations which, according to Begon (1999), respond in a similar way to abundance or shortage of resources.

The profiles of SDI and MDI data are clearly different at Barra Bonita and Lagoa Dourada, while those at Broa are between these extremes (Tab. IV). The variations in D, characteristic of each set of conditions are more marked in the mesotrophic state at Broa (where SD and CV are highest). No statistically significant linear correlation was found between any of the bacterial indexes.

At each site, no significant linear correlation was observed between values of mTSI with SDI, MDI or D, except between mTSI and MDI (r = - 0.9735, R^2 = 0.9477, P =0.001) in oligotrophic conditions (Lagoa Dourada) (Tab. III). This correlation is evident in the monthly variations of each index (Fig. 3).

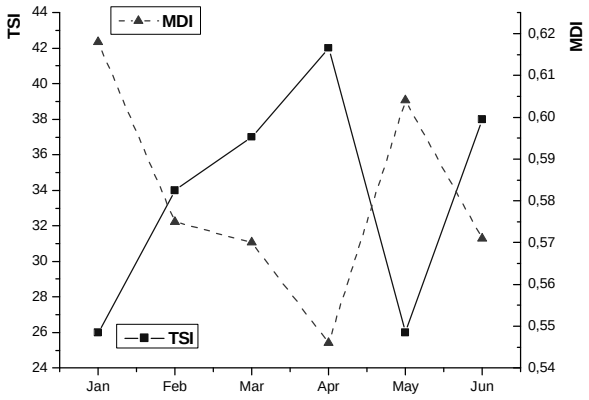

Figure 3: Monthly variations of the trophic state index (**TSI**) and of the morphological diversity index (**MDI**) in Lagoa Dourada (**LD**) during the sampling period.

Between the components of mTSI (TSI(Chla), TSI(P_T), and TSI(Sd), only TSI(Chla) showed a significant linear correlation (r = - 0.9506, R^2 = 0.9037, P = 0.0036) with MDI. This fact suggests that the variation in morphological diversity in Lagoa Dourada was mainly correlated with Chla concentration. This correlation is also clearly visible in the monthly variations of the index and the Chla (Fig. 4).

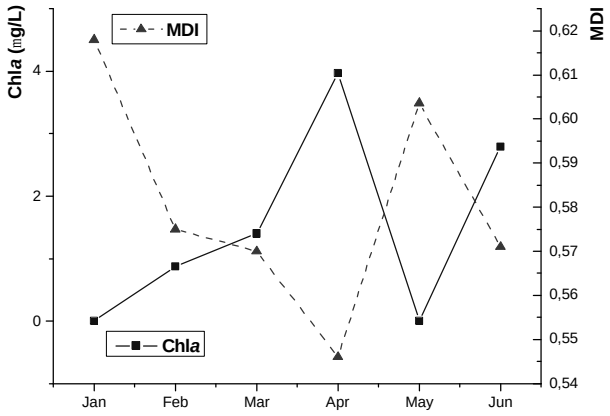

Figure 4: Monthly variations of the morphological diversity index (**MDI**) and of the concentration (mg/L) of chlorophyll a (**Chla**) in Lagoa Dourada (**LD**) during the sampling period.

A few works report the relative frequencies of bacterial morphotypes in freshwater, particularly in tropical or sub-tropical environments (e.g. Gomes et al., 1998). The present study is the first in which these data are treated as an index (MDI).

The MDI includes both the richness and evenness of morphotypes. In all three water-bodies, the same morphotypes (richness) were studied. Hence, any variation in MDI will result from changes in the relative frequencies (evenness) of the morphotypes. The sum of the elongated forms (bacilli, vibrios, filaments and spirilla) was higher in Lagoa Dourada (Fig.2) and this indicates that the higher value of MDI was directly related to the frequencies of these morphotypes (Racy, 2004).

Neither these data nor the literature offer elements for an unequivocal explanation of the predominance of elongated bacterial shapes in the oligotrophic environment. However, some hypotheses can be presented to explain the strong correlation between Chla and MDI in Lagoa Dourada.

In conditions of greater resource availability (eutrophic environment – Barra Bonita), where Chla concentration is major, growth of organisms with spherical, prolate spherical or spheroidal cells (cocci and coccobacilli) is favoured, and the bacterial density is high. Therefore, rounded shaped organisms have a more efficient reproductive strategy. On the other hand, when the resource availability is low (oligotrophic environment – Lagoa Dourada), where Chla concentration is minor and the bacterial density is smaller, growth of organisms with elongated forms is favoured. Thus, elongated shaped organisms have a strategy more suited to the resource scarcity.

Another hypothesis involves the surface/volume (S/V) ratio of bacteria and the availability of resources. Contradicting the general view that planktonic bacteria respond to food depletion by reducing their size and thus increasing their S/V ratio, Steinberger et al. (2002) showed that Pseudomonas aeruginosa reacted to the lack of nutrients in unsaturated biofilms by elongating their cells. Roszak & Colwell (1987) had published evidence supporting the theory proposed by Heinmets et al. (1953), that growth processes continue in the absence of cell division under stressful conditions. By this mechanism, the organism was able to increase its surface area, providing itself with greater contact with the medium and so enhancing its capacity to capture the scarce nutrients from the surroundings. Therefore, in Lagoa Dourada

the possibility of this pleomorphism, of one or more oligotrophic species, should not be discarded, in spite of the lack of experimental confirmation.

On the other hand, the comparison of the MDI values (Tab. IV) with the relative frequencies (Fig. 2) of the greater and more elongated forms (bacilli, vibrios, filaments and spirilla) indicates that the higher these frequencies, the greater the MDI value (Racy, 2004).

The Fig. 4 shows that the greater the Chla, the lower the bacterial morphological diversity. It is possible that large portion of Chla concentration comes from mixotrophic organisms (e.g. dinoflagellates such as Peridinium) that are abundant in Lagoa Dourada (Vieira et al., 2002).

They can sometimes consume bacterioplankton (e.g. Hadas & Berman, 1998; Graham et al., 2004) and the feeding behaviour of heterotrophic plankton appears to be associated with a preference for larger or growing prey (González et al., 1990; Hahn & Höfle, 1999). So, in this environment the mixotrophic organisms can, in certain periods, decrease their photosynthetic activity and start to act as heterotrophs, accelerating the cropping of certain types of bacteria (specially the more voluminous bacilli, vibrios, filaments and spirilla), reducing their relative frequency (Racy, 2004), and thus diminishing the morphological diversity.

This hypothesis is supported by several observations on the nutritional behaviour of mixotrophs (particularly in oligotrophic conditions), as well as the close relation between the abundance of bacteria and that of their predators ("top-down" effect) (Tittel et al., 2003; Jones, 2000; Roberts & Laybourn-Parry, 1999; Rothhaupt, 1996a; 1996b; Gasol & Vaqué, 1993).

Finally, it may be concluded from these results that density (D), like bacterial MDI is a biological tool capable of being used to distinguish the trophic state of the aquatic systems studied, simple to apply and of low operational cost, without requiring the use of different variables, as occurs in the determination of mTSI.

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