Variations in the number of active bacteria in the euphotic zone of a recently flooded reservoir

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ABSTRACT: Using the CTC (5-cyano-2,3-ditolyl tetrazolium chloride) method, we estimated the active fraction of bacterioplankton in the euphotic zone of the recently flooded Sep Reservoir, France, with the aim of evaluating its importance and dynamics in relation to temperature and some components of dissolved organic matter (DOM) (i.e. dissolved combined amino acids: DCAA; dissolved free monosaccharides: DFCHO; dissolved combined monosaccharides: DCCHO), and its metabolic significance in conjunction with a bacterial growth indicator: ^{14}C -glucose uptake. From our results, it appears that only a small fraction (0.04 to 3.23 %) of total bacterial count in this reservoir was metabolically active. Recorded correlations and multivariate regression analysis suggest that temporal variations in the number of active bacteria are mainly (80 %) governed by temperature, together with the high concentrations of those components of DOM, which were present in this ecosystem but which apparently were not always available for bacterial metabolism. We also found a positive relation between the number of active bacteria and the potential heterotrophic activity, which suggest that CTC-positive bacteria are responsible for the bulk of bacterial community metabolism in this recently formed reservoir.

KEY WORDS: Active bacteria · CTC · Dissolved organic matter · Temperature · Bacterial activity · New reservoir

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INTRODUCTION

In aquatic microbial ecology, determining bacterial densities is usually the first stage in the process of studying relations between bacteria and biochemical processes such as the degradation and recycling of organic matter (e.g. Richardot et al. 2000). The most common method for counting planktonic bacteria is epifluorescence microscopy, which uses fluorescent stains (Hobbie et al. 1977, Porter & Feig 1980) and provides an estimate of the total bacterial abundance in the environment, irrespective of their physiological state. It is now known that planktonic bacterial community consists of both metabolically active cells and of inactive cells (Dufour et al. 1990, Sommaruga &

Conde 1997). In most marine and lake ecosystems, it has been shown that the majority of bacterioplankton consist of cells that have a metabolic activity that is undetectable by the methods of autoradiographic labeling (Meyer-Reil 1978), flow cytometry (Jepras et al. 1995, Porter et al. 1995), or techniques using epifluorescence microscopy following specific cell staining (Zimmermann et al. 1978, Tabor & Neihof 1984, Rodriguez et al. 1992) or cell treatment with nalidixic acid (Kogure et al. 1979). Nevertheless, the determination of the quantitative importance of the metabolically active fraction in the bacterioplankton and the factors that control its dynamics is of major interest since this fraction is the basis of bacterial population activity (Rodriguez et al. 1992).

Recent studies have suggested that variability in bacterioplankton species composition is one of the main factors regulating temporal variability of the active

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fraction of bacterioplankton (e.g. Thom et al. 1993), whereas others have suggested temperature (Tabor & Neihof 1984, Robarts & Sephton 1988, Sommaruga & Conde 1997), lysis (Proctor & Fuhrman 1990), grazing (Gasol et al. 1995) and resource availability (del Giorgio & Scarborough 1995, Choi et al. 1999). In terms of resource availability, a wide variety of sources including phytoplankton excretion and lysis (Cole et al. 1988, Maurin et al. 1997) and grazing and release by zooplankton (e.g. Hygum et al. 1997) have been identified as producing dissolved organic matter (DOM) that fuels bacterioplankton metabolism. Inputs of allochthonous DOM and physical disaggregation of detrital particles (Hadas et al. 1990) are also likely to be significant energy sources for bacterial activities. However, nearly all these studies have been conducted in natural or stabilized artificial lake ecosystems. To our knowledge, no information is available on fluctuations in the number of active bacteria in recently flooded reservoirs that are expected to contain large quantities of terrestrial detritus and organic matter, which is known to efficiently regulate bacterial activity (Paterson et al. 1997).

The aim of this study was to determine the importance of the active fraction of bacterioplankton in a recently flooded reservoir, the main variables controlling their abundance (especially temperature and DOM) and their metabolic significance in conjunction with a growth indicator: ^{14}C -glucose uptake. The numbers of active bacteria were determined by electron transport system (ETS) activity assays, based on reduction of the tetrazolium salt CTC (5-cyano-2,3-ditolyl tetrazolium chloride), which is currently the most widely used method in aquatic ecology (e.g. Sherr et al. 1999).

MATERIALS AND METHODS

Sites and sampling. The Sep Reservoir, situated at an altitude of 500 m in the French Massif Central (46°2'N, 3°1'E), was built in 1994 by damming the Sep and Riaux streams. With an area of 33 ha and a mean depth of 14 m, this reservoir has a catchment of 27 km² whose vegetation consists of oak and beech forests and grasslands. The reservoir, which is oligomesotrophic in status, was completely filled for the first time in January 1995 and its mean residence time is 220 d. Temporal changes since flooding have shown evidence for a decrease in the proportion of heterotrophic and mixotrophic organisms in the planktonic biomass, these being progressively replaced by strictly autotrophs (Thouvenot et al. 2000).

Sampling was carried out at a permanent station situated at the deepest zone of the reservoir near the dam. Water samples from 1 m below the surface were

collected every 2 wk in April and weekly from May to August 1997 with a Van Dorn water sampler.

Physico-chemical parameters. Temperature and dissolved oxygen were measured *in situ* with a YSI-GRANT/3000 multiparametric probe. Chemical analyses, namely ammonium ($\text{NH}_3\text{-N}$), nitrates ($\text{NO}_3\text{-N}$), and orthophosphate ($\text{PO}_4\text{-P}$), were performed using standard methods (APHA 1992).

Duplicate DOM determinations were conducted. Water samples prefiltered through prewashed 0.2 μm pore size polycarbonate filters were used to determine the DOM content. Dissolved combined amino acids (DCAA) were determined by the 'micro BCA Protein Assay Reagent' Kit (Pierce). Bovine serum albumin (BSA) was used as a standard. Dissolved free amino acid (DFAA) analyses were performed with the o-phthalaldehyde method, as described by Parsons et al. (1984), using glycine as a standard. The coefficients of variation for the TDAA (total dissolved amino acids, DCAA and DFAA) analysis varied between 0.6 and 6.0%. Dissolved free monosaccharides (DFCHO) were determined according to Burney & Sieburth (1977) and Johnson & Sieburth (1977), using glucose as a standard. Total dissolved carbohydrate (TDCHO) concentrations were determined after hydrolysis with HCl (1 N; 100°C, 15 h). TDCHO minus DFCHO gave dissolved combined carbohydrates (DCCHO) (the coefficients of variation for the DCCHO analysis varied between 0.7 and 7.0%). The amount of organic carbon stemming from the protein and carbohydrate pool (C-TDAA + C-TDCHO) was calculated assuming that 1 mg of glucose is equivalent to 0.4 mg carbon, and 1 mg of BSA and glycine are equivalent to 0.5 and 0.16 mg carbon, respectively.

Biological variables. Total counts of bacterial cells were obtained from aliquots of subsamples fixed with formalin at a final concentration of 2%, after staining with DAPI (Porter & Feig 1980) and counting under epifluorescence microscopy. At least 500 bacterial cells were counted and measured in 20 to 50 fields of view of the microscope. The length and width of bacterial cells were estimated using an eyepiece micrometer. The number of respiring bacteria cells was determined by counting those showing an ETS activity (Zimmermann et al. 1978) using the procedure described by Rodriguez et al. (1992). Briefly, 0.5 ml of CTC stock solution was added to freshly collected water (10 ml). In a preliminary test, a final concentration of 0.75 mM was found to be sufficient to detect respiring bacteria. This concentration is within values used for pelagic systems (del Giorgio et al. 1997, Choi et al. 1999) and, according to Sherr et al. (1999), lower than the value (10 mM) above which the number of stained cells also tends to decline, presumably due to toxicity of CTC or its formazan products (Choi et al. 1999). The mixture

was incubated at room temperature (18°C) for about 2 h. Thereafter, the sample was filtered through a black nuclepore polycarbonate membrane (pore size, 0.2 µm), air dried and respirometrically active cells were counted microscopically. Our incubation time was chosen from preliminary tests and was within the shorter time period of incubation reported by many authors (Rodriguez et al. 1992, Choi et al. 1996, Lovejoy et al. 1996). Moreover, our incubation method, which consisted of small aliquots of liquid samples incubated with the CTC, seems more accurate according to Sherr et al. (1999). Indeed, it provides good results compared to the membrane incubation method of Karner & Furhman (1997) in which a large volume (~30 ml) of water is settled onto membrane filters, the filter surface is flooded with CTC solution, and the filter is incubated for a period of time.

Experiments on heterotrophic activity of microbial assemblages were run every 2 wk and estimated using [U-¹⁴C] glucose (specific activity = 293 mCi mmol⁻¹;

Amersham). At the sampled depth, serial increasing concentrations ranging from 0.5 to 2.7 µg C l⁻¹ of the labeled tracer were added to duplicate subsamples (30 ml) and 1 formalin-killed control and incubated *in situ* and in the dark for 3 h. Thereafter, particles were retained on 0.2 µm Sartorius filters, rinsed twice with 5 ml of deionized water, and the filters were stored in a scintillation cocktail. Radioactivity was counted with a LKB[®] counter. The maximum rates of substrate uptake (V_{max}), considered as the potential heterotrophic activity (PHA), were calculated using Michaelis-Menten kinetics and the Lineweaver Burke transformation (Wright & Hobbie 1965).

Statistical treatments mainly consisted of linear pairwise correlation analysis to establish the relationship between bacterial variables and environmental parameters. In addition, step-wise multiple regressions (with forward selection) were used to relate bacterial variables with the physical environment (i.e. temperature) and resource factors (i.e. DOM).

RESULTS

Fig. 1. Temporal variations of (A) temperature and (B) dissolved oxygen in the euphotic zone of Sep Reservoir, France. Dates given as mo/d/yr

The temperature at our sampling depth increased throughout the study, from <12 to ~26°C (Fig. 1A). Dissolved oxygen values changed slightly over the sampling time (Fig. 1B), the mean ($\pm$ SD) value being 8.03 ± 1.3 mg l⁻¹ (range = 5.58 to 10.55 mg l⁻¹). The mean concentrations of nitrates, ammonium and orthophosphate were 1.59 mg N l⁻¹ (0.87 to 2.3 mg N l⁻¹), 0.006 mg N l⁻¹ (0.003 to 0.009 mg N l⁻¹) and 0.030 mg P l⁻¹ (0.015 to 0.047 mg P l⁻¹), respectively.

The values of DFCHO varied about 7-fold, from 0.31 to 2.27 mg l⁻¹, whereas DCCHO varied about 40-fold, between 0.05 and 2.12 mg l⁻¹. For these 2 variables, the mean values were 1.08 ± 0.55 and 0.92 ± 0.54 mg l⁻¹, respectively. Temporal variations in the concentrations of DCCHO and DFCHO did not show a regular trend during the study. However, except for the period from late May to mid-June, late July and mid-August, when there was a clear positive correlation between the concentrations of DCCHO and DFCHO, they were mostly negatively correlated (Fig. 2A). Temporal changes in DCAA concentrations could be divided into 2 distinct periods. In the first phase, the DCAA concentrations varied between 4.1 and 5.2 mg l⁻¹. From 18 June onward, there was a quick change to higher concentrations, which reached peak values at the end of sampling (Fig. 2B). The mean DFAA content was 1.8 ± 1.0 mg l⁻¹.

Two periods of high concentrations were recorded, at the end of May and end of August, when the concentrations peaked at 3.7 and 4.2 mg l⁻¹, respectively (Fig. 2B). The amount of organic carbon derived from DOM made up of protein and carbohydrate pools fluctuated between 2.9 mg C l⁻¹ and 5.3 mg C l⁻¹ with a mean value of 4.1 mg C l⁻¹. Seasonally, their concentrations were about 3.0 mg C l⁻¹ during the first 3 sampling dates of the study; thereafter, they increased steadily throughout May up to a peak of 3.8 mg C l⁻¹, before declining to a constant value during June. From 9 July, the organic carbon concentrations increased strongly and reached the highest values measured during this study (Fig. 2B).

The bacterial population mostly consisted of free-living cells with a mean biovolume of 0.06 μm³. The density of CTC-positive bacteria fluctuated by about 2 orders of magnitude, from 0.10 to 10.20 × 10⁴ cells ml⁻¹ (mean = 4.12 ± 3.39 × 10⁴ cells ml⁻¹), whereas the density of total bacteria varied only between 1.41 and 4.49 × 10⁶ cells ml⁻¹ (mean = 2.93 ± 0.83 × 10⁶ cells ml⁻¹) (Fig. 2C). The abundance of bacteria counted using DAPI was highest at the start of the study, on 18 June and 13 August. The dynamics of CTC-active bacteria can be separated into 3 distinct periods. From the start of the study to 21 May, they were particularly infrequent (mean = 2.55 ± 1.16 × 10⁴ cells ml⁻¹); their number increased from the end of May and fluctuated between 2.3 and 3.1 × 10⁴ cells ml⁻¹. From 9 July, the abundance of CTC-active bacteria increased strongly and reached the highest values measured during this study (Fig. 2C). Active bacteria accounted for between 0.04 and 3.22% of total bacterial abundance and their seasonal variation was apparently independent of that of total bacterial abundance. The values of ¹⁴C-glucose uptake fluctuated between 1.3 and 7.4 × 10⁻² μg C l⁻¹ h⁻¹ (mean = 3.0 ± 1.8 × 10⁻² μg C l⁻¹ h⁻¹), the highest values being recorded in summer (Fig. 2A).

The abundance of CTC-positive bacteria was significantly correlated with the water temperature (Table 1). Bacterial uptake of ¹⁴C-glucose plus the DOM contents (DFAA, DCAA and TDCHO) were better correlated with the number of metabolically active bacteria than with total abundance (Table 1). The multiple regression analysis suggested that 80% of the

Fig. 2. Temporal variations of (A) dissolved free carbohydrate (DFCHO), dissolved combined carbohydrate (DCCHO) and ¹⁴C-glucose uptake rates (PHA: potential heterotrophic activity), (B) dissolved combined amino acids (DCAA), dissolved free amino acids (DFAA) and carbon content (from C-TDAA + C-TDCHO), (C) bacterial abundance and active bacteria (CTC-reducing bacteria), in the euphotic zone of Sep Reservoir. Dates given as mo/d/yr

Table 1. Variables that showed significant correlation with CTC-positive bacteria (n = number of samples)

	Coefficient correlation	p	n
Temperature	0.9	<0.001	17
V _{max} of glucose uptake	0.6	<0.01	9
C (TDAA+TDCHO)	0.9	<0.001	17
DFAA	0.5	<0.05	17
DCAA	0.8	<0.05	17

total variance observed in the number of CTC-positive bacteria could be explained by the temperature and the availability of resources, and particularly the quantity of DOM. When considered separately, the temperature and DOM explained 66 and 75 % of the variance in abundance of CTC-positive bacteria, respectively.

DISCUSSION

Methodological aspects and quantitative importance of CTC-positive cells

Despite the multitude of known methods used for distinguishing active bacteria from non-active ones within the bacterial community, none provide unambiguous counts of active bacteria in the water, the results even sometimes being contradictory. For example, Maki & Remsen (1981) concluded that counts of active bacteria by methods using the tetrazolium salt INT (2-p-iodophenyl-3p-nitrophenyl-5-phenyl tetrazolium chloride) or using nalidixic acid gave identical results, whereas Tabor & Neihof (1984) showed that, in general, the nalidixic acid method overestimated the density of active cells compared to an autoradiographic method and that using INT. Such divergences suggest prudence when comparing the results of studies relating to the number of active bacterial cells in various environments obtained using different methods. The method of estimation by autoradiography of active bacteria requires long incubation times and high concentrations of labeled nutrients, whereas with the nalidixic acid method, the problem is mainly distinguishing those cells that have actually increased in size after incubation. Methods using tetrazolium salts (formazan-positive) are easy to use (Zimmerman et al. 1978, Maki & Remsen 1981, Tabor & Neihof 1984, Rodriguez et al. 1992) and do not have the disadvantages mentioned above. There are however some methodological biases associated with these tetrazolium salt methods which can lead to an underestimate of the percentage of active bacteria. These are: (1) undetectable spots of formazan formed inside small-sized cells,

(2) the presence of cells with a low DNA content, which according to Choi et al. (1996) are less sensitive to CTC, (3) an overestimate of total bacterial abundance after staining with DAPI with notably the inclusion of some large DNA viruses (Sommaruga et al. 1995) and of 'ghost cells' (in the sense of Zweifel & Hagström 1995). In this study, the number of active bacteria was determined using the tetrazolium salt method, in particular the CTC method described by Rodriguez et al. (1992) because: (1) methods that use tetrazolium salts are easily applicable for routine purposes (Dufour & Colon 1992), (2) among the tetrazolium salts, INT is less directly coupled to respiratory activity than is the reduction of CTC (Smith & McFeters 1997), (3) CTC is currently the most widely used tetrazolium salt in studies of aquatic environments (Gasol et al. 1995, Choi et al. 1996, Lovejoy et al. 1996, Karner & Fuhrman 1997, Choi et al. 1999, Pinhassi et al. 1999, Sherr et al. 1999).

During this study, the number of CTC-positive bacteria in the surface waters of the Sep Reservoir varied from 0.10 to 10.20×10^4 cells ml⁻¹, corresponding to 0.04 – 3.22 % of the total bacterial density. Setting aside methodological differences, our results, like those of many other authors (Dufour et al. 1990, del Giorgio & Scarborough 1995, Sommaruga & Conde 1997), show that not all bacteria are metabolically active. In terms of the percentage of CTC-positive bacteria, our values are at the lower end of those reported in other studies (Table 2); they are similar to those reported by Rao et al. (1981) in Canadian lakes and by Lovejoy et al. (1996) in the Gulf of St. Lawrence (Canada). Relatively low values have also been reported from the Baltic Sea (Zimmermann et al. 1978) and Blanes Bay, USA (Gasol et al. 1995).

In general, in all instances where CTC has been used in natural water samples, the proportion of active cells has been low, typically between <1 and 35 % (del Giorgio & Scarborough 1995, Choi et al. 1996, del Giorgio et al. 1996, 1997, Lovejoy et al. 1996, this study). This does not imply the failure of CTC to detect all cells that are metabolically active in the plankton, but the result of the CTC reduction assay should be viewed and interpreted as identifying the most highly active cells in bacterioplankton populations or assemblages (Sherr et al. 1999). Indeed, the level of cell activity is what determines the detectability of respiring cells, since even bacteria in a starvation survival state must sustain certain functions, such as maintenance of the cell membrane integrity and active transport systems, and can carry out a low level of protein synthesis (Sherr et al. 1999). Choi et al. (1996, 1999) have shown that a portion, and sometimes a large fraction, of supposedly inactive bacteria cells can be induced to high metabolic activity by a change in environmental conditions (e.g. substrate availability, temperature increase).

Table 2. Range or mean values for percent of active bacteria in different water bodies using different methods

Location	% active cells	Method	Source
Westensee lake (Germany)	23–36	INT reduction method	Zimmermann et al. (1978)
Baltic sea (Kiel Firth, Germany)	12	INT reduction method	Zimmermann et al. (1978)
Baltic sea (Kiel Bight, Germany)	7	INT reduction method	Zimmermann et al. (1978)
Lake Huron (Canada)	0.5–3.2	INT reduction method	Rao et al. (1981)
Georgian parc (Canada)	1.2–5.7	INT reduction method	Rao et al. (1981)
Erié Lake (Canada)	3.9–7.2	INT reduction method	Rao et al. (1981)
Lake St-George (Canada)	3.4–3.5	INT reduction method	Rao et al. (1981)
Chesapeake Bay (MD, USA)	25–94	INT reduction method	Tabor & Neihof (1984)
Hartbeespoort Reservoir (South Africa)	16.4–20.4	Nalidixic acid method	Robarts & Sephton (1988)
Lake Geneva (France)	1–47	INT reduction method	Dufour et al. (1990)
Fish Pond (Ivory Coast)	28–71	INT reduction method	Dufour et al. (1990)
24 Canadian lakes (Canada)	15–33	CTC reduction method	del Giorgio & Scarborough (1995)
Blanes Bay (MD, USA)	6.4	CTC reduction method	Gasol et al. (1995)
14 Canadian lakes (Canada)	7.8–47.1	CTC reduction method	del Giorgio et al. (1996)
Gulf of St. Lawrence (Canada)	<1–2	CTC reduction method	Lovejoy et al. (1996)
Lake Rodo (Uruguay)	17–100	INT reduction method	Sommaruga & Conde (1997)
Sep Reservoir	0.04–3.23	CTC reduction method	This study

The creation of a new reservoir and flooding of a lake may initiate a series of changes in the plankton community related to alterations in hydrology and nutrient availability that frequently result from inputs of large amounts of newly flooded terrestrial organic matter that may also stimulate bacterial activity (Paterson et al. 1997). These effects almost always lead to the ecosystem evolving toward a new steady state of maturation with a trophic status generally lower than that observed immediately after flooding, and that may be achieved within years (Robarts et al. 1992, Paterson et al. 1997). It has been established over a broad range of aquatic systems that the number of active bacteria increases with the system's productivity (del Giorgio & Scarborough 1995). Indeed, according to those authors who have also used the CTC method during their investigation, the proportion of active cells may increase from values typical of ultra-oligotrophic open-ocean areas (<5%) to highly productive estuaries (>50%). The study of the temporal changes in the Sep Reservoir showed, firstly, a lowering in the DOM concentration with time (Richardot et al. 2000) and, secondly, a decrease in the proportion of heterotrophic organisms, such as bacteria, and mixotrophic organisms, which were progressively replaced by strictly autotrophic organisms (Thouvenot et al. 2000). The low number of CTC-active bacteria determined at this site during our study could in part be related to changes in this ecosystem.

Relations with the other parameters measured

As in other studies (Tabor & Neihof 1984, Robarts & Sephton 1988, Sommaruga & Conde 1997), the water temperature in the Sep Reservoir was significantly correlated with the number of metabolically active bacteria (Table 1). In the surface waters of the Gulf of St. Lawrence in Canada, the low proportions of CTC-active bacteria observed by Lovejoy et al. (1996) were between 1 and 2% during summer stratification and fell to <1% in winter. This was the same case in the Sep Reservoir, where active bacteria accounted for between 0.04 and 3.22% of total abundance but had apparently higher densities during the period of thermal stratification (Fig. 2C). Similarly, in a comparative review of published data, Sommaruga & Conde (1997) concluded that in conditions where resources are not limiting, warming the water leads to an increase in the abundance of metabolically active bacteria. For example, in Lake Rodo (Uruguay), those authors calculated that the numbers of CTC-positive bacteria were increased by more than an order of magnitude, parallel to an increase in temperature from 8 to 28°C. During this study, the water temperature at our sampling depth varied from <12 to ~26°C, leading to a ~100-fold increase in the abundance of CTC-positive bacteria. This observation agrees with that of Sommaruga & Conde (1997), and also puts the temperature dependence of our results in line with other recent reports

showing strong temperature dependence of bacterial growth rates at environmental temperatures of less than 20°C (e.g. Kirchman & Rich 1997). Thus, temperature appears to have had a profound effect on the pattern of induction of high ETS activity and of increased growth yields in the bacterial cells of our ecosystem.

The multiple regression analysis showed that 80% of the total variance associated with the numbers of CTC-positive bacteria could be explained by temperature and DOM and the correlation with the dissolved organic carbon stemming from the protein and carbohydrate pool (Table 1) show that, in this ecosystem, temperature is not the only factor involved in controlling the abundance of CTC-positive bacteria. Moreover, as in numerous studies that have already reported a good relation between metabolically active bacteria and bacterial activities, particularly bacterial ¹⁴C-glucose uptake (Robarts & Sephton 1988, Dufour et al. 1990), we also showed, in this study, a relation between active bacteria and the uptake of a directly usable component, namely glucose. Such a relation suggests that CTC-positive bacteria are responsible for the bulk of bacterial community metabolism in this recently formed reservoir. The statistical dependence between CTC-positive bacteria and DOM could have been caused solely by the increase in temperature, which appears to be the main factor explaining the fluctuations in the number of CTC-active bacteria, and which is also usually the controlling factor in the phenomena involved in the production of autochthonous organic matter readily assimilable by bacteria. Nevertheless, since the concentrations of TDAA and TDCHO in this reservoir were within the higher range measured in many ecosystems (Striquer-Soares & Chevolot 1996), the significant relationship between CTC-positive bacteria and supposedly readily useable DOM seems surprising. In fact, the high amount of organic matter present in this recently flooded reservoir was not from autochthonous production, but was mainly of allochthonous origin and difficult to biodegrade (Richardot et al. 2000). So, it is possible that, at times, the bio-availability of these compounds could also have been a limiting factor for the development of bacterioplankton. A previous study showed that DFAA and DCAA may contribute substantially to bacterial growth (Simon & Rosenstock 1992), and Choi et al. (1999) recorded a large increase in the percentage of CTC-positive bacteria with the addition of mixed amino acids. Moreover, Rosenstock & Simon (1993) reported an increased use of DCAA by bacterioplankton, when these components were resistant to the microbial degradation. Results from our observations demonstrate the role of organic matter and its assimilation in the regulation of the temporal variability in the number of active bacteria in the Sep Reservoir, and particularly

TDAA since statistical relations were more significant with DFAA and DCAA (Table 1).

To our knowledge, the significant relationship we recorded between organic matter and metabolically active bacteria is the first one in a recently flooded reservoir. In old and stabilized ecosystems, contrasting relationships between organic matter and metabolically active bacteria have been reported. Kogure et al. (1980) concluded from their survey of metabolically active bacteria in the water around Japan that the numbers increased in relation to the particulate and dissolved organic carbon concentrations. In Hartbeespoort Dam, Robarts & Sephton (1988) did not observe any correlation between the number of metabolically active bacteria and the dissolved organic carbon concentration. However, few *in situ* simultaneous estimates of DOM concentrations and active bacteria are sufficiently complete to permit accurate comparisons. The relation we observed between DOM and active bacteria agrees with recent reports by Pinhassi et al. (1999) and Choi et al. (1999), who recorded an increase in the number of viable bacteria (using the CTC method) in experimental mesocosms incubated at 15 and 20°C, respectively, and amended with organic substrates (i.e. amino acids, protein and carbohydrates).

From our results it appears that only a small fraction (0.04 to 3.23%) of the total bacterial count in the Sep Reservoir was metabolically active. Based on a recent study published by Choi et al. (1999), this fraction may be considered to be formed by bacterial cells with a high ETS activity since many more cells apparently become CTC-positive over time with the increase in temperature, and the high PHA during summer depended on the high DOM concentrations recorded in the water. These high DOM concentrations were probably linked with the recently flooded character of the reservoir. Other studies have demonstrated that bacterivorous protists graze preferentially on larger bacterial cells (Gonzalez et al. 1990), dividing cells (Sherr et al. 1992), and CTC-positive bacteria (del Giorgio et al. 1996). Thus, the action of those micro-organisms is likely to also control the abundance of CTC-positive bacteria and needs to be investigated in the future.

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