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Primary Research Paper

Bacterial production in the recently flooded Sep Reservoir: Diel changes in relation to dissolved carbohydrates and combined amino acids

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Abstract

The spatial distribution of bacterial abundance and production were measured every 4 h in a recently flooded oligo-mesotrophic reservoir (the Sep Reservoir, Puy-De-Dôme, France), in relation to concentrations of dissolved carbohydrates and combined amino acids. The concentration of dissolved organic matter (DOM) components in the recently flooded Sep Reservoir were higher than those measured in other lakes of similar trophic status. Short-term variations in the bacterial production in this new reservoir appeared cyclical and endogenous to bacterial communities. These results highlight the need for the evaluation of diel changes in bacterial production, if estimation of the daily production rate of bacteria is to be done accurately for a reliable model of carbon flow through bacterioplankton and ultimately through aquatic microbial food webs. Bacterial growth, measured over time and space, did not appear exclusively governed by DOM components from phytoplankton primary production.

Introduction

Dissolved organic matter (DOM) is a ubiquitous component of surface waters, and represents the main organic compartment in global aquatic carbon stocks (Hedges, 1992). DOM plays an important role in fueling microbial-based food webs (Findlay et al., 1986; Azam, 1998) and is nearly exclusively used by heterotrophic bacteria. An accurate quantification of the contribution of bacteria to DOM has been impeded by a poor understanding of the factors that regulate the degree to which DOM substrates consumed by bacteria are respired or converted into biomass (del Giorgio & Cole, 1998).

Bacterial metabolism depends on the quality and quantity of supplied DOM (Eiler et al., 2003; Perez et al., 2003; Kramer et al., 2005; Young et al., 2005). Previous studies of resource limitation in bacteria, looking at variables such as bacterial production, suggested that bacterial growth in aquatic systems could be limited by the supply of organic carbon substrates from the phytoplankton compartment (Cole et al., 1982; White et al., 1991; Sell & Overbeck, 1992). In addition to algal carbon, there are a wide variety of organic substrates found in aquatic systems (Azam, 1998), which include terrestrial derived DOM that bacteria can use for growth (Moran & Hodson, 1990; Tranvik, 1992). Diel changes in plankton are probably connected with periodic change in phytoplankton activity or to changes in the diel activity of zooplankton, either directly by grazing or indirectly by consumption of algae and

excretion by zooplankton (Straskrabova & Fuksa, 1982; Jørgensen & Bosselmann, 1988).

To assess the significance of labile DOM within the microbial food web, we need to better understand the *in situ* interaction between DOM and the growth activity of its main users, heterotrophic bacteria. Bacterial cycling of reactive DOM constituents has been followed, based on the study of analytically recognizable compounds such as amino acids (Rosenstock & Simon, 1993; Weiss & Simon, 1999; Kirchman, 2003) or carbohydrates (Jørgensen & Jensen, 1994; Tranvik & Jørgensen, 1995; Hanisch et al., 1996; Rich et al., 1996; Jørgensen et al., 1998; Skoog et al., 1999; Weiss & Simon, 1999; Kirchman, 2003). However, almost all of these studies and others dealing with diel variations in bacterioplankton, are related to natural or stabilized artificial lake ecosystems where phyto- and zoo-plankton activities are the dominant source of DOM. In contrast to established aquatic systems, diurnal changes may not be as influential on bacterial activities in recently flooded reservoirs, since they are expected to contain large quantities of terrestrial detritus and organic matter, also known to effectively regulate bacterial production (Paterson et al., 1997; Lennon & Pfaff, 2005).

This study was designed to analyze short-term fluctuations of bacteria abundance and production in relation to concentrations of dissolved carbohydrates and combined amino acids in a model system represented here by the recently flooded Sep Reservoir.

Materials and methods

Study site and sampling

The Sep Reservoir resulted from a dam built across the Sep Stream for the summertime irrigation of an agricultural zone of the Haute Morge, ''Massif Central'', France (ca. 46° N, 3° E). The Sep reservoir was built in 1994 and completely filled for the first time in January 1995. However, it was emptied in the summer, usually from July to September, in 1995 and 1996 to prevent deoxygenation in the deep layer. At its full supply level, the reservoir contains 4.7×10^6 m^3 of water and has a surface area of 33 ha, with a mean and a maximum depth of 14 and 37 m, respectively. This reservoir has a catchment of 27 km^2 whose vegetation consists of oak and beech forests and grasslands. The Sep reservoir is generally classified as an oligo to mesotrophic lake, based on the annual pigment and nutrient concentrations, following Organization for Economic Co-operation and Development (OECD) recommendations (OECD, 1982). During the period of the present study (i.e. July 1997), the flow rate in the reservoir was low (ca. 0.5 m^3 s^{-1}) and the flow rate from the reservoir was maintained at approximately the same level. Consequently, the maximum depth at our sampling area was a constant 20 m. The calculated residence time of waters in the reservoir was approximately 200 days.

Water samples were collected every 4 h over a period of 48 h (from July 23 at 10:00 to July 25 at 10:00) at the deepest zone of the reservoir, using a horizontal Van Dorm bottle. Sampling depths were 1 and 7 m below the surface and at 1 m above the sediment. The sampling depths were chosen to be representative of the epilimnion (1 m below the surface), metalimnion (7 m below the surface), and hypolimnion (1 m above the sediment). During the study and several days before, the weather was calm and sunny during the day with a period of darkness between ~22:00 and 06:00.

Physico-chemical variables

Duplicate DCAA, TDCHO and DFCHO determinations were conducted. Water samples prefiltered through 0.2 μm pore size polycarbonate filters were used to determine the dissolved organic matter content. Dissolved combined amino acid (DCAA) concentrations were determined using the Micro BCA Protein Assay Reagent Kit (Pierce), and bovine serum albumin (BSA) was used as a standard. The coefficients of variation for DCAA 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 (Glu) 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 DCHO analysis varied between 0.7 and 7.0%). Organic carbon concentrations were calculated assume to 1 mg Glu corresponds to 0.4 mg carbon, and 1 mg BSA coresponds to 0.5 mg carbon (Richardot et al., 2000). Blanks with carbon-free distilled water were processed in parallel, and the value obtained in each native sample was corrected to its respective blank. All glassware for analysis was acid-cleaned and furnaced at 550 °C for 5 h before use and samples were handled with powder-free gloved hands to avoid contamination.

Biological variables

Chlorophyll *a* (Chl *a*) values were obtained using 100 ml of water filtered through a GF/C glass fiber filter. Concentrations were determined spectrophotometrically after overnight dark extraction at 4 °C in 90% acetone, according to SCOR-UNESCO (1966). Primary production (PP) was measured *in situ* by means of the ^{14}C incorporation method (Steemann Nielsen, 1952), using water sub-samples (100 ml) containing essentially phytoplankton cells screened through 160 μm mesh size. Abundance and production of heterotrophic bacteria were determined as describe in Jugnia et al. (2000) using DAPI and (^{3}H-methyl) thymidine incorporation methods, respectively.

Statistical treatments

Statistical treatments consisted mainly of correlation analysis to establish the empirical relationships between variables. The differences between daytime and nighttime values of the variables under study were tested for significance ($p < 0.05$) using the Student *t*-test.

Results

Physico-chemical variables

TDCHO concentrations varied from 0.36 to 1.86 mg C l^{-1}, the mean value being at 0.82 ± 0.43 mg C l^{-1} (Fig. 1a). During the survey, TDCHO concentrations peaked in the surface water between 14:00 and 06:00, declined between 06:00 and 22:00, and then increased towards the end of the study (Fig. 1a). At 7 m below the surface, TDCHO concentrations varied slightly with a peak at 18:00 during the second day. At 1 m above sediment no consistent trend was evident in the diel variations of TDCHO concentrations.

DFCHO concentrations ranged between 0.07 and 1.38 mg C l^{-1} (mean $\pm$ SD = 0.40 ± 0.36 mg C l^{-1}). The highest and the lowest mean concentrations of DFCHO were recorded in the surface and bottom water, respectively. Over the sampling period, concentrations of these carbohydrates did not show any significant trend in diel variations (Fig. 1b). DCCHO concentrations fluctuated between 0.04 and 0.89 mg C l^{-1} (mean $\pm$ SD = 0.42 ± 0.17 mg C l^{-1}) and the highest concentrations generally occurred in the surface water (1 m). Variations in DCCHO concentrations were less than those observed with DFCHO, this was most notable at 1 and 7 m below the surface during the last 24 h of the study (Fig. 1c). However, no significant diel variation was registered (*t*-test, $p > 0.05$).

Regardless of the increase seen at 18:00 in the sample taken at 1 m above sediment during the first day, only minor diel variations were observed in the DCAA concentrations (Fig. 1d). These ranged from 1.63 to 3.02 mg C l^{-1} with a peak amount of 3.02 mg C l^{-1}. There were only minor vertical differences and high values were in most cases registered in the metalimnion as with Chl *a* (Jugnia et al., 2000). No significant difference was noted between the mean concentration obtained from the light and dark time periods.

Biological variables

Phytoplankton

Results of phtoplankton biomass (Chl *a*) and activity (PP) are presented in detail in Jugnia et al. (2000). Briefly, throughout the study, the highest Chl *a* concentrations were almost always recorded in the metalimnion, where the values were notably higher than in the epilimnion and much higher than in the hypolimnion (Fig. 2a). The highest values for photosynthetic activity were recorded in the epilimnion at 0 and 1 m below the surface. In the metalimnion, almost all the values were lower than 1 μg C l^{-1} h^{-1},

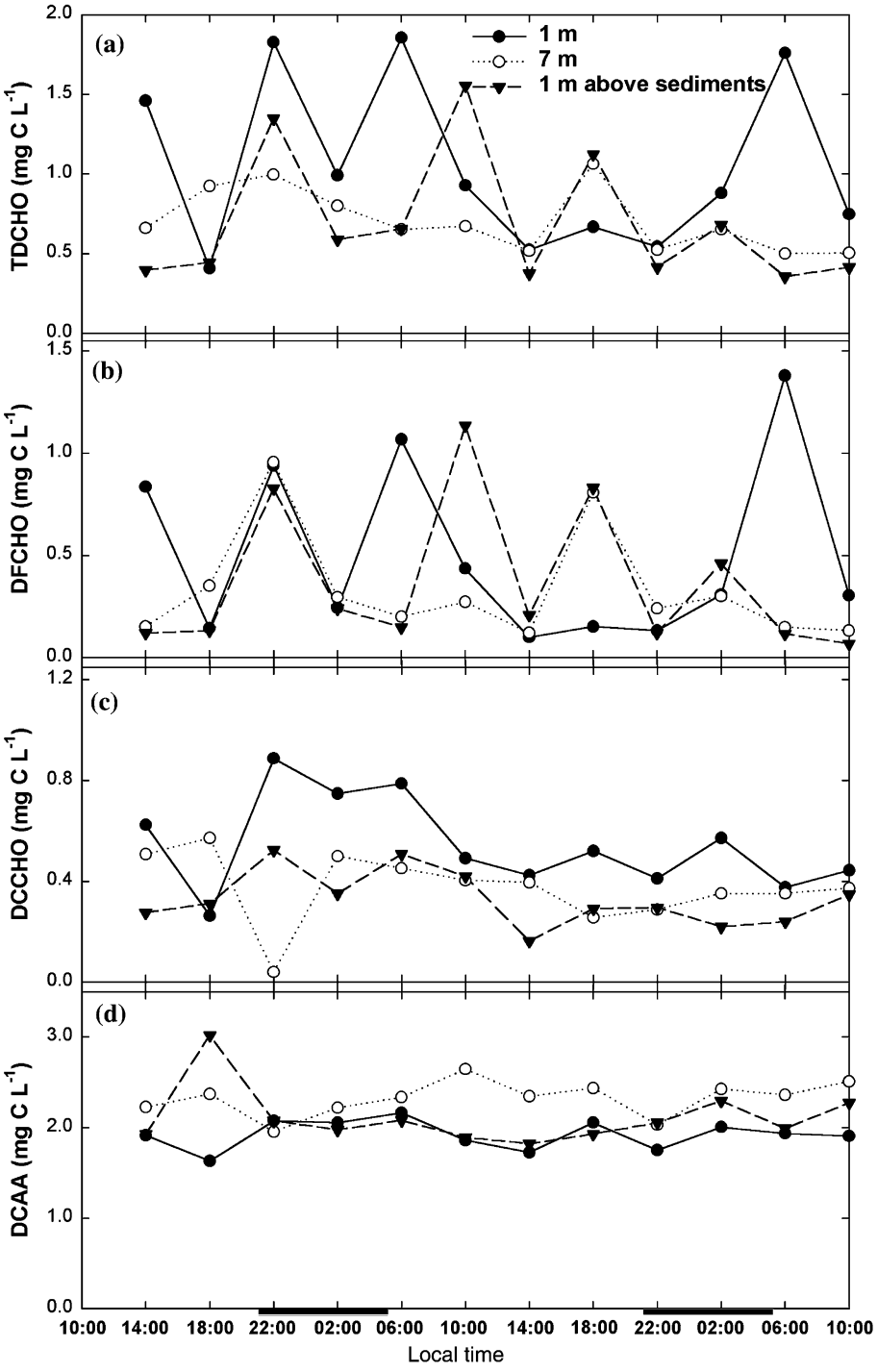

Figure 1. Spatio-temporal variation of (a) Total dissolved carbohydrate (TDCHO), (b) Dissolved free monosaccharides (DFCHO), (c) Dissolved combined carbohydrates (DCCHO) and (d) Dissolved combined amino acids (DCAA) in the Sep Reservoir during a 48-h survey, 23–25 July 1997. The horizontal black line represents the period of darkness.

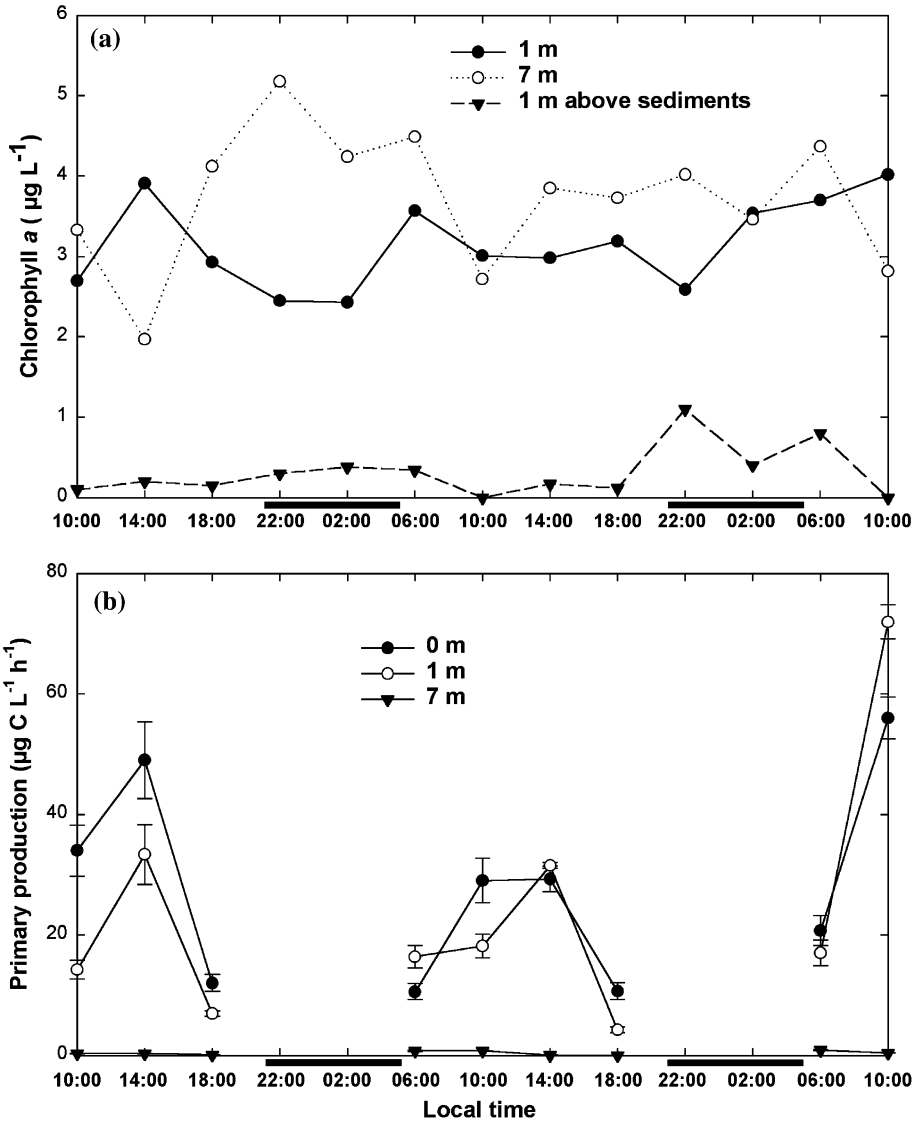

Figure 2. Spatio-temporal variation of (a) chlorophyll *a* concentration and (b) primary production in the Sep Reservoir during a 48-h survey, 23–25 July 1997. The horizontal black line represents the period of darkness. Data of this figure are from Jugnia et al. (2000).

whereas no photosynthetic activity was detected in the hypolimnion (Fig. 2b).

Bacterial abundance and production

Bacterial density varied about 8-fold, from 0.4×10^6 cells ml^{-1} (at 1 m above the sediment) to 3.39×10^6 cells ml^{-1} (at 1 m below the surface), with a mean value of $1.67\pm0.79\times10^6$ cells ml^{-1} (Fig. 3a). In terms of depth, values were similar at 1 and 7 m below the surface, where they were much higher than at 1 m above the sediment. The temporal variations in bacterial abundance seemed to be greater during the first 24 h of the study (Fig. 3a) and follows the same trend as seen with the DCCHO concentrations (Fig. 1c). There was no significant diel variation in this bacterial abundance (*t*-test, $p>0.05$) at any of the depths sampled.

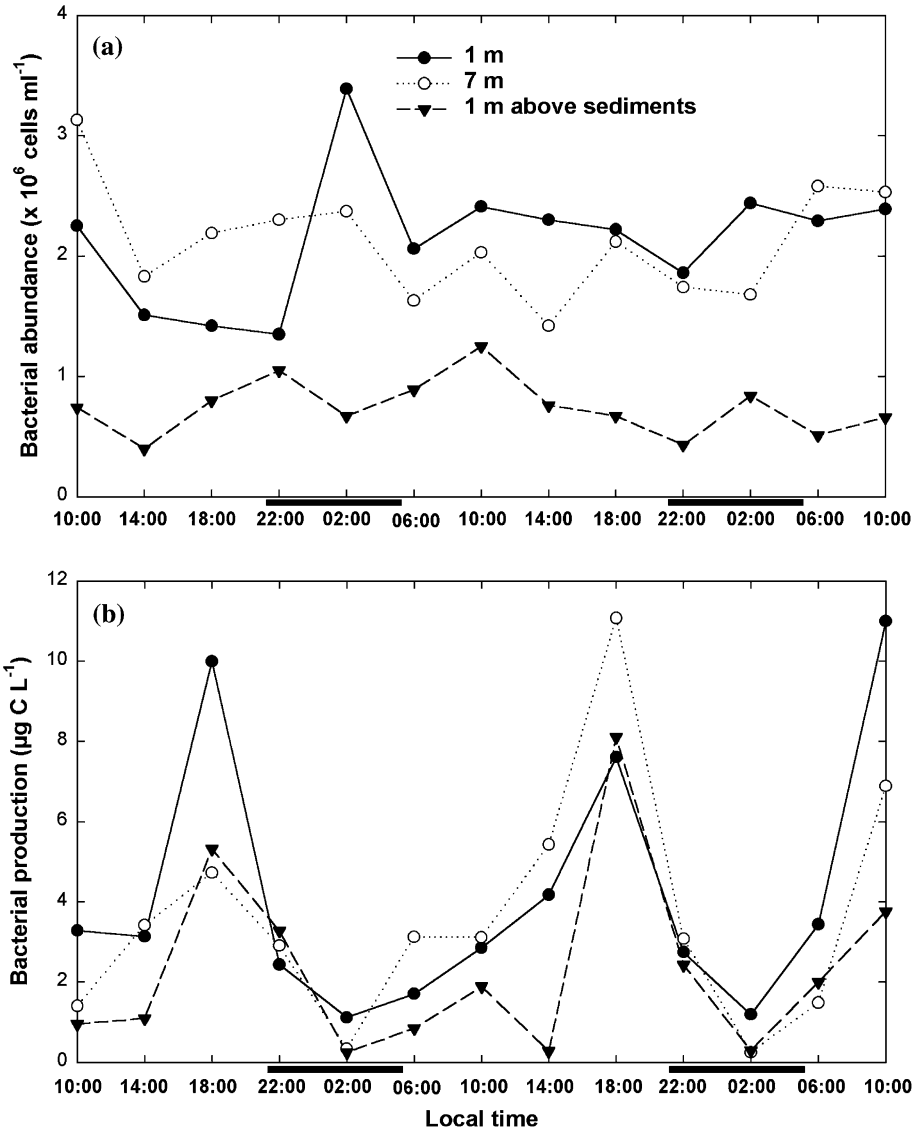

Figure 3. Spatio-temporal variation of (a) bacterial abundance and (b) production in the Sep Reservoir during a 48-h survey, 23–25 July 1997. The horizontal black line represents the period of darkness.

Bacterial production (BP) varied between 0.25 and 11.08 μg C l^{-1} h^{-1}, the mean value being 3.47 ± 2.88 μg C l^{-1} h^{-1}. The mean values decreased with depth, due mainly to the differences observed on 23 July at 18:00 and on 25 July at 10:00; the rest of the time the vertical variations were slight (Fig. 3b). The temporal variations at all sampling depths exhibited a trend that appeared to increase from the onset of daylight up to 18:00, and thereafter decreased to reach minimum values at 02:00 (Fig. 3b).

Discussion

During the study, both inputs and outputs from the reservoir were apparently constant and the main environmental impact on the biological variables under study was the diel cycle (Jugnia et al., 2000).

Concentrations, role and origin of DOM

In a newly-flooded reservoir, DOM seems to play a key role in controlling bacterial communities

through resources (Paterson et al., 1997; Richardot et al., 2000). The DOM concentrations in the recently flooded Sep Reservoir were higher than those measured in other lakes of similar trophic status, i.e. oligotrophic to oligo-mesotrophic (Richardot et al., 1999, 2000). The DCAA concentrations were in fact higher than any cited in the literature, which are usually between 0.1 and 2.9 mg C l^{-1} (Striquer-Soares & Chevolot, 1996). The carbohydrate concentrations, particularly those recorded 1 year after reservoir flooding, were similar to those encountered in lakes of a higher trophic status than the Sep Reservoir, such as the eutrophic lake Pluβsee where the DCCHO concentrations varied from 0.1 to 6 mg C l^{-1} (Münster, 1985), or aquatic ecosystems subjected to high allochthonous inputs (Curtis, 1998). The submersion of terrestrial vegetation during reservoir filling almost certainly explains the high concentrations of DOM. After flooding of an experimental lake, Paterson et al. (1997) also observed a large increase in dissolved allochthonous organic nitrogen and carbon as a result of the decomposition of flooded terrestrial organic matter.

The DOM components that we considered belonging to the labile pool of DOM are generally thought to consist mainly of sugars, amino acids, peptides and other simple compounds. Labile DOM has been shown to support the majority of bacterial production and turns over rapidly, in a time span of hours to days (Moran & Hodson, 1990). Therefore, variations in the concentration of the DOM components investigated could be easily detected within a study with a time scale of hours. If the DOM components measured were principally from the phytoplankton compartment and represented the main source of substrate fuelling the BP, then fluctuations in BP as well as in phytoplankton variables and the DOM components might be expected to exhibit similar patterns. This would have been interpreted as indicating a close coupling between bacterial metabolism and the local production of photosythetically derived DOM, in accordance with conclusions from previous comparative studies (Cole et al., 1988).

Changes in dissolved carbohydrate concentrations were observed in the present study and must reflect high assimilation or release rates, or both. During the study, conditions and particularly input to the reservoir were relatively constant and observed changes undoubtedly reflect intrinsic phenomena occurring within the system. Dissolved carbohydrate concentration was positively but not significantly correlated with the amount of Chl *a*, suggesting at least partial autochthonous production of DOM by the phytoplankton compartment. This agreed with previous studies showing that algae exude large amounts of carbohydrates (Mopper et al., 1995; Biddanda & Benner, 1997). There was an inverse correlation ($r = -0.5$, $p < 0.05$) between DCAA and Chl *a* concentrations and both the highest DCAA and Chl *a* concentrations were recorded at the depth 7 m below the surface. This was an indication that DCAA was principally released by senescent algal cells, ruptured cells as a result of herbivorous grazing found at 7 m below the surface, or more likely from nitrogenous waste from protozoan, zooplankton, and higher trophic levels (i.e. could come from hydrolyzation of fecal pellets). The accumulation of DCCA at that depth, which represented the boundary between the epi- and hypo-limnion, could be caused by a temporary reduction in the rate of organic matter sedimentation. This rate probably decreased in this zone of the water column because of the thermocline.

It is well established that in pelagic waters, dissolved carbohydrates and DCAA inputs from sources other than the phytoplankton compartment can support bacterial growth (Richardot et al., 2001; Rosenstock & Simon, 2001; Cherrier & Bauer, 2004; Young et al., 2005). These sources, including meso- and micro-zooplankton excretion, sloppy feeding and allochtonous DOM, can greatly alter the relative importance of algae as a source of nutrition for bacteria (Gocke et al., 2004) eliminating any correlation between DOM components and phytoplankton primary production. Moreover, within the refractory pool of DOM composed mostly of higher molecular weight humic acids, some components may be altered photochemically to produce more bioavailable compounds (Wetzel et al., 1995; Moran et al., 2000) and several studies have suggested that humic substances are more important components of the biodegradable DOM pool than previously thought (Volk et al., 1997). All this would explain the observed lack of correlation between DOM component concentrations, and either diel variations or differences between sampling depths. A

study conducted from 1996 to 1998 in this system indicated that polysaccharides and DCCHO from an allochtonous source controlled BP (Richardot et al., 2000). Despite the importance of DOM at a pluriannual scale, diel fluctuations of polysaccharides during this study could not be related to the periodicity in BP, suggesting the presence of high concentrations of DOM components from other sources including viral lysis of bacterial and algal cells, excretion from protozoan, zooplankton and higher trophic level.

Diel variation of bacterial production in relation to dissolved organic matter

The bacterial abundance varied 2–3 fold over during the first 24 h of the study. This seems too large to be accounted for by *in situ* bacterial production, but it is difficult to confidently explain this dynamic. One possible explanation could be that sporadic mixing occurred between two masses of water with different bacterial abundances. Our results also indicated that bacterial production in the Sep Reservoir varied greatly over time. Thymidine uptake rates in particular exhibited cyclical changes that suggested the synchronization of division in bacteria communities. In freshwater ecosystems, it is well known that the division of planktonic single-celled organisms (primarily dinoflagellates) can be synchronized *in situ* whereby every 24 h, a portion of the cell divides at a fairly precise time. For example, Pollingher & Serruya (1976) reported that the division of *Peridinium* in Lake Kinneret (Israel) takes place at night between 01:00 and 07:00, with a peak between 02:00 and 04:00. The synchronized diel patterns of bacterial growth observed during this study have also been previously reported in a eutrophic lake (Lake Aydat – France), in a humus-rich lake (Lake Cromwell – Canada) (Sime-Ngando et al., 1991) and in a mesotrophic lake (Lake Mondsee – Austria) (Psenner & Sommaruga, 1992). Riemann & Sondergaard (1984) attribute such diurnal changes in bacterial activity to factors such as phytoplankton production, zooplankton excretion and sloppy feeding, and mixing processes in the system. According to Kurath & Morita (1983), the synchronous division of bacterioplankton can be explained by the presence of bacteria in a latent state whose development is induced by cyclical environmental changes, such as an increase in the concentration and quality of substrates resulting from diurnal algal activity. For Turley & Lochte (1986), the bacterial community and its different components may be adapted to exploiting rhythms in the production of dissolved organic matter by photosynthetic organisms. Overall, the apparent cyclical changes observed in bacterial production during this study indicated that it is essential to evaluate the diel changes in the BP, if an accurate estimation of both the daily production rate of bacteria and carbon passing through bacterioplankton is to be achieved. This is of great importance in the establishment of a model of organic matter and energy flows within the microbial food web in aquatic systems.

From our study, despite the fact that PP peaks at 14:00 and bacterial production reaches a maximum at 18:00, we believe the higher thymidine incorporation rate observed in the evening might be ascribed to PP. Indeed, bacterial activity generally exhibit daily cycles with peaks at sunrise, sunset and at night, markedly out of phase with primary production (Bell, 1984; Riemann & Sondergaard, 1984; Di Siervi et al., 1995). Such behavior is not inexplicable because DNA synthesis does not show an immediate response but rather a time lag response to substrate supply (Di Siervi et al., 1995). However, although PP stimulated BP in the system, there was no evidence from our results that PP governs BP through time and space. Indeed, unlike PP there was no difference in BP measured at different depths except during the first day (18:00) and at the end of the study (10:00). Moreover, using a bacterial growth yield of 17%, the mean carbon demand of bacterioplankton during this study represented 84% of the PP (Jugnia et al., 2000), using the unlikely assumption that the PP was entirely available for the bacterioplankton. All of these different aspects point to the role of DOM components originating from sources other than phytoplankton primary production in fueling bacterial activity in the Sep reservoir, especially in the bottom water where PP was undetectable (Fig. 1).

We conclude that DOM components originating from sources other than phytoplankton primary production may be important in sustaining bacterial growth on a short-time scale in a recently flooded reservoir. This does not preclude an effect

on bacterial community growth rates by DOM from the phytoplankton compartment. This is an issue that needs further investigation.

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