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NOTE

Bacterioplankton responses to bottom-up and top-down controls in a West African reservoir (Sélingué, Mali)

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ABSTRACT: We conducted experiments to determine whether bacterial growth, with or without predators, is limited by inorganic (N and P) and organic (C) substrates in Sélingué, a mesotrophic reservoir located in Mali, West Africa. Significant increases (relative to controls) in bacterial cell volumes and thymidine incorporation rates were observed after 24 h incubation only for samples amended with the combination +PC and +CNP. The data revealed a colimitation of bacterioplankton growth by organic carbon during the dry season. Flow cytometry discriminated 3 groups of bacteria (Bact I, Bact II, Bact III) differing in increases in nucleic acid content and cell size. The Bact I group, comprising cells with low nucleic acid content and of small size, was the dominant population in all experiments. In the absence of bacterial predators, only the Bact II group showed significant differences between the control and the +PC and +CNP treatments, indicating that this bacterial group was the most sensitive to nutrient additions. The Bact II group, corresponding to cells with high DNA content, are active members of the bacterioplankton community. The Bact III group did not increase in any treatment but the proportions of these cells always increased in the presence of bacterial predators. These cells may be, at least partly, grazing-resistant bacteria.

KEY WORDS: Bacteria · Nutrient · Predation · Flow cytometry · West Africa

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INTRODUCTION

Nutrient availability ('bottom-up control') and protozoan predation ('top-down control') or viral lysis are the more important factors regulating bacterial communities. Traditionally, organic carbon has been considered the main factor limiting the growth of pelagic heterotrophic bacteria. However, studies on nutrient limitation of such communities have shown that mineral limitation of growth rate is widespread in various marine and freshwater ecosystems (Torreton et al. 2000, Carlsson & Caron 2001, Chrzanowski & Grover 2001). In temperate regions, bacterial dynamics are also influenced by environmental factors, like temperature (White et al. 1991). Bacterivory by heterophic nanoflagellates (HNF) has been identified as one of the main loss factors affecting bacteria, and has been shown to balance bacterial production and thus to regulate bacterial biomass in a large number of pelagic ecosystems (Solic & Krstulovic 1994, Christaki et al. 1999, Ferrier-Pagès & Furla 2001). Size-selective grazing coupled with resource availability was recognized as a shaping force in both the taxonomic and phenotypic structures of bacterial communities (Jürgens & Matz 2002).

However, although factors controlling bacterial communities of temperate and some tropical areas have been studied, we are unaware of such studies in West African aquatic ecosystems. A multidisciplinary program began in 2000 to examine the ecological func-

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tioning of Sélingué, a major reservoir in Mali, West Africa. A limnological study investigated the effects of climate and hydrology on the trophic status of this aquatic system from water samples collected biweekly from November 2000 to November 2001 from a station representative of the northern part of the water body (Arfi 2003). Here we present bacterial responses to nutrient enrichment based on bioassay experiments performed in May 2001 on water collected in Sélingué reservoir. Analysis by flow cytometry revealed contrasting responses of different bacterial populations with and without bacterial predators.

MATERIALS AND METHODS

Sélingué reservoir is located in the southwest of Mali, West Africa (11° 38′ N, 8° 14′ W) forming an inverted Y with an approximate length of 80 km. The water column is stratified from March to May as a result of cooling induced by the 'harmattan' (North-East trade winds; Arfi 2003). The reservoir was sampled daily from 16 to 31 May 2001 in the framework of a multidisciplinary program conducted by the IRD (Research Institute for Development) and its partners. Among the 3 sites investigated during this program, a station called 'Carrière', representative of the hydrological regime of the northern part of the reservoir (Arfi 2003), was sampled for bioassay experiments. Water was sampled from the surface to 2 m using a sampling tube in order to get water from a layer corresponding to a large part of the euphotic zone. We conducted 2 bioassay series to compare the responses of the bacterial community to inorganic and organic enrichment with (Series A, using <50 µm-filtered water) and without (Series B, using <1 µm-filtered water) bacterial predators and phytoplankton. Subsamples (200 ml) were transferred into acid-washed 250 ml polycarbonate bottles. At time zero (t_0), inorganic nitrogen (20 µM as NH_4Cl), inorganic phosphorus (2 µM as NaH_2PO_4), or organic carbon (100 µM as glucose) were added alone or in combination: no addition for control, +N, +P, +C, +NP, +PC, +NC, +CNP. All treatments were performed in triplicate with a total of 24 bottles per experiment. The bottles were placed in an incubator at a light intensity of 35 µmol quanta m^{-2} s^{-1} PAR (photosynthetically active radiation), measured using a Li-Cor 193 spherical quantum sensor (4π). This PAR value corresponded to a depth close to 3 m depth (bottom of the euphotic zone) during the survey. This light level is low enough to avoid a dramatic increase in phytoplankton growth in response to enrichment, but enables a moderate density of phytoplankton to avoid a large change in dissolved organic carbon supply from phytoplankton exudates. The light cycle was that of natural daylight (12:12); the temperature in the incubator was controlled and stayed close to the *in situ* surface water temperature in the reservoir (close to 30°C). Subsamples were removed after 12 h (t_{12}) and 24 h (t_{24}) incubation for microbial measurements. Growth rates of bacterioplankton in each experimental replicate were calculated as $\mu = (\ln N_f - \ln N_i)/t$, with t as incubation time and N_f and N_i as concentration of bacteria at the end and at the beginning of each incubation time, respectively.

Subsamples for determination of bacterioplankton were fixed with buffered formalin (2% final concentration) and stained with DAPI fluorochrome (Porter & Feig 1980). Mean bacterial volumes were determined by measurement of up to 100 cells using an Olympus DP 50 camera mounted on an Olympus BX 60 epifluorescence microscope. Cell volumes were computed using the formula described by Blackburn et al. (1998) based on the area, the longest length and the radius of the object. Net bacterial production was measured by (methyl-^{3}H) thymidine incorporation into cold trichloroacetic acid (TCA) precipitate (Fuhrman & Azam 1980). Duplicates and 1 control ($t = 0$) were incubated with (methyl-^{3}H)-thymidine (47 Ci $mmol^{-1}$, Amersham) in the dark at *in situ* temperature. Radioactivity was counted by the liquid scintillation procedure and results were expressed as nmol of incorporated thymidine l^{-1} h^{-1}.

Flow cytometry analyses of bacterial community were performed on the basis of SYBR-Green I (Molecular Probes) staining of bacterial cells according to the method described by Marie et al. (1997). Subsamples were fixed with buffered formalin and immediately stored in liquid nitrogen until analysis. For each subsample, 3 replicate counts were performed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled argon laser (488 nm, 15 mW). Stained bacterial cells, excited at 488 nm, were enumerated according to their right-angle light scatter (RALS) and green fluorescence (FL1) collected at 530/30 nm. Fluorescent beads (0.94 µm; Polysciences) were systematically added to each sample. We discriminated 3 bacterial cell populations (Bact I, Bact II, Bact III) differing in increases in fluorescence intensity (FL1) and scatter values (SSC).

The monotetrazolium redox dye CTC (Polysciences) was used for fluorescence microscopic detection of respiring cells following Rodriguez et al. (1992). Duplicate samples were incubated with a final CTC concentration of 5 mM for 1 h in the dark and at *in situ* temperature. Incubations were terminated by addition of 2% formalin. DAPI (see above) was then added to counterstain the entire bacterial population. CTC-positive cells were detected with a 450 nm excitation filter, a 510 nm beam splitter, and a 520 nm emission

filter combination with an Olympus BX 60 epifluorescence microscope.

Heterotrophic nanoflagellates (HNF) were enumerated from 4 ml subsamples filtered onto 0.8 µm black polycarbonate filters. The flagellates were stained using the DAPI fluorochrome (final concentration of 20 µg l^{-1}) for 15 min and counted by epifluorescence microscopy (magnification ×1200).

Data were analyzed using the statistical package SigmaStat (Jandel Corporation). A Kruskal-Wallis test (1-way ANOVA on ranks) was used to follow variations in the different treatments over the incubation period. For comparison of the mean values of each variable response to the different treatments, Student's *t*-test was applied to log-transformed data.

RESULTS AND DISCUSSION

Sélingué reservoir constitutes a typical artificial water body located in Mali, a very sensitive Sahelian area in West Africa in terms of water resources. The prime purposes of the reservoir are to supply the capital, Bamako, with electricity, to provide water for large rice-fields (Anne et al. 1991), and to supply fresh fish for human consumption (annual yield >5000 t, Laë & Weigel 1994). Few ecological works concern Sélingué reservoir, and these were only conducted after filling the reservoir in 1980 (Lelek & Tobias 1985, Anne et al. 1994). There was a lack of knowledge about the present trophic status of the water body after 2 decades of functioning as a multipurpose reservoir, but on the basis of inorganic nutrient and chlorophyll *a* concentrations recorded during a 1 yr survey, Arfi (2003) showed that Sélingué reservoir is oligotrophic during the high-water period, and mesotrophic during the low-water period. Our experiments were performed in May 2001 during the low-water period, with NH_4-N concentrations below 2 µmol l^{-1} and reactive orthophosphate concentrations ranging from the detection limit of 0.05 µmol l^{-1} to 0.18 µmol l^{-1}. Water temperatures were always high, with a mean value of 29.9°C in the surface water. Chlorophyll *a* concentrations were close to 20 µg l^{-1} in the surface waters.

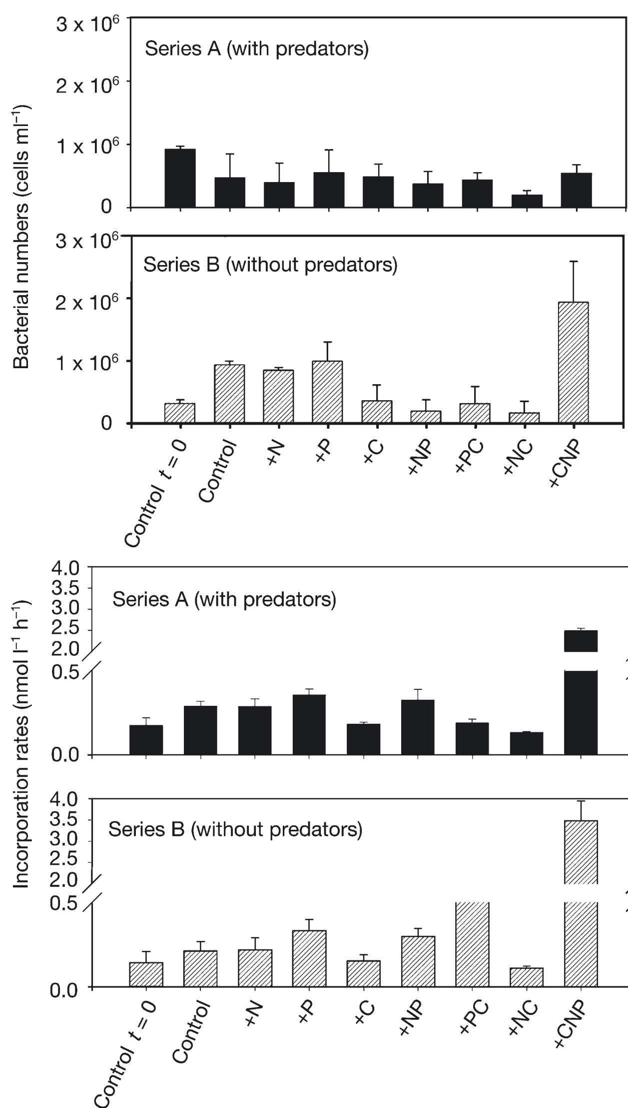

Fig. 1. Changes in bacterial abundance and thymidine incorporation rates following different enrichment treatments between beginning of experiment (control at $t = 0$) and end of incubation (24 h) conducted with (Series A) and without (Series B) bacterial predators

Table 1. Chlorophyll *a* concentration and heterotrophic nanoflagellate (HNF) abundance at time zero (t_0) and after 24 h (t_{24}) in controls and treatments with +C, +P, +PC, +CNP in Series A (with predators). Means and (SD); n = 3

Time	Chlorophyll *a* (µg l^{-1})		HNF concentration (ind. ml^{-1})	
t_0	24.4	(0.3)	7800	(1526)
t_{24}				
Control	21.9	(0.2)	6190	(516)
+C	23.8	(0.3)	7230	(1860)
+P	21.4	(0.4)	8430	(1124)
+PC	19.8	(0.3)	11600	(2546)
+CNP	20.5	(0.4)	11400	(1100)

During the 24 h experiment, chlorophyll *a* concentrations and HNF abundances did not increase significantly in the control or in the different treatments tested ($p > 0.05$, ANOVA; Table 1). In Series A treatments with bacterial predators, no increase in bacterial numbers was observed after 12 h (data not shown) or after 24 h incubation, whatever the nutrient addition (Fig. 1). Conversely, increases in thymidine incorpora-

tion rates occurred with addition of organic carbon coupled with inorganic nutrients (+CNP) ($p < 0.001$; Fig. 1). Average thymidine incorporation rate was high (2.48 nmol l^{-1} h^{-1} at t_{24}, n = 3) compared with the low values for the control (mean of 0.288 nmol l^{-1} h^{-1}). In Series B without bacterial predators, bacterial abundance increased significantly in the +CNP treatment (at t_{24}, $p = 0.047$, with an abundance increase of 208%). Thymidine incorporation rates increased significantly with the +PC and +CNP treatments (Fig. 1; $p < 0.001$ at t_{24}). Higher thymidine incorporation rates were observed under +CNP conditions, with a mean of 3.47 nmol l^{-1} h^{-1} compared to 0.211 nmol l^{-1} h^{-1} for the control (increase of 1648%). Thus, the bacterial community dynamics in Sélingué reservoir were not affected by a single addition of phosphate, ammonium or organic carbon. Growth of the bacterial assemblage was only stimulated with +PC and +CNP additions at t_{24}, with significant increases in bacterial numbers and thymidine incorporation compared to the controls. These stimulations of bacterial assemblages indicated colimitation by nutrient availability, especially with organic carbon, as also demonstrated by Carlsson & Caron (2001) in a small oligotrophic dimictic lake (Massachusetts, USA). Conversely, Wang & Priscu (1994) demonstrated that growth of bacterioplankton collected from a eutrophic lake (USA) could be stimulated by direct addition of inorganic P and N. However, the colimitation by organic carbon suggests that bacterial utilization of phosphorus or nitrogen was limited by the availability of endo- or exogenous organic carbon. In this context, phytoplankton exudates (carbon-rich photosynthetate; Riemann et al. 1982) can play a substantial role in controlling bacterial growth only when there is sufficient inorganic P and N. A first consequence of a colimitation of bacterial growth by CNP may be the limitation or the suppression of competition with phytoplankton for inorganic nutrients, at least when degradable organic C concentrations or fluxes are limited. This means that if inorganic nutrients inputs occur, one can expect primarily phytoplankton to benefit from this input. Preliminary microscopic examination of phytoplankton assemblages in samples collected in May 2001 revealed the presence of numerous cyanobacteria belonging to different genera: *Anabeana*, *Lyngbia*, *Microcystis*, *Merismopedia* and *Cylindrospermopsis* (Bouvy et al. 2001, L. Ten Hage pers. comm.).

Another consequence of colimitation by organic carbon will be that the microbial loop is less active. The first indication of a low turnover of a bacterial community is given by their net growth rates. Indeed, in the present study, net growth rates always exhibited negative values in the presence of bacterial predators (Series A) whatever the treatment. Without bacterial predators (Series B), positive growth rates were noted (except with +NC), with values ranging from 0.0034 h^{-1} (with +NP) to 0.076 h^{-1} (with +CNP). The highest growth rate values were with +CNP addition corresponding to a low turnover time of total bacterial assemblage (9.2 h) compared to the *in situ* generation time of 5 h reported by Bouvy et al. (1998) for shallow eutrophic reservoirs located on the Côte d'Ivoire. Secondly, the very low proportion of CTC-positive cells also indicated that most of the bacterial cells were inactive or exhibited a very low level of respiratory activity. Indeed, CTC-positive cells were only present in significant numbers in the bioassays with PC and CNP enrichment after 24 h incubation, without the presence of predators (Series B). At $t_{24,}$ the percentage of positive cells increased to up to 6.9% of total cells with the CNP treatment, and to 38.1% of total cells with the PC treatment (coefficient of variation <50% among the microscopic fields). With the other treatments, only a few cells were CTC-positive and their proportion in relation to the total counts was very low (<1%) with a high coefficient of variation among the microscopic fields counted (>200%).

The results obtained through flow cytometry confirm the existence of different bacterial populations in the

Table 2. Proportion (%) of total cell count comprised by different bacterial groups (Bact I, Bact II and Bact III) detected by flow cytometry following various treatments with (Series A) and without (Series B) bacterial predators. **Bold** indicates significant difference from control value t_{24} (t-test, $p < 0.05$)

Group	Control	+N	+P	+C	+NP	+PC	+NC	+CNP
Series A								
Bact I	97.25	96.26	94.55	97.22	94.07	93.58	96.52	91.74
Bact II	1.80	2.43	3.83	1.76	4.35	5.19	2.56	7.68
Bact III	0.95	1.31	1.62	1.02	1.58	1.23	0.92	0.59
Series B								
Bact I	98.23	97.52	93.97	97.45	96.27	82.94	98.65	88.39
Bact II	1.45	1.68	4.59	1.99	2.89	**15.96**	1.15	**11.34**
Bact III	0.32	0.80	1.44	0.56	0.84	1.09	0.20	0.27

community (Li et al. 1995, Troussellier et al. 1999, Gasol & Del Giorgio 2000), and showed contrasting responses to changes in bottom-up and top-down control (Table 2). We discriminated 3 groups of bacteria (Bact I, Bact II, Bact III), that differed in their increases in fluorescence intensity (FL1) and scatter values (SSC). Cytograms representing the FL1 and SSC distributions for the 3 groups are shown in Fig. 2 for the controls and for the CNP addition after 24 h incubation. The Bact I group was comprised of cells with low nucleic acid content and of small size. They formed the dominant population in all experiments (between 83 and 99% of the total bacterial community) and showed very limited changes in the various treatments. The Bact II group, consisting of bacterial cells of higher volume and nucleic acid content, showed the most important changes, with a significant increase in +PC and +CNP treatments compared to the control (Table 2, Fig. 2). This increase was only observed when bacterial predators were removed (Series B, Table 2), as was the case for the CTC-responsive cells. Turnover time of the Bact II group was estimated as 4.3 h with +CNP addition, and this value was lower than the turnover time estimated for the total bacterial cells (13 h) with the same treatment. This population represents that fraction of the community that grows following nutrient addition, and that was preferentially grazed by protozoans. Therefore, the Bact II population defined in this study corresponds to HDNA (high DNA content) cells, which are considered active and dynamic members of the bacterioplankton community (Gasol et al. 1999). Abundances of the Bact III group remained very low, with percentages always less than 2% of the total community. Nutrients did not significantly affect the growth of these bacterial cells, which were characterized by higher nucleic acid content and larger size (Fig. 3). However, in the presence of predators (Series A), the percentages of Bact III cells were significantly higher ($p < 0.03$) than the percentages without grazers (Series B). These cells may, at least partly, be grazing-resistant bacteria. This observation was confirmed by the highest bacterial size classes (close to 0.9 µm^3) and the high mean biovolume (0.168 µm^3; Table 3) observed at t_{24} with +CNP (obtained by epifluorescence microscopy). Therefore, with this treatment, 20 size classes were determined with predators (Series A) whereas only 7 size classes and 10 size classes were observed in the control and in +C treatment, respectively. In Series B (without predators), the number of size classes was lower than in Series A (Fig. 3). With the +CNP treatment, only 10 size classes were determined, with the largest size class reaching 0.6 µm^3. The presence of the highest bacterial size classes in the presence of bacterial predators compared to the spectrum of bacterial size classes observed without bacterial grazers (Fig. 3) indicated the possibility of escaping grazing by flagellates. When protozoan species show highest grazing rates for a distinct prey size class (Fenchel 1980), bacterial assemblages have a possibility of escaping from grazing by a shift to inedible or less vulnerable size classes (Posch et al. 1999). Our data confirm that cell size distribution is an important factor in predator-prey relationship regulation (Jürgens & Güde 1994).

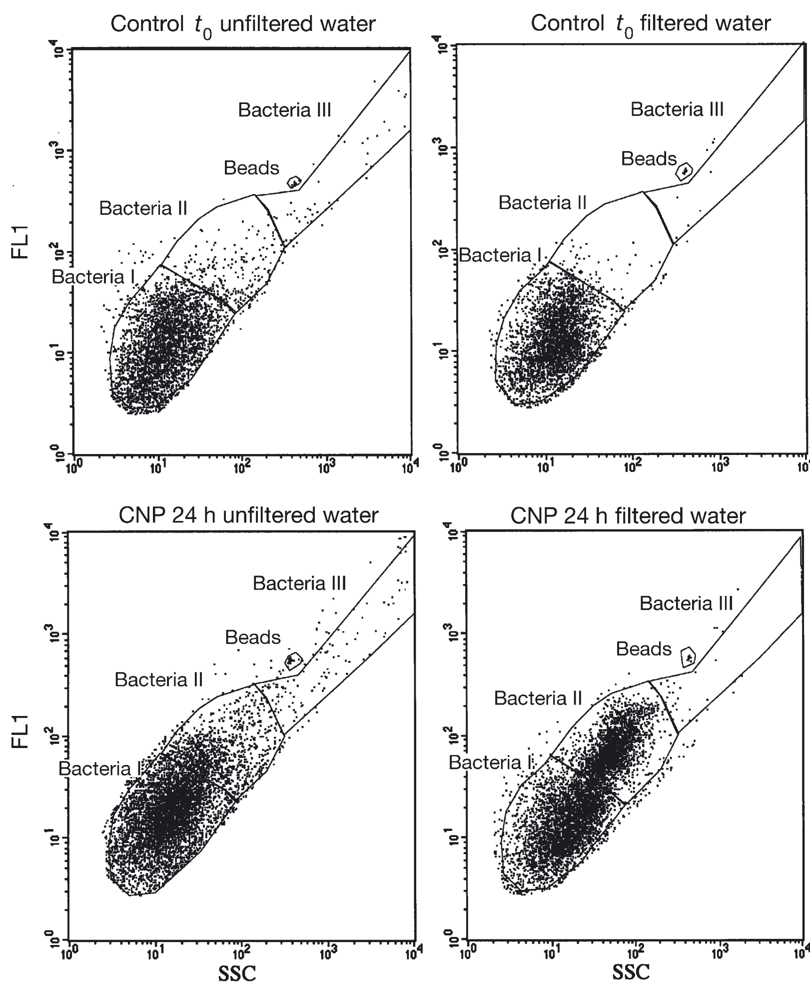

Fig. 2. Flow cytometry analysis of water samples (Series A and Series B) at $t = 0$ (control) and after 24 h incubation with CNP nutrient enrichment. Light scatter (SSC) and fluorescence (FL1) values differentiated 3 bacterial groups (Bact I, II and III). Microbeads (0.96 µm) were used as internal standard

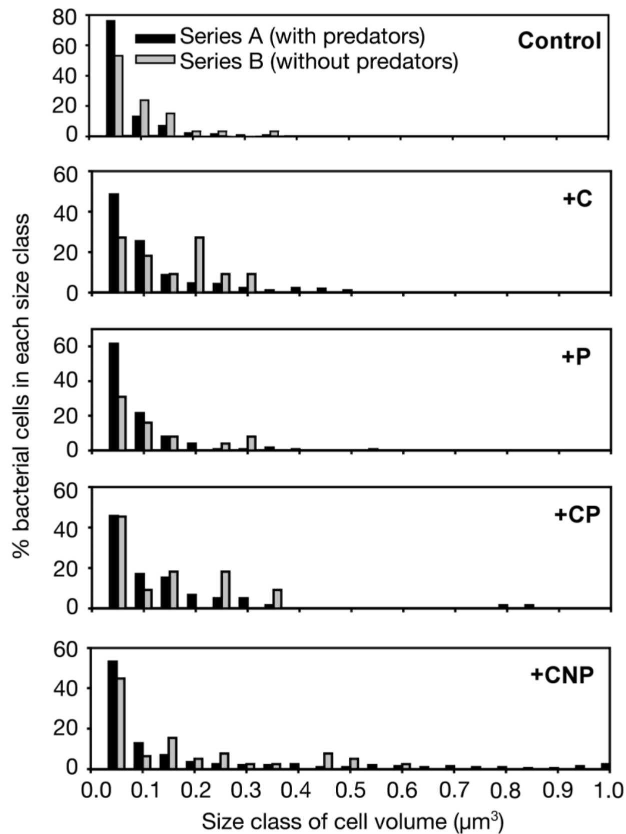

Fig. 3. Size class distribution of cell volume of bacterial communities at t = 0 (control) and after 24 h incubation with +C, +P, +PC and +CNP in Series A (with bacterial predators) and Series B (without bacterial predators)

Table 3. Bacterial biovolumes at t_0 and t_{24} for the different treatments with (Series A) and without (Series B) predators

Time	Biovolume (µm³) Mean (SD)	n
Series A		
t_0	0.037 (0.038)	82
t_{24}		
Control	0.045 (0.053)	152
+C	0.089 (0.111)	274
+P	0.061 (0.077)	126
+PC	0.106 (0.112)	116
+CNP	0.168 (0.264)	203
Series B		
t_0	0.041 (0.041)	60
t_{24}		
Control	0.062 (0.074)	68
+C	0.116 (0.079)	44
+P	0.082 (0.091)	67
+PC	0.098 (0.106)	87
+CNP	0.141 (0.162)	81

The results obtained in this study on the bacterial groups of the Sélingué bacterial community were consistent with different key processes that are known to shape the dynamics of bacterial communities in other aquatic ecosystem: (1) only a limited and variable fraction of the bacterial community remains active and grows in response to CNP addition (Smith & del Giorgio 2003), (2) planktonic grazers remove active bacterial cells in a selective way (Del Giorgio et al. 1996), and (3) the proportion of grazing-resistant bacteria (e.g. elongated cells) increases as a consequence of grazing pressure exerted by heterotrophic flagellates (Jürgens & Güde 1994). Compared to the 'static' view that produces OECD (Organization for Economic Cooperation and Development)-like indexes, bioassays appear to achieve a good understanding of microbial dynamics, especially when faced with increased nutrient input. In the case of Sélingué reservoir, our results indicate a potentially important role of increased nutrients that will mainly benefit phytoplanktonic populations if nutrients are mainly of inorganic nature, in a context where growth of bacterial assemblages appears also to be limited by the availability of organic carbon.

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