# Community composition and activity of prokaryotes associated to detrital particles in two contrasting lake ecosystems

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## Abstract

The composition, distribution and extracellular enzyme activities of bacteria attached to small (2–50 μm in size) transparent exopolymer and Coomassie-stained proteinaceous particles (TEP and CSP) were examined in two lakes of different trophic status located in the Massif Central of France. TEP concentrations ($10^4$–$10^6$ particle per L) were significantly higher in the more productive lake and were significantly related to chlorophyll *a* concentrations. The majority of TEP and CSP were colonized by bacteria that constituted 2.6% and 7.4% of the total 4′,6-diamidino-2-phenylindole-stained bacteria in lakes Pavin and Aydat, respectively. In both lakes, the composition of particle-associated bacteria was different from that of free-living bacteria, the *Betaproteobacteria* and *Bacteroidetes* (i.e. former *Cytophaga–Flavobacteria* group) being the dominant groups on particles. We also found that 2–5 μm TEP were more colonized than 2–5 μm CSP in the two lakes, and that TEP colonization was higher in the less productive lake. Measurements of Leucine aminopeptidase and α-glucosidase activities in fractionated lake water (0.2–1.2, 1.2–5 and >5 μm fractions) indicated that proteolytic activity was always higher and that particle-associated bacteria have higher enzymatic activities than free-living bacteria. The glycolytic activities in the 1.2–5 and >5 μm fractions were related to the abundance of TEP. We conclude that small freshwater detrital organic particles constitute microhabitats with high bacterial activities in pelagic environments and, undoubtedly, present significant ecological implications for the prokaryotic community structure and function in aquatic ecosystems.

## Introduction

More than half of the bacterioplankton in aquatic ecosystems can be attached to detrital particles (Simon, 1987; Riemann *et al.*, 2000). The composition and abundance of these particles influence the density, biomass and production of the attached bacteria (Simon, 1987; Kepkay, 1994; Carrias *et al.*, 2002). The attached communities can account for a large proportion of the total bacterial production in lakes (Simon, 1987) and can be responsible for up to 75% of the total hydrolytic activity (Middelboe *et al.*, 1995). Both protozoa and metazoa can consume particles, including the attached bacteria (Shimeta, 1993). Therefore, detrital particles can constitute a link to higher trophic levels, and greatly influence the fluxes of matter in pelagic systems, especially during sedimentation and mixing events (Logan *et al.*, 1995). Recent studies have demonstrated the numerical importance and the ubiquity of small-sized (<50 μm) detrital particles in the pelagic zone of marine and freshwater systems (Alldredge *et al.*, 1993; Passow & Alldredge, 1994; Mostajir *et al.*, 1995b; Schuster & Herndl, 1995; Long & Azam, 1996; Mari & Kiørboe, 1996; Carrias *et al.*, 2002). These particles provide surface areas for bacterial colonization and could enhance the aggregation of large solid particles and therefore promote the sedimentation of particulate carbon through the water column (Passow, 2002; Verdugo *et al.*, 2004).

Three types of pico- and nano-sized detrital organic particles are generally distinguished in pelagic ecosystems, namely transparent exopolymeric particles (TEP) stained with an acidic solution of Alcian Blue (Passow & Alldredge, 1994; Mari & Kiørboe, 1996), proteinaceous particles

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stained by Coomassie Brillant Blue (Coomassie-stained particles: CSP) (Long & Azam, 1996), and DAPI Yellow particles (DYP) stained with 4′,6-diamidino-2-phenyl-indole (DAPI) (Mostajir *et al*., 1995a). TEP consist of a matrix of colloidal fibrils and are produced from dissolved carbohydrate polymers exuded by phytoplankton and bacteria (Passow & Alldredge, 1994; Passow, 2000). DYP are almost exclusively organic, enzyme-degradable matter and could include phytoplankton-derived detritus, particles released from protozoa, and the agglomeration of colloids produced from dissolved organic carbon (DOC) adsorption on bubbles (Mostajir *et al*., 1995a). Cell lysis, cell death or adsorption of protein onto particles could produce CSP (Long & Azam, 1996). The few data on pico- and nano-sized detrital particle colonization have revealed that the different types of particles contain attached bacteria (Passow & Alldredge, 1994; Mari & Kiørboe, 1996; Carrias *et al*., 2002), representing 1–20% of the total bacterial counts. Observations in both freshwater and marine ecosystems have indicated that TEP are always colonized by bacteria (Carrias *et al*., 2002; Passow, 2002). In addition, bacterial colonization of pelagic detrital particles is a function of the nature of the particle and of the productivity of the system (Carrias *et al*., 2002), suggesting potential differences in the composition of bacteria attached to different types of small-sized particles. According to Brachvogel *et al*. (2001), bacteria attached to microaggregates are dominated by *Betaproteobacteria* or bacteria of the *Bacteroidetes* group. These two bacterial phylotypes are also an important component of bacteria attached to lake snow aggregates (Weiss *et al*., 1996; Schweitzer *et al*., 2001).

To the best of our knowledge, data about the composition of freshwater bacteria attached to detrital particles are lacking, and no comparison of particle-associated bacterial communities between proteinaceous and polysaccharide particles in lakes is available. Bacteria attached to large detrital particles are known to produce high levels of extracellular enzymes in culture (Riemann *et al*., 2000; Junge *et al*., 2002), and enzymatic activities in lake snow are higher than in the surrounding waters (Grossart & Simon, 1998a). We therefore hypothesize that particles such as TEP and CSP could greatly influence the composition and activity of particle-associated bacteria in the field.

To test this hypothesis, we analysed the main genetic groups of prokaryotes attached to different types of detrital particles in two lakes of different trophic status. We wanted to address the following three questions. (i) Is there a difference in the composition and the enzymatic activity between free-living and particle-attached bacteria? (ii) Is there a difference in the composition of attached bacteria related to the composition and size of the particles? (ii) Are these differences in bacterial composition related to the trophic level of the lake?

## Materials and methods

### Study site and sampling

The study was conducted in two lakes of different trophic status, the lakes Pavin and Aydat situated in the Massif Central of France. Lake Pavin (45°29′41″N, 02°53′12″E, altitude: 1197 m) is a meromictic and dimictic oligo-mesotrophic lake with partial overturns. It is a volcanic mountain lake characterized by its depth (maximum depth = 92 m) and its low surface and drainage basin areas (44 and $50 \times 10^4$ m$^2$, respectively). Lake Aydat (45°39′48″N, 02°59′04″E) is a small (area = 60.3 ha, maximum depth = 15.5 m) eutrophic, dimictic lake, located at an altitude of 825 m.

Water samples were collected once per month from March to May 2002 with a 10 L Van Dorn bottle at the deepest points of the lakes. In each lake, three different depths located in the epi-, meta- and hypolimnion (5, 10, 30 and 1, 7, 13 m for lakes Pavin and Aydat, respectively) were sampled. The water temperature and dissolved oxygen were measured *in situ* with an YSI GRANT 3800 probe.

### Phytoplankton analysis

Chlorophyll *a* concentrations were determined spectrophotometrically from samples collected on Whatman GF/C filters (Dassel, Germany). Pigments were extracted in 90% acetone, and concentrations calculated with SCOR-UNESCO equations (SCOR/UNESCO 1966). Microscopic counts of epilimnetic phytoplankton (1 m depth for Lake Aydat, 5 and 10 m for Lake Pavin) were performed on Lugol's iodine (Sigma, St Quentin, Fallavier, France) fixed samples, using a Wild 40 inverted microscope (Leica Microsystems, Rueil-Malmaison, France) at a magnification of $\times 400$ (Utermöhl, 1958).

### Prokaryotic community composition and detrital particle analysis

Water samples were fixed with 4% (volume in volume, v/v) formalin, allowing hybridization of the probes (Moter & Göbel, 2000) and the preservation of detrital particles (Passow & Alldredge, 1994; Passow, 2002). Within 12 h of fixation, fixed samples were filtered in triplicates at a low vacuum pressure ($< 100$ mmHg) onto 0.2 μm polycarbonate filters. Filters were then rinsed with sterile phosphate-buffered saline (PBS; MWG-Biotech, Champlon, France) solution dehydrated with ethanol. These preparations were stored at $-25$ °C in the dark until further processing within 2 days.

We used combined protocols to observe particles and attached prokaryotic cells. The prokaryotic community composition (PCC) was analysed with fluorescent *in situ* hybridization (FISH) using group-specific rRNA oligonucleotidic probes (MWG-Biotech) according to Moter and Göbel (2000). The following probes were used: EUB338 for


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*Eubacteria*, ALF1b and BET42a for the *Alpha* and *Beta* subclasses of *Proteobacteria*, and CF319a for members of the *Cytophaga–Flavobacterium* group (now renamed *Bacteroidetes*, group *Sphingobacteria–Flavobacteria*). The probe ARCH915 was used for the *Archaea* domain. A number of *Gammaproteobacteria* are almost certainly detected by the BET42a probe. Therefore, the *Beta* subclass of *Proteobacteria* presented in this study may contain *Gammaproteobacteria*. Probes were labelled with the indocarbocyanine fluorescent dye Cy3 (MWG-Biotech). The hybridization buffer consisted of 5 M NaCl, 1 M Tris-HCl (pH = 7.4), a variable concentration of formamide (20% v/v for the ALF1b and ARCH915 probes, 35% v/v for the other probes) and 1 μL of a 10% sodium dodecyl sulphate (SDS) solution. Prior to hybridization, the filters were cut into small sections. These sections were incubated at 46 °C for 90 min with 80 μL of hybridization buffer containing a variable concentration of the respective fluorescent probe (50 ng $\mu L^{-1}$ of EUB338 and 100 ng $\mu L^{-1}$ of ALF1b, BET42a, CF319a and ARCH915). Filters were then incubated in a washing buffer composed of 1 mM Tris-HCl, a variable concentration of NaCl (250 mM final concentration for ARCH915, 225 mM for ALF1b, and 80 mM for the other probes) and a SDS (10% v/v) solution, at 48 °C for 10–15 min in the dark. Filters were then stained with sterile 1 μg $L^{-1}$ (final concentration) 4′,6-diamidino-2-phenylindole (DAPI) for 15 min to evaluate the abundance of DAPI-stained bacteria.

Transparent exopolymer particles and Coomassie-stained particles were visualized on separate filter sections. We used slight modifications of the methods of Passow & Alldredge (1994) and of Long & Azam (1996) to obtain quantitative microscopic analysis of TEP and CSP, respectively. TEP were stained on filter sections with an aqueous solution of 0.03% Alcian Blue (Sigma) and 0.06% acetic acid (pH = 2.5). The filter sections were fully covered with the dye (500 μL) for about 5 s. CSP were stained for 30 s with Coomassie Brilliant Blue G-250 (0.04% final concentration, 500 μL, pH = 7.4). All filter sections were then rinsed with sterile distilled water, air-dried, mounted onto slides with Citifluor oil, and stored at −25 °C in the dark. As a control, filter sections were prepared without DAPI staining and without Alcian Blue or Coomassie Brilliant Blue staining. DYP were also considered during this study. The abundance of these particles and their bacterial colonization can be easily evaluated with the DAPI procedure (Mostajir *et al.*, 1995a). We estimated that only 10% of DYP were colonized by bacteria, making it difficult or impossible to count probe-labelled bacteria attached to these particles. Consequently, DYP particles were excluded from the analysis.

Slides were examined using an epifluorescence microscope (Leitz Laborlux) equipped with a 1250× objective lens, a Sony 3CCD colour video camera (model DWC-950P; Clichy, France), a Sony video recorder, and a Leica Q500 personal computer with image analysis software. This is a highly sensitive system, which was helpful for the low signal intensity of some preparations. Image acquisition was done as soon as possible after staining and as follows: a first image was taken under green-light excitation to visualize probe-labelled bacteria, a second under UV light to visualize DAPI-stained bacteria, and a third under visible light to count and measure CSP or TEP.

Free bacteria and bacteria associated with at least 40 TEP or CSP per slide were counted by switching between the images recorded under visible and UV light. Free and attached bacteria stained with the specific probes were counted by switching between the images recorded under UV and green light. The abundance of particles and associated bacteria were expressed in two size classes of particles (2–5 and 5–50 μm). However, because of the low accuracy of the methods used for the smallest particles (Mari & Kiørboe, 1996; Carrias *et al.*, 2002), a third class size, 0.2–2.0 μm, was excluded from analysis. Unhybridized, DAPI-stained filter sections indicated that red fluorescent picocyanobacteria were negligible during the study period. In addition, counts of DAPI-stained bacteria or probe-labelled bacteria before and after staining of TEP or CSP were not significantly different. Owing to the very low number of *Archaea* found during this study, this community was omitted.

## Hydrolytic activity

Potential ectoenzyme activities by bacterial extracellular enzymes were measured in fractionated (0.2–1.2, 1.2–5 and >5 μm) water samples incubated at 20 °C in the dark for 2 or 6 h according to the protocol of Hoppe (1983). Six milliliter subsamples were filtered through a set of 5, 1.2 and 0.2 μm pore-size membrane filters (Millipore) using a low-vacuum (<300 mmHg) pressure. Enzymatic activities were estimated on duplicate filters for each size fraction. Filters were placed in plastic sterile tubes with 6 mL Volvic™ water (Société des eaux de Volvic, Puy de Dôme, France). The fluorochrome-labelled substrates, 4-methylumbelliferyl α-glucoside (4MUG) and L-leucine-β-naphtylamide (LEU), were then added to evaluate the α-glucosidase and leucine aminopeptidase activities, respectively. We used a 2.5–10 μM concentration range of 4MUG and a 3–30 μM concentration range of LEU to establish substrate saturation curves and then calculate the apparent $V_{max}$. Fluorescence emission was recorded at regular time intervals on a Kontron SFM-25 spectrofluorometer (Kontron Instruments, Zürich, Switzerland) (excitation wavelengths of 365 and 340 nm; emission wavelengths of 460 and 410 nm for the 4MUG and the β-naphthylamide, respectively). Calibration of the fluorometer was performed with a standard solution of the substrates, and kinetic parameters of enzyme activities ($K_m$, $V_{max}$) were calculated. Cell-specific activities

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were estimated in each size fraction, assuming that attached bacteria were present in the 1.2–5 μm and >5 μm size fractions but that the 0.2–1.2 μm fraction only contained free-living bacteria. Based on a previous study in the same lakes (Carrias *et al.*, 2002), we estimated that the bacterial density on particles (number of bacteria per unit area of particle) was the highest on 2 − 5 μm particles, especially for TEP. Consequently, we deliberately defined a 1.2–5 μm fraction in which we expect a high potential enzymatic activity of attached bacteria. However, as noted by some authors (Riemann *et al.*, 2000; Carrias *et al.*, 2001), size fractionation of samples involves a bias that cannot be ignored. Thus, we cannot rule out the possibility that some free-living bacteria were trapped on the 1.2 μm filters, especially in samples from the eutrophic Lake Aydat. This can result in overestimation of the activities of attached bacteria. In contrast, some bacteria attached to small particles could pass through the 1.2 μm filter, resulting in an underestimation of the contribution of attached bacteria. Despite these biases, which cannot be entirely avoided, we consider that our survey is of great interest in better understanding bacterial enzymatic responses to changes in composition and abundance of detrital particles.

### Statistical analysis

The equality of the variances and the normality of the residuals were tested by a Shapiro–Wilk test. Data were transformed following the Taylor procedure when the assumptions of ANOVA were not satisfied. One-way ANOVA was used to test differences (i) in the composition of attached bacteria between particle types and between lakes, and (ii) in enzymatic activities between size fractions and lakes. Potential relationships between the variables under study were tested using Pearson's coefficient correlations.

## Results

### Environmental characteristics

In both lakes, water-column temperatures were low (5 and 7 °C in the lakes Pavin and Aydat, respectively) and homogeneous in March. Thermal stratification became established at the end of May, with values close to 12 and 14 °C in surface waters of lakes Pavin and Aydat, respectively. Oxygen concentrations in Lake Pavin were high during the study, ranging from 9 to 12 mg $L^{-1}$. Similar values were recorded in the epi- and metalimnion of Lake Aydat. In contrast, anoxic conditions were observed in the hypolimnion of Lake Aydat in May. Chlorophyll *a* concentrations and total bacterial abundance were significantly different between lakes (Table 1), indicative of the different trophic status of these ecosystems. Highest chlorophyll *a* values (6.15 and 27.5 μg $L^{-1}$ in Lake Pavin and Lake Aydat, respectively) were recorded during the mixing period.

### Particle concentrations

The abundance of TEP was significantly higher in Lake Aydat than in Lake Pavin, whereas no difference in CSP abundance was found between lakes (Table 1). The abundance of both particles decreased with depth in March and April. In May, values of TEP and CSP were the highest in the metalimnion of the two lakes. Generally, the chlorophyll *a* concentration and particle abundance followed a similar seasonal pattern. However, only TEP concentrations were correlated with chlorophyll *a* (Pearson's $r = 0.774$, $P = 0.003$) and bacterial abundance ($r = 0.663$, $P = 0.007$), indicating the greater dependence of these particles on lake trophic status (Carrias *et al.*, 2002).

**Table 1.** Mean values and comparisons between lakes (one-way ANOVA) of chlorophyll *a* concentrations, bacterial abundance and detrital organic particle concentrations during spring 2002

| | Lake Pavin | | Lake Aydat | | ANOVA | |
|---|---|---|---|---|---|---|
| | Mean ± SD | Range | Mean ± SD | Range | *F* | *P* |
| Chl *a* (μg $L^{-1}$) | 4.5 ± 3.0 | 1.8–9.6 | 13.3 ± 6.1 | 6.1–27.5 | 17.2 | 0.001 |
| Bacteria ($10^6$ $mL^{-1}$) | 3.8 ± 1.4 | 2.3–6.9 | 7.2 ± 2.0 | 2.0–10.5 | 21.11 | 0.001 |
| 2–5 μm size class | | | | | | |
| CSP ($10^6$ $L^{-1}$) | 0.4 ± 0.2 | 0.1–0.8 | 2.1 ± 0.8 | 0.7–5.9 | 0.00 | NS |
| TEP ($10^5$ $L^{-1}$) | 0.06 ± 0.1 | 0.01–0.3 | 1.3 ± 0.4 | 0.4–2.1 | 69.58 | <0.001 |
| 5–50 μm size class | | | | | | |
| CSP ($10^6$ $L^{-1}$) | 1.4 ± 0.8 | 0.4–3.3 | 1.7 ± 0.7 | 0.3–2.7 | 0.27 | NS |
| TEP ($10^5$ $L^{-1}$) | 2.6 ± 2.5 | 0.7–8.5 | 25.4 ± 8.1 | 8.1–39.1 | 59.09 | <0.001 |
| 2–50 μm size class | | | | | | |
| CSP ($10^6$ $L^{-1}$) | 1.8 ± 1.0 | 0.5 ± 4.1 | 3.8 ± 1.5 | 0.4–3.3 | 0.20 | NS |
| TEP ($10^5$ $L^{-1}$) | 2.7 ± 2.6 | 0.7–8.8 | 26.7 ± 8.6 | 8.6–41.2 | 59.09 | <0.001 |

NS, not significant; Chl, chlorophyll *a*; CSP, Coomassie-stained particles; TEP, transparent exopolymeric particles.
The number of degrees of freedom is 13 for all comparisons.


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## Overall bacterial colonization

There was no significant difference between the two lakes in the relative abundance of colonized particles. All TEP, and 90% (Lake Pavin) and 94% (Lake Aydat) of CSP were colonized by bacteria as measured by the DAPI procedure. Bacteria associated with particles constituted 2.6% and 7.4% of the total DAPI-stained bacteria in lakes Pavin and Aydat, respectively. In the two lakes, 75% of attached bacteria were characterized as *Eubacteria*, while free-living *Eubacteria* accounted for only 50% and 57% of free-living DAPI-stained bacteria in lakes Pavin and Aydat, respectively (Table 2). Significant differences in the abundance of each group of *Eubacteria* were observed between the free and attached fractions in Lake Pavin, whereas no difference was observed in Lake Aydat, except for the *Betaproteobacteria* (Table 2). The mean relative abundances of each group of attached bacteria, expressed as a percentage of DAPI-stained bacteria or *Eubacteria*, were similar in both lakes (Table 2).

## Differences in the composition of attached bacteria

For each size class (2–5 and 5–50 μm) of both TEP and CSP we compared (ANOVA) the number of attached bacteria per particle between lakes. The numbers of *Alphaproteobacteria*, *Betaproteobacteria* and *Bacteroidetes* per particle on 2–5 and 5–50 μm TEP were significantly higher ($P<0.01$) in Lake Pavin than in Lake Aydat (Fig. 1). The same difference between lakes was observed for the number of attached bacteria per CSP, but the relationship was significant for the 2–5 μm particle size class only ($P<0.01$).

In both lakes, the number of attached bacteria per TEP in each bacterial group was higher ($P<0.05$) for the 2–5 μm particles than for the 5–50 μm particles. The number of attached bacteria per CSP was also higher for the 2–5 μm size class of CSP in Lake Aydat.

The comparison clearly indicated that 2–5 μm TEP are more colonized than 2–5 μm CSP in both lakes ($P<0.05$ for Lake Pavin and $P<0.005$ for Lake Aydat). In contrast, the difference in bacterial colonization of 5–50 μm particles was not related to the nature of the particle in either lake.

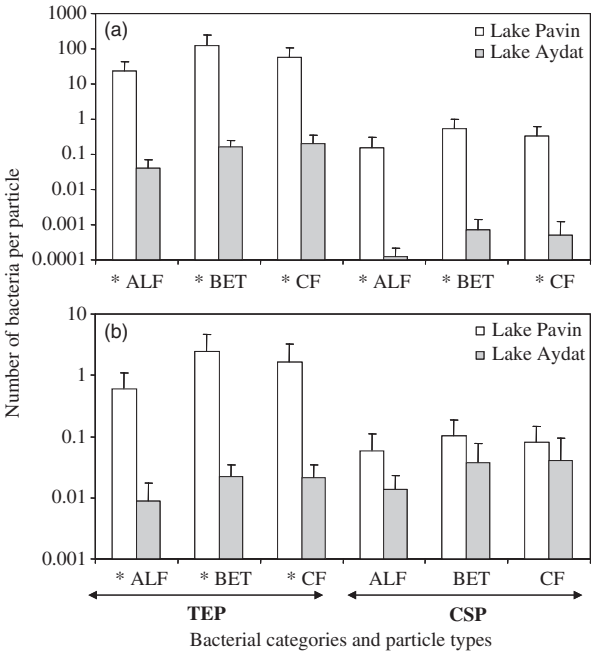


**Fig. 1.** Mean number of bacteria per particle for the (a) 2–5 μm and (b) 5–50 μm size fractions of detrital organic particles in lakes Pavin and Aydat during spring 2002. ALF: *Alphaproteobacteria*; BET: *Betaproteobacteria*; CF: *Cytophaga–Flavobacterium*; TEP: transparent exopolymer particles; CSP: Coomassie-stained particles. *Significant difference ($P<0.01$) between lakes.

## Glycolytic and proteolytic activities in fractionated water samples

The $V_{max}$ values of leucine aminopeptidase and α-glucosidase activities varied from 0.5 to 21.3 nM $L^{-1}$ $h^{-1}$ and from

**Table 2.** Comparison (one-way ANOVA) between free and attached fractions of the various bacterial groups expressed as mean abundances or percentages of DAPI- or EUB338-stained bacteria in lakes Pavin and Aydat

| | Lake Pavin | | | Lake Aydat | | |
|---|---|---|---|---|---|---|
| | Free | Attached | *P* | Free | Attached | *P* |
| Total DAPI cells (cells $mL^{-1}$) | $3.8 \pm 1.4 \times 10^6$ | $9.9 \pm 3.6 \times 10^4$ | <0.0001 | $7.2 \pm 2.0 \times 10^6$ | $53.3 \pm 12.4 \times 10^4$ | <0.0001 |
| EUB338 (cells $mL^{-1}$) | $1.9 \pm 0.4 \times 10^6$ | $7.5 \pm 0.9 \times 10^4$ | 0.043 | $4.1 \pm 1.1 \times 10^6$ | $40.0 \pm 2.6 \times 10^4$ | 0.003 |
| EUB338/DAPI (%) | 49.4 ± 11.8 | 76.6 ± 9.3 | 0.043 | 56.6 ± 14.8 | 75.2 ± 5.0 | 0.003 |
| ALF1b (cells $mL^{-1}$) | $4.2 \pm 1.2 \times 10^5$ | $1.1 \pm 0.2 \times 10^4$ | <0.0001 | $7.0 \pm 2.4 \times 10^5$ | $6.2 \pm 1.0 \times 10^4$ | NS |
| ALF1b/EUB338 (%) | 21.9 ± 6.4 | 15.3 ± 2.1 | <0.0001 | 17.2 ± 5.9 | 15.5 ± 9.1 | NS |
| BET42a (cells $mL^{-1}$) | $5.5 \pm 0.9 \times 10^5$ | $3.4 \pm 0.4 \times 10^4$ | <0.0001 | $1.3 \pm 0.2 \times 10^5$ | $15.8 \pm 2.3 \times 10^4$ | <0.0001 |
| BET42a/EUB338 (%) | 29.1 ± 4.9 | 45.7 ± 6.1 | <0.0001 | 30.7 ± 4.2 | 39.5 ± 5.7 | <0.0001 |
| CF319a (cells $mL^{-1}$) | $4.7 \pm 0.6 \times 10^5$ | $2.4 \pm 0.4 \times 10^4$ | 0.008 | $1.7 \pm 0.2 \times 10^5$ | $15.4 \pm 2.7 \times 10^4$ | NS |
| CF319a/EUB338 (%) | 24.7 ± 3.1 | 32.2 ± 5.3 | 0.008 | 41.2 ± 5.8 | 38.6 ± 6.8 | NS |

Mean values (± SD) were calculated from data of different dates and depths.
NS, not significant.

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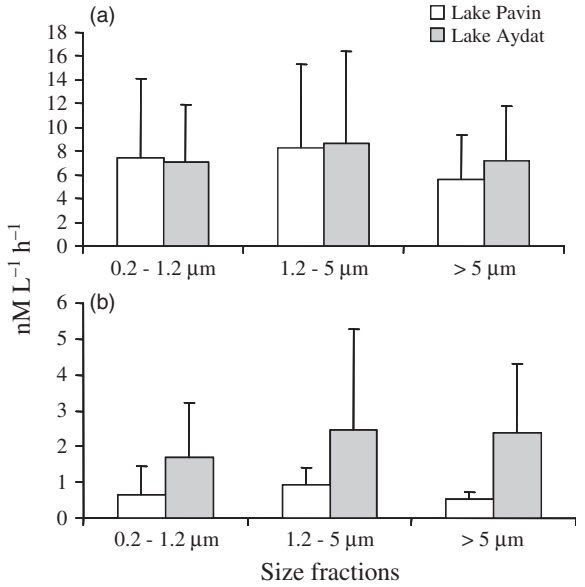


**Fig. 2.** Mean values of potential (a) leucine aminopeptidase and (b) α-glucosidase activities ($V_{max}$) in fractionated lake water from lakes Pavin and Aydat. Mean values (± SD) were calculated from data of different dates and depths.

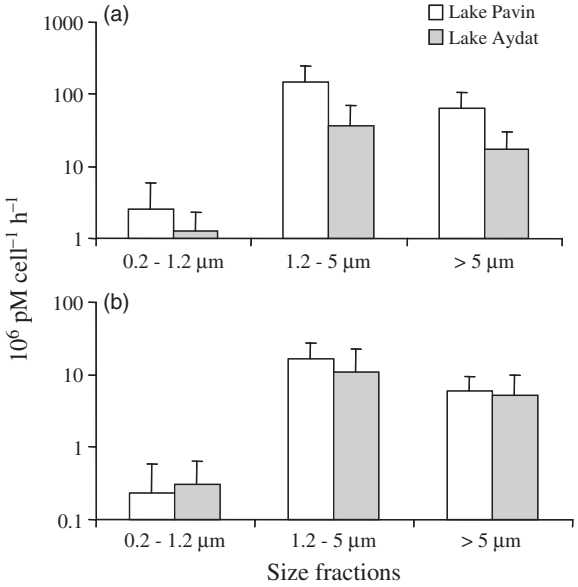


**Fig. 3.** As Fig. 2, but for cell-specific enzymatic activity.

0.1 to 8.8 nM L$^{-1}$ h$^{-1}$, respectively. As expected, most activity was associated with the 1.2–5 µm fractions (Fig. 2). On average, glycolytic activities were three times lower than proteolytic activities. No significant differences in the $V_{max}$ of proteolytic activity between lakes and between fractions were found. Mean values of glycolytic activity were lower in Lake Pavin than in Lake Aydat, but significant differences between lakes were found only in the > 5 µM lake-water fraction (one-way ANOVA, $F = 7.72$, df = 12, $P = 0.018$). Leucine uptake affinity ($K_m$) had values four times lower in Lake Pavin (7.8 ± 14.9 µM) than in Lake Aydat (28.0 ± 48.0 µM). In each lake, values of $K_m$ for leucine uptake were on average higher in the 1.2–5 µM fraction (12.4 ± 25.3 µM in Lake Pavin and 42.5 ± 74.3 µM in Lake Aydat) than in the 0.2–1.2 µM fraction (7.7 ± 7.3 µM in Lake Pavin and 21.1 ± 36.3 µM in Lake Aydat) and the > 5 µM fraction (3.32 ± 3.7 µM in Lake Pavin and 21.1 ± 19.8 µM in Lake Aydat). The $K_m$ values of α-glucosidase activity were lower than the $K_m$ of leucine aminopeptidase activity, mean values reaching 3.15 ± 4.8 µM in Lake Pavin and 8.2 ± 10.6 µM in Lake Aydat. In both lakes, glucose uptake affinity had the lowest values in the 0.2–1.2 µm fraction (1.4 ± 2.6 µM in Lake Pavin and 5.5 ± 5.4 µM in Lake Aydat). On a per cell basis, values for leucine uptake were one order of magnitude higher than those estimated for glucose uptake. Cell-specific hydrolysis rates in the 1.2–5 µM and > 5 µM fractions were significantly (ANOVA, $P < 0.001$) higher than in the 0.2–1.2 µm fraction (Fig. 3). Although mean values of specific activity in the individual fractions were usually much higher in Lake Pavin than in Lake Aydat (Fig. 3), differences between lakes were not statistically significant. In both lakes, enzymatic activity decreased with depth on most dates.

Correlation analysis indicated no relationship between the abundance of proteinaceous particles, i.e. CSP, and proteolytic activities (total or cell-specific activities). In contrast, the concentrations of 2–5 and 5–50 µm TEP were related to the $V_{max}$ of glycolytic activities in the 1.2–5 µm fraction ($r = 0.599$, $P = 0.03$ for the 2–5 µm TEP size class) and the > 5 µm fraction ($r = 0.868$, $P < 0.001$ for the 5–50 µm TEP size class). Neither the glycolytic nor the proteolytic activities were related to the abundances of free and attached bacteria, nor to the number of attached bacteria per particle.

## Discussion

The abundances of TEP and CSP measured during this study were similar to those reported for the same lakes during spring 2000 (Carrias *et al.*, 2002). The spatio-temporal distribution of the values for the two lakes was also similar, indicating a common origin of these particles in these two lakes of the French Central Massif. Hence, abundances of CSP were not significantly different between lakes, whereas those of TEP were almost 10 times higher in the eutrophic than in the oligo-mesotrophic lake (Table 1). Abundances of TEP in the mesotrophic Lake Constance (Brachvogel *et al.*, 2001) are intermediate between those recorded in Lake Pavin and in Lake Aydat. In the eutrophic Lake Kinneret (Berman & Viner-Mozzini, 2001), values are slightly higher than in Lake Aydat. These differences clearly


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indicate that TEP are closely related to the productivity or trophic status of the lake. In marine environments, abundances of TEP usually fluctuate from $10^3$ to $10^4$ particles per mL according to the gradient of productivity (Passow, 2002). As noted during spring 2000 (Carrias *et al.*, 2002), we also observed a strong correlation between TEP abundance and chlorophyll *a* concentrations. The formation of TEP in pelagic ecosystems is largely dependent on phytoplankton, which constitutes the main source of the precursors of these particles (Alldredge *et al.*, 1993; Passow *et al.*, 1994). This close link between TEP and phytoplanktonic cells has also been observed in marine waters (Passow & Alldredge, 1994; Schuster & Herndl, 1995) and in mesocosm experiments (Passow & Alldredge, 1994). These particles are probably generated abiotically from polysaccharide precursors released from diatoms as dissolved and colloidal matter (Passow, 2000; Zhou *et al.*, 1998). The origin and the formation of CSP are quite different from those of TEP. No relationship between the abundance of CSP and the concentrations of chlorophyll *a* was found during our study. Proteinaceous particles appear less closely related to the productivity of the lakes, which supports the assumption that their origin is probably multiple (Long & Azam, 1996). However, the concentrations of these particles were on average higher in Lake Aydat than in Lake Pavin both during this study (Table 1) and during the study carried out in 2000 (Carrias *et al.*, 2002). In contrast to our results, Berman and Viner-Mozzini (2001) found a positive relationship between CSP and chlorophyll *a* in Lake Kinneret, but no correlations were found between TEP abundance and either chlorophyll *a* concentration or phytoplankton biomass in this lake. Many reports, however, have indicated that TEP are related to the dynamics of diatoms (Passow, 2002). Very little data on CSP abundance and dynamics in the field are available (Long & Azam, 1996; Berman & Viner-Mozzini, 2001; Carrias *et al.*, 2002), and the formation and the role of these particles remain largely unknown.

The major aim of this study was to examine the composition of particle-associated bacteria in two lakes of different trophic status. The composition of the bacterial community within aggregates (marine and lake snow) differs from that in the surrounding water (DeLong *et al.*, 1993; Knoll *et al.*, 2001), suggesting that the bacterial community attached to small TEP and CSP also differs from free-living cells. Our results indicated that the proportion of *Eubacteria* in lakes Pavin and Aydat is different between the free-living and attached fractions. The contribution of *Eubacteria* to particle-associated and DAPI-stainable bacteria averaged 75% in the two lakes, whereas their contribution to free-living bacteria was only 50–57% (Table 2). In Lake Constance, 40 to 80% of the DAPI-stainable bacteria were detected by the probe EUB338. This suggests that other typical freshwater groups such as *Actinobacteria* (Glöckner *et al.*, 2000) are an important group in lakes. This group can dominate bacterial communities in some lakes (Urbach *et al.*, 2001; Van der Gucht *et al.*, 2005). Because this study was restricted to the *Proteobacteria* and the *Bacteroidetes* groups, we cannot exclude the possibility that *Actinobacteria* were well represented in our samples. We found high proportions of *Betaproteobacteria* in particle-associated bacteria in the two lakes compared with free-living cells. These bacteria constitute 34% and 30% of DAPI-stainable bacteria in lakes Pavin and Aydat, respectively. However, because of the possibility of detecting *Gammaproteobacteria* with the BET42*a* probe, the abundance of *Betaproteobacteria* may be overestimated. In contrast to the case for *Betaproteobacteria*, the contribution of *Alphaproteobacteria* to particle-associated bacteria was lower than their contribution to free-living cells, but this difference was significant in Lake Pavin only. In this lake, the proportion of *Cytophaga–Flavobacteria* is also significantly higher on particles than in the surrounding water. Based on our results, we conclude that the composition of particle-associated bacteria is different from that of free-living bacteria, and that this difference is more pronounced in the oligomesotrophic Lake Pavin.

In Lake Constance, the contribution of the *Betaproteobacteria* to DAPI-stainable bacteria on lake snow aggregates varied from 27% to 42% (Weiss *et al.*, 1996), and the >8 μm microaggregates were predominantly colonized by *Betaproteobacteria* and *Cytophaga–Flavobacteria* group (Brachvogel *et al.*, 2001). In marine sediments, Ravenschlag *et al.* (Ravenschlag *et al.*, 2000) reported high proportions of *Cytophaga–Flavobacteria* in the top 5 cm. High proportions of the *Cytophaga–Flavobacteria* group were also found on biofilm and aggregates in a river and an estuary (Böckelmann *et al.*, 2000; Brümmer *et al.*, 2000; Selje & Simon, 2003). In addition, an experimental study on bacterial colonization of diatom microaggregates from Lake Constance clearly showed that the composition of the free-living bacteria differed from that on microaggregates (Knoll *et al.*, 2001). Interestingly, the dominant groups of particle-associated bacteria found in this experimental investigation are the same as those found in the field during our study. Therefore, even though studies on the composition of bacteria associated with small particles are lacking, we can speculate that the high proportion of *Betaproteobacteria* and/or *Cytophaga–Flavobacteria* on detrital organic particles is a general trend in pelagic ecosystems. The dominance of the *Cytophaga–Flavobacteria* group on marine or lake snow or detritus could be explained by a better ability of these bacterial communities to degrade refractory particulate organic matter (Kirchman, 2001; Knoll *et al.*, 2001). In lakes Pavin and Aydat, the proportions of the *Cytophaga–Flavobacteria* group were slightly higher in the hypolimnion. If we assume that particles in the hypolimnium are the oldest (Schweitzer *et al.*, 2001), then our results could support the

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idea that *Cytophaga–Flavobacteria* communities preferably colonize old refractory organic particles.

Based on the contribution of each bacterial group to DAPI- or EUB338-stainable bacteria (Table 2), we did not find any significant difference in the composition of free-living or particle-associated bacterial communities between lakes. However, the numbers of *Alphaproteobacteria*, *Betaproteobacteria* and *Cytophaga–Flavobacterium* per particle on 2–5 μm TEP and CSP and on 5–50 μm TEP were higher in the oligomesotrophic lake than in the eutrophic one, indicating that small particles are an important support for bacteria in oligotrophic waters. The different colonization of particles by bacteria in the two lakes suggests differences in the quality of the particles, which is in contrast to our previous statement that particles have a similar origin in the two lakes. We also found that 2–5 μm TEP were more colonized than 2–5 μm CSP in both lakes, indicating that small TEP are more suitable substrates for bacteria than small CSP (Carrias *et al*., 2002).

These findings are supported by the measurement of enzymatic activities in size-fractionated water. Accordingly, the concentration of TEP was significantly related to the $V_{max}$ of α-glucosidase activity measured in the 1.2–5 μm and $>5$ μm fractions, whereas no relationship between CSP and proteolytic activities was found. In addition, as noted by some authors (Middelboe *et al*., 1995; Riemann *et al*., 2000; Lehman & O'Connell, 2002), cell-specific hydrolysis rates in the attached bacterial fractions (1.2–5 μm and $>5$ μm fractions) were significantly higher than those in the free-living bacterial fraction (0.2–1.2 μm fraction, Fig. 3). As indicated previously, the activity of attached bacteria was probably overestimated owing to the presence of free-living bacteria in the 1.2–5 μm fraction. This bias is, however, of minor importance in the $>5$ μm fraction. This supports the idea that particle colonization was accomplished by specialized bacteria of high hydrolytic capacity. Nevertheless, various other factors could enhance the cell-specific activities in the fractions of particle-associated bacteria. As suggested by Huston & Deming (2002), the enhanced rates of enzymatic activity associated with particles may reflect a greater contribution of metabolically active attached bacteria rather than an increase in enzyme production by the whole particle-associated bacteria. Another factor controlling the increase in enzymatic activity in the attached bacterial fractions may be grazing activity. Enzymes released by the grazing of bacterivorous protists may contribute to the observed high values of enzymatic activity in the attached bacterial fractions. $V_{max}$ values as well cell-specific activities in lakes Pavin and Aydat were on average highest in the 1.2–5 μm size fraction, in which bacterivores are often dominant (Carrias *et al*., 1996). In addition, a recent study carried out in Lake Pavin indicated that the abundance of heterotrophic nanoflagellates is strongly related to the concentrations of TEP (unpublished data). These results show that freshwater TEP constitute habitats of high bacterial productivity, which probably significantly enhance the bacterivory of nanoflagellates in these nanometre-scale environments.

## Conclusion

The role of TEP and CSP in the formation of organic aggregates in the pelagic environment is well documented (Kiørboe & Hansen, 1993; Logan *et al*., 1995; Passow & Alldredge, 1995a; Grossart *et al*., 1997; Bhaskar *et al*., 2005). In contrast, few reports have considered the importance of these particles as microbial microhabitats (Berman & Viner-Mozzini, 2001; Verdugo *et al*., 2004). The present study indicates that (i) small TEP are more appropriate habitats than CSP for bacterial colonization in lakes, (ii) the bacterial community composition associated with TEP and CSP is different from that of free-living bacteria in the surrounding water, this difference being more pronounced in the less productive lake, and (iii) attached bacteria express high cell-specific enzymatic activities and are probably of major importance in biogeochemical processes in the food web. On the whole, our field data show that freshwater detrital particles, particularly TEP, interact strongly with microbial communities and biogeochemical cycles in pelagic ecosystems.

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## References

Alldredge AL, Passow U & Logan BE (1993) The abundance and significance of a class of large, transparent organic particles in the ocean. *Deep-Sea Res* **40**: 1131–1140.

Berman T & Viner-Mozzini Y (2001) Abundance and characteristics of polysaccharide and proteinaceous particles in Lake Kinneret. *Aquat Microb Ecol* **24**: 255–264.

Bhaskar PV, Grossart HP, Bhosle NB & Simon M (2005) Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin. *FEMS Microb Ecol* **53**: 255–264.

Böckelmann U, Manz W, Neu TR & Szewzyk U (2000) Characterization of the microbial community of lotic organic aggregates (river snow) in the Elbe river of German by cultivation and molecular methods. *FEMS Microb Ecol* **33**: 157–170.

Brachvogel T, Schweitzer B & Simon M (2001) Dynamics and bacterial colonization of microaggregates in a large mesotrophic lake. *Aquat Microb Ecol* **26**: 23–35.



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Brümmer IHM, Fehr W & Wagner-Döbler I (2000) Biofilm community structure in polluted rivers: abundance of dominant phylogenetic groups over a complete annual cycle. *Appl Environ Microbiol* **66**: 3789–3796.

Carrias J-F, Amblard C & Bourdier G (1996) Protistan bacterivory in an oligomesotrophic lake: importance of attached ciliates and flagellates. *Microb Ecol* **31**: 249–268.

Carrias J-F, Thouvenot A, Amblard C & Sime-Ngando T (2001) Dynamics and growth estimates of planktonic protists during early spring in Lake Pavin (France). *Aquat Microb Ecol* **24**: 163–174.

Carrias JF, Serre JP, Sime-Ngando T & Amblard C (2002) Distribution, size, and bacterial colonization of pico- and nano-detrital organic particles (DOP) in two lakes of different trophic status. *Limnol Oceanogr* **47**: 1202–1209.

DeLong EF, Franks DG & Alldredge AL (1993) Phylogenetic diversity of aggregate attached vs. free-living marine bacterial assemblages. *Limnol Oceanogr* **38**: 924–934.

Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A & Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl Environ Microbiol* **66**: 5053–5065.

Grossart HP & Simon M (1998a) Bacterial colonization and microbial decompo-sition of limnetic organic aggregates (lake snow). *Aquat Microb Ecol* **15**: 127–140.

Grossart HP, Simon M & Logan BE (1997) Formation of macroscopic organic aggregates (lake snow) in a large lake: the significance of transparent exopolymer particles, phytoplankton and zooplankton. *Limnol Oceanogr* **42**: 1651–1659.

Hoppe HG (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar Ecol Prog Ser* **11**: 299–308.

Huston AL & Deming JW (2002) Relationships between microbial extracellular enzymatic activity and suspended and sinking particulate organic matter: seasonal transformations in the North Water. *Deep-Sea Res* **49**: 5211–5225.

Junge K, Imhoff JF, Staley JT & Deming JW (2002) Phylogenetic diversity of numerically important arctic sea-ice bacteria cultured at subzero temperature. *Microb Ecol* **43**: 315–328.

Kepkay PE (1994) Particle aggregation and the biological reactivity of colloids. *Mar Ecol Prog Ser* **109**: 293–304.

Kiørboe T & Hansen JLS (1993) Phytoplankton aggregate formation: observations of patterns and mechanisms of cell sticking and the significance of exopolymeric material. *J Plankton Res* **15**: 993–1018.

Kirchman DL (2001) The ecology of *Cytophaga–Flavobacteria* in aquatic environments. *FEMS Microbiol Ecol* **39**: 91–100.

Knoll S, Zwisler W & Simon M (2001) Bacterial colonization of early stages of limnetic diatom microaggregates. *Aquat Microb Ecol* **25**: 141–150.

Lehman RM & O'Connell SP (2002) Comparison of extracellular enzyme activities and communities composition of attached and free-living bacteria in porous medium columns. *Appl Environ Microbiol* **68**: 1569–1575.

Logan BE, Passow U, Alldredge AL, Grossart HP & Simon M (1995) Mass sedimentation of diatom blooms as large aggregates is driven by coagulation of transparent exopolymer particles (TEP). *Deep-Sea Res* **42**: 203–214.

Long RA & Azam F (1996) Abundant protein-containing particles in the sea. *Aquat Microb Ecol* **10**: 213–221.

Mari X & Kiørboe T (1996) Abundance, size distribution and bacterial colonization of transparent exopolymeric particles (TEP) during spring in the Kattegat. *J Plankton Res* **18**: 969–986.

Middelboe M, Sondergaard M, Letarte Y & Borch NH (1995) Attached and free-living Bacteria: production and polymer hydrolysis during a diatom bloom. *Microb Ecol* **29**: 231–248.

Mostajir B, Dolan JR & Rassoulzadegan F (1995a) Seasonal variations of pico- and nano-detrital particles (DAPI Yellow Particles, DYP) in the Ligurian Sea (NW Mediterranean). *Aquat Microb Ecol* **9**: 267–277.

Mostajir B, Dolan JR & Rassoulzadegan F (1995b) A simple method for the quantification of a class of labile marine pico- and nano-sized detritus: DAPI Yellow Particles (DYP). *Aquat Microb Ecol* **9**: 259–266.

Moter A & Göbel UB (2000) Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *J Microbiol Methods* **41**: 85–112.

Passow U (2000) Formation of transparent exopolymer particles, TEP, from dissolved precursor material. *Mar Ecol Prog Ser* **192**: 1–11.

Passow U (2002) Transparent exopolymer particles (TEP) in aquatic environments. *Prog Oceanogr* **55**: 287–333.

Passow U & Alldredge AL (1994) Distribution, size and bacterial colonization of transparent exopolymer particles (TEP) in the ocean. *Mar Ecol Prog Ser* **113**: 185–198.

Passow U, Alldredge AL & Logan BE (1994) The role of particulate carbohydrate exudates in the flocculation of diatom blooms. *Deep-Sea Res* **41**: 335–357.

Passow U & Alldredge AL (1995a) Aggregation of a diatom bloom in a meso-cosm: the role of transparent exopolymer particles (TEP). *Deep-Sea Res* **42**: 99–109.

Ravenschlag K, Sahm K & Amann R (2000) Quantitative molecular analysis of the microbial community in marine artic sediments (Svalbard). *Appl Environ Microbiol* **67**: 387–395.

Riemann L, Steward GF & Azam F (2000) Dynamics of bacterial community composition and activity in a mesocosm diatom bloom. *Appl Environ Microb* **66**: 578–587.

Schuster S & Herndl JG (1995) Formation and significance of transparent exopolymer particles in the Northern Adriatic Sea. *Mar Ecol Prog Ser* **124**: 227–236.

Schweitzer B, Huber I, Amann R, Ludwig W & Simon M (2001) α- and β- Proteobacteria control the consumption and release of amino acids on lake snow aggregates. *Appl Environ Microb* **67**: 632–645.

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SCOR/UNESCO (1966) Determination of photosynthetic pigments in seawater. *Monographs on oceanographic methodology* **1**: 11–18. UNESCO, Paris.

Selje N & Simon M (2003) Composition and dynamics of particle-associated and free-living bacterial communities in the Weser estuary, Germany. *Aquat Microb Ecol* **30**: 221–237.

Shimeta J (1993) Diffusional encounter of submicrometer particles and small cells by suspension feeders. *Limnol Oceanogr* **38**: 456–465.

Simon M (1987) Biomass and production of small and large free-living and attached bacteria in Lake Constance. *Limnol Oceanogr* **32**: 591–607.

Urbach E, Vergin KL, Young L & Morse A (2001) Unusual bacterioplankton community composition structure in ultra-oligotrophic Crater Lake. *Limnol Oceanogr* **46**: 557–572.

Utermöhl H (1958) Zur vervollkommung der quantitativen Phytoplankton. *Methodik Mitt Int Ver Limnol* **9**: 1–38.

Van der Gucht K, Vandekerckhove T, Vloemans N, Cousin S, Muylaert K, Sabbe K, Gillis M, Declerk S, De Meester L & Vyverman W (2005) Characterisation of bacterial communities in four freshwater lakes differing in nutrient load and food web structure. *FEMS Microb Ecol* **53**: 205–220.

Verdugo P, Allredge AL, Azam F, Kirchman DL, Passow U & Santschi PH (2004) The oceanic gel phase: a bridge in the DOM-POM continuum. *Marine Chemistry* **92**: 67–85.

Weiss P, Schweitzer B, Amann R & Simon M (1996) Identification in situ and dynamics of Bacteria on limnetic organic aggregates (lake snow). *Appl Environ Microb* **62**: 1998–2005.

Zhou J, Mopper K & Passow U (1998) The role of surface-active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnol Oceanogr* **43**: 1860–1871.



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