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Distribution, size, and bacterial colonization of pico- and nano-detrital organic particles (DOP) in two lakes of different trophic status

Abstract—Abundance, size spectra, and bacterial colonization of three types of freshwater pico- and nano-detrital organic particles (DOP), namely transparent exopolymeric particles (TEP), Coomassie-stained proteinaceous particles (CSP), and 4′,6-diamidino-2-phenylindole (DAPI) yellow particles (DYP), were examined by epifluorescence microscope equipped with a color camera and a video recorder. The study was conducted during spring in the oligomesotrophic Lake Pavin and the eutrophic Lake Aydat, France. Pico- and nano-DOP were abundant (10^5–10^8 particles L^{-1}) and exhibited significant variations in morphometric characteristics and numerical density within and among lakes, which is consistent with comparable studies known mostly from marine systems. Surface area and abundance of pico- and nano-DYP and nano-TEP were significantly higher in Lake Aydat than in Lake Pavin. Both these variables significantly increased with chlorophyll *a* concentration and the abundance of total heterotrophic bacteria. Bacterial colonization of DOP was highly related to the nature of the particle. Attached bacterial counts were on the order of 10^7–10^8 bacteria L^{-1}, representing 1–17% of the total bacterial counts. Overall, we conclude that nonliving particulate organic matter are ubiquitous and abundantly occurring components in lakes where, at least, they serve as support for the attachment and as substrate for the metabolism of bacteria, the latter role being likely dependent on the composition of the particles and the trophic status of the milieu.

Recent studies have revealed that small-sized (e.g., pico- and nano-sized) detrital organic particles (DOP) are a ubiquitous and abundant component of the nonliving particulate organic matter in the pelagic zone of marine environments (Passow and Alldredge 1994; Mostajir et al. 1995*b*; Schuster and Herndl 1995; Mari and Kiørboe 1996; Mari and Burd 1998). These particles actually include transparent exopolymeric particles (TEP) stained with an acidic solution of Alcian Blue (Alldredge et al. 1993; Passow and Alldredge 1994), proteinaceous particles stained by Coomassie Brilliant Blue (Coomassie-stained particles, CSP) (Long and Azam 1996), and 4′,6-diamidino-2-phenylindole (DAPI)-stained yellow particles (DYP) (Mostajir et al. 1995*a*).

TEP were described for the first time by Wiebe and Pomeroy (1972). These detrital particles consist of a matrix of colloidal fibrils (Leppard and Burnison 1990) and are produced from dissolved carbohydrate polymers exuded by phytoplankton and bacteria (Alldredge et al. 1993; Passow and Alldredge 1994; Passow 2000). DYP are almost exclusively organic, enzyme-degradable matter (Mostajir et al. 1995*a*). The sources of DYP could include phytoplankton-derived detritus, particles released from protozoa, and agglomeration of colloids produced from dissolved organic carbon (DOC) adsorption on bubbles (Mostajir et al. 1995*b*). Little is known about the origins of CSP. However, it is likely that various mechanisms such as cell lysis, cell death, or adsorption of protein onto particles could produce CSP (Long and Azam 1996). Detrital organic particles serve as substrate for bacterial growth (Kepkay 1994; Passow and Alldredge 1994; Smith et al. 1995) and may be consumed by protozoa and metazoa (Shimeta 1993; Alldredge et al. 1993). TEP may coagulate with both detrital and living (e.g., diatoms) particles into larger aggregates (i.e., marine or lake snow) (Passow et al. 1994; Passow and Alldredge 1995), and thereby potentially influence food web structure and particulate flux in aquatic ecosystems (Logan et al. 1995).

Relatively few investigations have focused on the size distribution and bacterioplankton colonization of nonliving organic particles (Passow and Alldredge 1994; Mostajir et al. 1995*b*; Schuster and Herndl 1995; Mari and Kiørboe 1996; Mari and Burd 1998; Worm and Søndergaard 1998), most of them being mainly concerned with marine TEP. Worm and Søndergaard (1998) used a neutral solution of Alcian Blue to stain transparent particles in the eutrophic Lake Frederiksborg Slotssø, Denmark. The abundance, size spectra, and bacterial colonization of their Alcian Blue-stained particles were close to those of marine TEP. Logan et al. (1995) reported high concentrations of TEP in Lake Constance and indicated that the formation of sinking aggregates following blooms of mucous-producing diatoms is primarily controlled by TEP. To our knowledge, there is no comparable study conducted in other lakes and data about freshwater DYP and CSP are lacking. Data on the numerical importance, size, and bacterial colonization of DOP are therefore necessary to evaluate the significance of these particles in the transformation and flux of organic matter in freshwaters. Indeed, DOP are presumably important in the food web structure, the carbon fluxes, and the formation of lake snow aggregates, which are fairly similar to marine snow aggregates (Grossart and Simon 1993; Grossart et al. 1997).

The scope of this study was to estimate the abundance of small-sized DYP, TEP, and CSP in two temperate lakes of different trophic status. The abundance of these particles could vary according to lake productivity. Schuster and Herndl (1995) indicated that TEP densities tended to increase with increasing productivity in the Adriatic Sea. We also determine the size spectra and bacterial colonization of the different DOP under study. More specifically, our objectives were to answer the following questions. (1) Are abundance, bacterial colonization, and size distribution of freshwater DOP similar to those of marine systems? (2) Are there marked differences in abundance, size, and bacterial colonization according to the nature of these particles? (3) Are the occurrence of the particles and the attached bacteria related to lake trophy?

Materials and methods—Study sites and sampling: The study was conducted in two lakes of different trophic status

situated in the Massif Central of France (ca. 46°N, 3°E). Lake Pavin (altitude = 1,197 m) is a meromictic and dimictic oligomesotrophic lake with partial overturns. It is a volcanic mountain lake characterized by its deepness (maximum depth = 92 m), its low area (44×10^4 m^2) and its low drainage basin area (50×10^4 m^2). Lake Aydat is a small (area = 60.3 ha, maximum depth = 15.5 m) eutrophic, dimictic lake located at 825 m altitude. For more details on the morphometric characteristics of the lakes, see Amblard and Bourdier (1990) and Sime-Ngando and Hartmann (1991).

A total of 18 water samples were collected from 8 March to 15 May 2000 with a 10-liter Van Dorn bottle in a central location in the lakes. In each lake, three different depths (1, 7, 13, and 5, 10, 30 m for Lakes Aydat and Pavin, respectively) corresponding to the epi-, meta-, and hypolimnion were sampled. Water temperature and dissolved oxygen were measured with a YSI GRANT 3800 meter and probe.

Fixation, staining, and enumeration of bacteria and DOP: Samples for particle counts were fixed with formalin. The final concentrations were 0.5% to preserve TEP or CSP and 3% to preserve DYP (Passow et Alldredge 1994; Mostajir et al. 1995*a*).

DYP samples and associated bacteria were analyzed according to Mostajir et al. (1995*a*). 2–5-ml subsamples were stained with DAPI (final concentration = 0.25 μg ml^{-1}) and filtered onto black Nuclepore filters using low vacuum. The filter was mounted with immersion oil between a glass slide and a cover slip. Preparations were made within 24 h after the sampling date and stored at −25°C. Particles were counted within 2 weeks after the sampling date with an epifluorescence microscope (Leitz Laborlux) equipped with a ×1,250 objective lens, a Sony 3CCD color video camera (model DWC-950P), a Sony video recorder, and a Leica Q500 personal computer. Counts were performed on video images using the image analysis Leica Qwin software. At least 15 fields per filter were recorded under UV light to visualize the yellow fluorescence of DYP. A total of 120–820 DYP were sized and enumerated on each slide by image analysis. Free-living bacteria and bacteria attached to at least 100 DYP were also counted.

Subsamples (2–5 ml) were double-stained with DAPI and the polysaccharide-specific Alcian Blue to enumerate TEP and bacteria attached to TEP (Passow and Alldredge 1994). Subsamples were first stained with DAPI (0.25 μg ml^{-1}), filtered onto 0.2-μm Nuclepore filters, and then stained with Alcian Blue. Staining was performed on damp filters for 5 s using an aqueous solution of 0.03% Alcian Blue and 0.06% acetic acid. The filter was removed, mounted on a glass slide with immersion oil, and stored at room temperature in the dark. Slides were examined within a week using the Leitz Laborlux microscope. Two to four transects were scanned for each filter under visible light at ×1,250 magnification and TEP (40 to 500 particles) were sized and counted. Bacteria associated with at least 40 TEP per slide were counted by switching between visible and UV light.

CSP samples and associated bacteria were processed with a method almost identical to the method described for TEP. Subsamples (2–5 ml) were double-stained with DAPI and Coomassie Brilliant Blue G-250 (final concentration = 0.04%), a protein stain. The filters were prepared as described above for TEP counts. CSP (40–400) from each slide were enumerated and sized on three to five transects under visible light, and bacteria associated with 40 CSP were counted.

Size classes, size spectra, and bacterial colonization of DOP: For each of the sampling dates and depths, DOP were separated in different size classes according to their greatest linear dimensions (GLD): 0.2–2, 2–5, 5–10, 10–15, and 15–20 μm for DYP; 2–5, 5–10, 10–15, and 15–20 μm for TEP and CSP. The 0.2–2-μm size fraction for TEP and CSP was excluded from analysis because the applied methods do not allow the enumeration of the smallest particles due to a high interference between visible light and the pores of the Nuclepore filters. The averages of GLD, the cross-sectional area, the equivalent spherical diameter, and the number of attached bacteria were calculated for each size class. The surface area of each size class of the three different types of DOP was estimated by multiplying the mean surface area by 2 (to account for the area of both sides of the particles) and by the number of particles in each size class. Cumulative area of DOP was estimated by summing surface areas of the different size classes. The total number of attached bacteria were obtained by the sum of attached bacteria in each size class.

Because (1) abundances and surface areas depend on the size range of particles considered and (2) the size distribution, although formally correct, may be biased because of variations in the bin sizes, we also present our data as size spectra. For the size spectra analysis, each type of DOP was assigned to three to six logarithmically increasing size intervals; that is, the first interval is half the length of the second interval, and so on (McCave 1984). The size spectra of the different types of particles were then described for each sampling date and depth by the power relationship $dN/dl = \mathrm{k}l^{-(\beta+1)}$, where the constant k depends on the concentration of particles and β describes the size spectra; the smaller β is, the smaller the fraction of small particles (McCave 1984; Passow and Alldredge 1994; Mari and Kiørboe 1996; Mari and Burd 1998; Worm and Søndergaard 1998). In practice, for each size interval, the particle concentration (dN) was normalized to the length of the interval (dl) and plotted against the arithmetic average length (l). The parameters k and β were estimated by regression after logarithmic transformation. All the regressions were statistically significant for DYP ($P < 0.05$, for 80% of the samples $P < 0.01$), whereas 80 and 45% of the regressions were significant for CSP and TEP, respectively.

In order to compare the bacterial colonization of the different types of DOP under study, the number of attached bacteria was related to the DOP size for each sample. This relationship has been described by the power law $n = \mathrm{a}d^{\mathrm{b}}$ (Mari and Kiørboe 1996) where n is the number of attached bacteria per DOP, d is the equivalent spherical DOP diameter, and a and b are constants for a given sample. These constants were obtained from log-log plots of n versus d. Regressions were statistically significant ($P < 0.05$) for 100, 88, and 77% of the samples of TEP, CSP, and DYP, respectively.

Chlorophyll *a* concentrations and phytoplankton and nanoflagellate counts: In the two lakes, chlorophyll *a* (Chl *a*) was determined spectrophotometrically from samples collected on Whatman GF/C filters. Pigments were extracted with 90% acetone, and Chl *a* concentrations were calculated from the equations of Scor-Unesco (1966).

Microscopic enumeration of epilimnetic phytoplankton was performed on Lugol's iodine-fixed samples using a Wild M40 inverted microscope at a magnification of ×400. Biomass was calculated from the mean biovolume of each species measured on the basis of a few hundred individuals by considering that 10^6 $\mu m^3 = 1$ μg (wet weight) and assuming that organic cell carbon represents 12% of wet weight biomass.

Nanoflagellate (nonpigmented and pigmented) cell samples (50 ml) were fixed with 1% (final concentration) glutaraldehyde. Subsamples for counts were stained with primulin (Caron 1983) and collected onto black 0.8-μm pore size Nuclepore filters. Preparations were made within 24 h after the sampling date and stored at −25°C to minimize the loss of autofluorescence. Slides were examined at ×1,250 magnification by epifluorescence microscopy in a dark room using the Leitz Laborlux epifluorescence microscope. A total of 300–600 nanoflagellates from each slide was counted on several transects (SD < 10%) and the trophic status of cells (pigmented or nonpigmented) was noted.

Data analysis: Normal distribution of data was checked by a Kolmogorov–Smirov test. Differences in biological or DOP variables between and within lakes were tested by one-way analysis of variance (ANOVA). The coefficients a were tested for difference in bacterial colonization of the three types of DOP in each lake and between the two lakes. Similarly, the constants β were tested for the differences in the DOP size spectra. Potential relationships between DOP and biological variables were analyzed by Pearson correlations or linear regressions. All statistical analyses were performed using MINITAB 12.

Results and discussion—Ambient environmental conditions: After a period of almost complete homogeneity of the water column temperature in March, a clear thermal stratification became established in May with values close to 15°C in surface waters of the two lakes (Fig. 1). Dissolved oxygen peaked (~12 mg L^{-1}) in May in the metalimnion of the two lakes. Oxygen concentrations in the hypolimnion of Lake Pavin were substantially high during this study (9 mg L^{-1}). In contrast, microaerophilic conditions (<1 mg O_2 L^{-1}) were observed in the hypolimnion of Lake Aydat in May.

Chl *a* concentrations were significantly higher in Lake Aydat than in Lake Pavin (Table 1). Maximum values (9.4 and 32.2 μg L^{-1} in Lake Pavin and Lake Aydat, respectively) were recorded during mixis and coincided with the highest values of phytoplankton biomasses (1.4 and 5.5 μg C L^{-1} in Lake Pavin and Lake Aydat, respectively) determined by inverted microscopy. In both lakes, phytoplankton communities were largely dominated by large-size diatoms (*Aulacoseira italica* and *Asterionnella formosa*). They accounted for 47–98% of phytoplankton biomass, and their proportions decreased in the two lakes throughout the study. The abundances of heterotrophic bacteria were also significantly higher in Lake Aydat than in Lake Pavin (Table 1). Although abundances of nanoflagellates were higher in Lake Aydat than in Lake Pavin, both lakes did not differ significantly for their pigmented and nonpigmented flagellate contents (Table 1), suggesting that the spring densities of these protists were only slightly related to lake trophy.

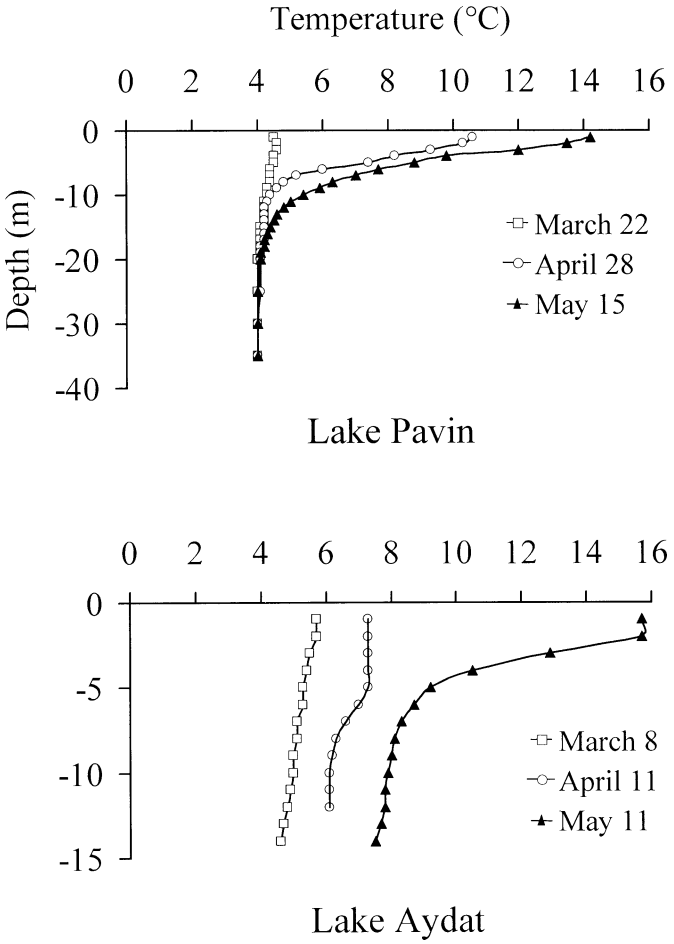

Fig. 1. Temperature profiles in Lakes Pavin and Aydat during spring 2000.

Accuracy of microscopic analysis of DOP: Based on triplicate subsamples, particle abundance always had a coefficient of variation (CV) <12%. These CVs were lower for DYP (CV = 8–9%) than for TEP or CSP (CV = 9–12%). The counting procedure used for analyzing TEP and CSP in this study did not allow the characterization of picoparticles (<2 μm) because of the interference between visible light and the pores of the black polycarbonate filters. This inconvenient is avoided when using the filter–transfer–freeze (FTF) technique (Hewes and Holm-Hansen 1983; Passow and Alldredge 1994), where DOP are transferred from the polycarbonate filter onto a glass slide before microscopic observation. However, in preliminary experiments, we found that this transfer was inefficient for our samples, similar to the report by Schuster and Herndl (1995) in Adriatic Sea.

In the majority of the studies on pelagic DOP, morphometric characteristics of the particles (length, width, diam-

Table 1. Values of some biological parameters and characteristics of DOP in Lakes Pavin and Aydat during spring 2000. Results of one-way ANOVA for differences between lakes are indicated: ns, not significant; df, degrees of freedom. HNF, heterotrophic nanoflagellates; ANF, autotrophic nanoflagellates.

	Lake Pavin		Lake Aydat		ANOVA		
	Mean±SD	Range	Mean±SD	Range	df	*F*	*P*
Chl *a* (μg L^{-1})	5.4±3.3	1.6–9.4	18.8±7.6	11.7–32.2	17	27.71	<0.001
Phytoplankton biomass (μg C L^{-1})*	0.53±0.77	0.08–1.42	3.57±2.76	0.41–5.48	5	3.36	ns
Bacterial abundance (10^6 ml^{-1})	1.3±0.2	1.0–1.5	3.0±1.1	1.5–4.5	17	33.06	<0.001
HNF abundance (10^3 ml^{-1})	1.3±0.5	0.7–2.3	1.9±0.8	0.7–3.3	17	3.24	ns
ANF abundance (10^3 ml^{-1})	1.6±1.3	0.5–4.2	4.0±5.2	0.4–16.1	17	1.62	ns
DYP (0.2–20 μm)							
Abundance (10^7 L^{-1})	13.1±9.6	4.3–33.8	48.5±20.6	11.8–77.5	17	22.89	<0.001
Cumulative surface area (mm^2 L^{-1})	422.3±211.5	125.1–700.9	1793.0±820.6	643.7–3339.1	17	32.56	<0.001
TEP (2–20 μm)							
Abundance (10^5 L^{-1})	3.2±2.6	0.3–7.6	20.1±6.8	11.6–31.1	17	37.74	<0.001
Cumulative surface area (mm^2 L^{-1})	35.5±28.7	4.6–85.9	192.8±94.0	105.4–354.4	17	33.08	<0.001
CSP (2–20 μm)							
Abundance (10^6 L^{-1})	1.5±0.9	0.4–3.6	2.2±0.8	0.6–3.1	17	1.63	ns
Cumulative surface area (mm^2 L^{-1})	72.6±24.4	26.5–98.3	127.6±70.3	38.3–244.4	17	3.87	ns

[a] Mean values for epilimnetic samples.

eter, individual surface area) were estimated assuming simple geometric shapes (Schuster and Herndl 1995; Long and Azam 1996; Worm and Søndergaard 1998). The use of an image analysis system during this study allows precise measurements of individual surfaces of the particles, which exhibited very diverse and complex shapes (Web Appendix 1: http://www.aslo.org/lo/toc/vol_47/issue_4/1202a1.pdf). However, because of flatness, surface area of particles retained on the filter by filtration and analyzed microscopically should be extrapolated to nature with care.

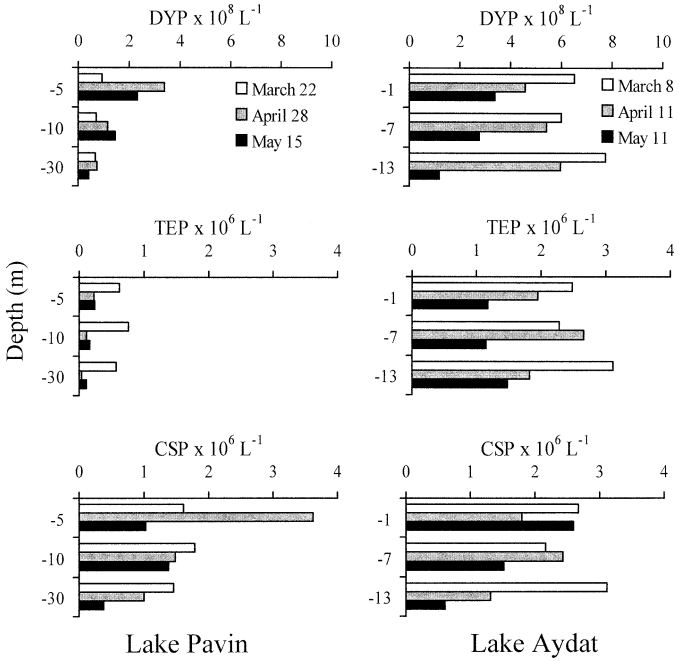

Fig. 2. Spatiotemporal distribution of the densities of DAPI yellow particles (DYP), transparent exopolymeric particles (TEP), and Coomassie-stained particles (CSP) in Lakes Pavin and Aydat: 0.2–20-μm DYP, 2–20-μm TEP, and 2–20-μm CSP were considered.

DYP are composed of organic materials (Mostajir et al. 1995*a*) and may therefore contain both polysaccharides and protein. Thus, DOP categories distinguished in this study probably overlap. However, proteinaceous and polysaccharidic nanoparticles averaged only 9.7 and 7.2% of nano-DYP abundance in Lakes Pavin and Aydat, respectively. This indicates that most of the DYP under study were distinct from TEP and CSP. Microscopic observations of double-staining samples indeed revealed that DYP were distinguished from TEP or CSP. In addition, DYP of each size class in both lakes were on average more elongated than TEP and CSP, and the mean surface areas of each size class of DYP were lower than those of TEP and CSP. It is thus likely that the composition of the three types of DOP were different, although picoparticles (<2 μm) are excluded from the above comparisons.

Abundance and surface area of DOP: Abundance and cumulative surface area of DYP were significantly higher in Lake Aydat than in Lake Pavin (Table 1). In the two lakes, DYP abundance decreased with depth during thermal stratification in May (Fig. 2). Values of numerical density or surface area of these particles were one order of magnitude higher than those recorded in marine environment by Mostajir et al. (1995*b*). Our counting method could explain this difference. Indeed, the use of an image analyzer allows a precise enumeration of particles in the 0.2–2-μm size fraction, which represented the majority of DYP during this study. Moreover, the marine system studied by Mostajir et al. (1995*b*) appears less productive than Lakes Pavin and Aydat, which can also explain the noted differences.

TEP, which originate from dissolved and particulate organic matter, generally increase with the productivity of aquatic ecosystems (Søndergaard and Middelboe 1995; Schuster and Herndl 1995). Our results corroborate this pattern because the abundance and surface area of TEP in the eutrophic lake were significantly higher than those recorded in the oligomesotrophic lake (Table 1). Values recorded in

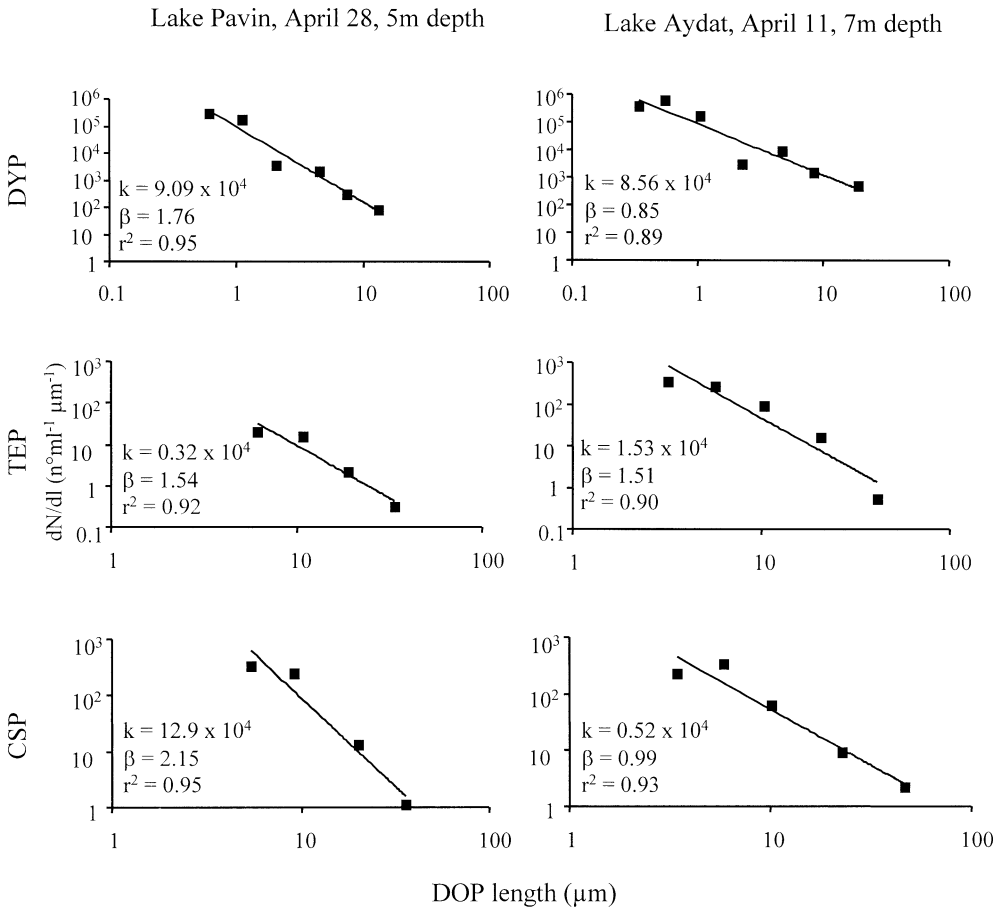

Fig. 3. Examples of differential DOP size distribution in Lakes Pavin and Aydat during spring 2000. Regression line: $dN/dl = kl^{-(\beta+1)}$. See text for explanation.

both lakes for these variables are in agreement with those reported along a trophic gradient in the northern Adriatic sea by Schuster and Herndl (1995). Similarly, nano-TEP abundance in Lake Pavin was close to that estimated by Logan et al. (1995) during a diatom bloom in the mesotrophic Lake Constance. On the other hand, very high polysaccharidic particle densities (10^8 L^{-1}) have been recorded in a Danish eutrophic lake by Worm and Søndergaard (1998), likely because these authors used a neutral rather than acidic solution of Alcian Blue. Alcian Blue complexes specifically with dissociated carboxyl and sulfate groups of acidic mucoplysaccharides (Parker and Diboll 1966), and it is likely that enhancing the pH of the stain solution increases the number of binding sites on particles. Estimates of the abundance and surface area of marine TEP reported by Passow and Alldredge (1994), Mari and Kiørboe (1996), and Mari and Burd (1998) are also generally higher than ours, mainly because these authors considered the 0.2–2.0-μm size fraction of TEP in their studies.

In both Lakes Aydat and Pavin, the abundance of nano-TEP strongly decreased with the installation of thermal stratification (Fig. 2), indicating that the formation of pelagic TEP is likely accelerated by water turbulence. This was shown for marine TEP in laboratory experiments (Schuster and Herndl 1995).

In contrast to DYP and TEP, no difference in abundance or surface area was found between lakes for CSP (Table 1). This suggests that lake trophy is probably not an essential factor for CSP occurrence. CSP abundances reported from marine coastal waters by Long and Azam (1996) are one to two orders of magnitude higher than those we reported in Lakes Pavin and Aydat. However, few descriptions of pelagic CSP are sufficiently complete to permit comparisons among the various pelagic habitats.

During this study, we found that the structure of DOP is slightly different between lakes. Indeed, the relative contribution of nano-TEP and nano-CSP to total abundance and total surface area of nano-DOP were much higher in the oligomesotrophic Lake Pavin than in the eutrophic Lake Aydat.

Size spectra of DOP: Our estimates of β, the exponent of the power law relationship (Fig. 3), revealed that the size spectra of DYP was significantly (ANOVA, $F_{17} = 5.97$, $P = 0.027$) different between lakes (mean β were 1.3 ± 0.24 and 1.02 ± 0.22 in Lakes Pavin and Aydat, respectively), whereas no significant difference was found for the size spectra of TEP ($\beta = 2.02 \pm 0.67$ and 1.75 ± 0.65) and CSP ($\beta = 1.32 \pm 0.63$ and 1.60 ± 0.69). We speculated that DYP degradation is probably more intense in the eutrophic lake, resulting in a lower proportion of small particles (i.e.,

lower values of β), which is corroborated by the greater colonization by bacteria of nano-DYP in Lake Aydat than Lake Pavin (Table 2). However, as noted by Mostajir et al. (1995*b*), both physical and biological mechanisms are involved in the origins and fates of DYP. Thus, absorption of DOC on bubbles during mixis could influence the size spectra of DYP and explain the noted difference. Assuming that TEP and CSP are mainly produced by phytoplankton, we hypothesized that the lack of significant differences in the values of β for these particles probably reflected the similar ecological conditions (i.e., end of the spring diatom bloom) in the two lakes. In addition, the number of bacteria per unit area of the different size classes of TEP and CSP is quite similar between lakes (Table 2).

Overall, values of β are low and always <3 (range 0.63–2.95), indicating that DOP size distribution was not in steady state (McCave 1984). Our estimates of β for TEP are very close to the values (mean $= 2.3 \pm 0.76$) reported by Mari and Kiørboe (1996) in marine waters, but higher than those (0.6 ± 0.45) recorded by Passow and Alldredge (1994) during a bloom of marine diatoms. However, as noted by these authors during the late floccing stage of a marine diatom bloom, the size distributions of TEP from the two lakes did not always follow a power law distribution; only 45% of the regressions were significant for this DOP type. Unfortunately, comparative studies for DYP and CSP are lacking.

Relationships between DOP and phytoplankton: Based on all the data from Lake Aydat and Lake Pavin, the abundance and cumulative surface area of 0.2–2-μm DYP and nano-TEP increased significantly with Chl *a* (Table 3). Variations in Chl *a* concentrations explained >70% of the variance in the total number and total surface area of nano-TEP and 25–30% of the variance in the total number and total surface area of DYP. In addition, abundance and cumulative surface area of nano-TEP were also correlated ($P < 0.05$) with the epilimnetic phytoplankton biomass (Pearson's $r = 0.896$ and 0.815 for nano-TEP abundance and area, respectively; $n = 6$). These correlations highlight the finding that TEP are primarily generated by phytoplankton (Passow and Alldredge 1994), whereas DYP probably have various origins (Mostajir et al. 1995*a*,*b*). Abundance and cumulative surface area of DYP and TEP were also correlated with total bacterial density (Table 3). In contrast, no significant relationship was found between the abundance or the cumulative surface area of any of the studied DOP and nanoflagellated communities. The sparse data available from marine environments suggest that DYP (Mostajir et al. 1995*b*) and TEP (Passow and Alldredge 1994; Schuster and Herndl 1995) tend to increase with increasing productivity. In a mesocosm study, Passow and Alldredge (1995) found that the abundance of TEP was a linear function of Chl *a* during a diatom bloom. Concerning CSP, our results indicated that the surface area of these protein-containing particles was related to Chl *a*, but their abundance was not (Table 3). However, when considering the different size classes, both the abundance and surface area of CSP in the 10–20-μm size fraction were correlated ($P < 0.001$) with Chl *a* concentration (Pearson's $r = 0.838$–0.898). This suggests that large-size protein-containing particles were mainly produced by phytoplankton and may have

Table 2. Average values for bacterial colonization of DOP in Lakes Pavin and Aydat during spring 2000. nd, not determined; Pav, Lake Pavin; Ayd, Lake Aydat.

Size interval	Bacteria per particle						Bacterial per unit area (N per μm^2)						Number of attached bacteria ($\times 10^3$ ml^{-1})					
	DYP		TEP		CSP		DYP		TEP		CSP		DYP		TEP		CSP	
	Pav	Ayd	Pav	Ayd	Pav	Ayd	Pav	Ayd	Pav	Ayd	Pav	Ayd	Pav	Ayd	Pav	Ayd	Pav	Ayd
0.2–2	0.04	0.03	nd	nd	nd	nd	0.04	0.03	nd	nd	nd	nd	4.37	12.70	nd	nd	nd	nd
2–5	0.52	1.04	3.05	2.58	1.84	3.03	0.11	0.25	0.32	0.31	0.26	0.36	9.62	42.04	0.04	0.89	1.00	1.38
5–10	1.88	4.51	6.18	5.18	3.27	3.98	0.09	0.22	0.21	0.23	0.15	0.16	3.90	61.01	0.81	4.27	2.65	4.84
10–15	3.08	8.81	10.47	8.90	5.39	7.08	0.09	0.20	0.17	0.13	0.11	0.08	2.40	26.70	1.21	4.45	0.64	1.91
15–20	9.00	16.5	16.28	14.85	13.17	15.64	0.11	0.14	0.12	0.11	0.13	0.17	1.33	21.58	0.90	4.55	0.72	1.25

Table 3. Significant Pearson's correlation coefficients (r) between DOP and biological variables. All data from Lake Pavin and Lake Aydat were used (n=18). Ab, abundance; CSA, cumulative surface area.

	Pico- and nano-DYP		Nano-TEP		Nano-CSP	
Biological variables	Ab	CSA	Ab	CSA	Ab	CSA
Chl *a*	0.506*	0.532*	0.799***	0.833***	ns	0.570**
Total bacterial abundance	0.770***	0.827***	0.730***	0.679**	ns	ns

* $P < 0.05$; ** $P < 0.01$; *** $P \leq 0.001$.

a different origin than small-sized CSP. The fact that various mechanisms of cell lysis or death lead to the production of CSP (Long and Azam 1996) is thus in good agreement with our results. However, to test this hypothesis, future studies should address the origins of CSP.

DOP-associated bacteria: A potential source of error in estimating DOP-associated bacteria is free bacteria retained beneath or on the particle during filtration. We estimated the number of such retained bacteria from the filter area, the sample volume, the surface area of the particles, and the observed concentration of total bacteria. The number of these retained bacteria represented, for all samples, <1% (mean = 0.53%) of the total number of bacteria. This would add only about 10% (range 5–17%) of attached bacteria to DOP and was thus ignored.

In the two lakes, double-staining (Web Appendix 1: http://www.aslo.org/lo/toc/vol_xx/issue_x/xxxxa1.pdf) indicated that all nano-TEP and 82% of nano-CSP were colonized by bacteria, whereas the DAPI-staining protocol revealed that 30 and 60% of nano-DYP were colonized by bacteria in Lake Pavin and Lake Aydat, respectively. In contrast, <3% of pico-DYP were colonized. There was no significant difference between the two lakes in the relative abundance of colonized particles. TEP appeared as a minor component of DOP (nano-TEP accounted for only 1.5 and 3.3% of nano-DYP abundance in Lakes Pavin and Aydat, respectively) but apparently offered suitable sites for bacterial attachment. In both lakes, the average numbers of bacteria per unit area of TEP were similar, with the highest values occurring in the 2–10-μm size class (Table 2). In Lake Pavin, bacterial density on 2–10-μm TEP was higher than those calculated for 2–10-μm DYP and 2–10-μm CSP. Difference in bacterial colonization of DOP was also confirmed by comparing the slope (a) of the regression lines between the number of bacteria per particle and the equivalent spherical diameter of the particles (Table 4). In both lakes, the values of constant a, determined for DYP, were significantly lower (ANOVA) than those determined for TEP ($F_{14} = 45.49$, $P < 0.001$ in Lake Aydat; $F_{16} = 19.65$, $P < 0.001$ in Lake Pavin) and CSP ($F_{12} = 6.41$, $P = 0.028$ in Lake Aydat; $F_{16} = 15.34$, $P = 0.001$ in Lake Pavin), indicating a more important colonization of TEP and CSP. The highest values of constant a were obtained for nano-TEP in Lake Pavin and for CSP in Lake Aydat (Table 4). Our results thus suggested that nano-TEP are more suitable substrates for bacteria in an oligotrophic environment, whereas nano-CSP are more colonized in productive waters. That nitrogen-rich substrates like amino acids can potentially adhere to TEP (Decho 1990) probably explains the strong bacterial colonization of these particles in Lake Pavin. In contrast, nano-CSP, particularly the 2–5-μm size class (Table 2), appeared to be a good substrates for bacteria in eutrophic waters. In addition, 2–15-μm DYP exhibited twofold more attached bacteria in Lake Aydat than in Lake Pavin. However, difference in the slope of the regression lines was not statistically significant because of the high variability between samples. The above comparisons indicate that bacterial colonization of freshwater DOP during spring are highly related to the nature of the particle.

Bacteria attached to nano-particles averaged 1.9 and 6.0% of total bacteria in Lake Pavin and Lake Aydat, respectively, corresponding to about 10^7–10^8 bacteria L^{-1} associated with nano-sized detrital organic particles. Our estimated values of TEP-associated bacteria (average 0.3 and 0.8% of total bacterial abundance in Lake Pavin and Lake Aydat, respectively) are at the lower end of the values (0–20%) recorded in marine systems (Passow and Alldredge 1994; Schuster and Herndl 1995; Mari and Kiørboe 1996). Our study was, however, limited to spring, a period of low abundance of attached bacteria in Lake Aydat compared to the summer period (Marvalin et al. 1989). As noted by Passow and Alldredge (1994), it is obvious that studies that have not considered bacteria attached to nonliving organic particles have underestimated the relative contribution of attached cells in the plankton. Because of differences in particle abundance, the majority of bacteria attached to nano-particles (86.5 and

Table 4. Average values (±SD) of the constants a and b from the equation $n = ad^b$, where n is the number of bacteria per particle and d is the equivalent spherical diameter of the target particle. The constants were determined on each sampling date and depth by regressions between n and d. According to the lake and the particle type, mean values were calculated from different numbers of significant regressions as indicated in parenthesis.

	DYP		TEP		CSP	
	a	b	a	b	a	b
Lake Aydat	0.138±0.106(6)	2.207±0.504	0.537±0.115(9)	1.280±0.130	0.688±0.519(7)	1.225±0.394
Lake Pavin	0.065±0.021 (8)	2.296±0.360	0.591±0.334(9)	1.318±0.203	0.371±0.218(9)	1.452±0.359

68.4% in Lakes Aydat and Pavin, respectively) was associated with DYP (Table 2). Based on all samples from Lake Pavin and Lake Aydat, the abundance of bacteria associated with each of the three DOP types increased significantly with the total abundance of bacteria and with the total surface area of the respective particles ($0.651 <$ Pearson's $r < 0.962$, $P < 0.005$). We conclude that bacterial colonization of freshwater DOP is a function of the nature of the particle and of lake trophy.

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