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Alcian Blue-stained particles in a eutrophic lake

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Abstract. We used a neutral solution of Alcian Blue to stain transparent particles in eutrophic Lake Frederiksborg Slotssø, Denmark. Alcian Blue-stained particles (ABSP) appeared to be similar to the so-called transparent exopolymer particles (TEP) identified with an acidic solution of Alcian Blue. Our results on the abundance, size distribution and bacterial colonization of ABSP therefore reflect general patterns of TEP. The abundance of ABSP in the size range 3–162 µm and retained by 3 µm pore size filters averaged $3.6 \pm 2.49 \times 10^5 \text{ ml}^{-1}$ (\pm SD), which is among the highest concentrations reported for comparable size spectra of TEP. On average, 35% of ABSP (by number) were colonized by bacteria and 8.6×10^5 bacteria ml^{-1} lake water were attached to ABSP, which corresponds to 7% of the total bacterial abundance.

Introduction

In recent years, high abundance of transparent microscopic particles in aquatic systems has been reported, whether identified as (i) transparent exopolymer particles (TEP) stained with polysaccharide-specific Alcian Blue (Alldredge *et al.*, 1993), (ii) DAPI Yellow Particles (DYP) stained with 4',6-diamidino-2-phenylindole (DAPI) (Mostajir *et al.*, 1995) or (iii) Coomassie Stained Particles (CSP) known to contain protein (Long and Azam, 1996). At present, most knowledge about transparent microscopic particles concerns TEP in marine systems.

TEP are usually between 1 µm and 1 mm in length, and formed through coagulation and aggregation of dissolved and particulate matter. Being sticky and often abundantly present in the water column, TEP contribute to glue together smaller particles into larger aggregates known as marine or lake snow (Kiørboe and Hansen, 1993; Passow *et al.*, 1994; Logan *et al.*, 1995). TEP thereby influence the biological and physical dynamics of aquatic systems, as the formation of larger particles is a prerequisite for rapid sedimentation (Stokes Law), for instance when diatom blooms collapse (Passow *et al.*, 1994; Logan *et al.*, 1995). TEP constitute a potential food resource for bacteria, in particular those attached (Kepkay, 1994; Passow and Alldredge, 1995a; Smith *et al.*, 1995). Other heterotrophic organisms also feed on microscopic detritus (Biddanda and Pomeroy, 1988; Posch and Arndt, 1996). The distribution of TEP-associated bacteria is reported in a wide range from zero (Schuster and Herndl, 1995) to 69% (Passow *et al.*, 1994) of total bacterial abundance. In general, however, ~5% of the bacterioplankton is associated with TEP (Mari and Kiørboe, 1996).

Here, we present an average estimate of abundance and bacterial colonization of transparent microscopic particles in a eutrophic lake with high levels of particulate and dissolved organic carbon.

Method

During July and August 1995, microscopic transparent particles were studied in the eutrophic Lake Frederiksborg Slotssø, Denmark. The lake is shallow (average depth 3.1 m) and covers an area of 0.2 km² (Andersen and Jacobsen, 1979). Parallel measures of chlorophyll, temperature, particulate and dissolved organic carbon, bacterial biomass and activity during the period are presented elsewhere (Worm and Søndergaard, 1997).

Fourteen samples of surface water from 0 and 0.5 m depth were taken from the middle of the lake and mixed in equal volumes. Double staining with Alcian Blue and the fluorochrome (DAPI) allowed visualization of transparent particles (Alldredge *et al.*, 1993) and bacteria (Porter and Feig, 1980) by transmission and epifluorescence microscopy, respectively.

Volumes of 0.5 ml DAPI-stained samples (Sigma; final concentration 0.1 µg ml⁻¹) were diluted with ~3 ml of 0.2 µm pre-filtered Milli-Q water and filtered onto 3 µm pore size polycarbonate filters (Uni-Pore or Poretics). Particles collected on the 3 µm filters were subsequently stained with a few drops of 0.2 µm pre-filtered Alcian Blue 8 GX (Sigma; final concentration 0.05%). The stock solution of Alcian Blue was not acidified. Alcian Blue-stained particles (ABSP) are, therefore, not strictly identical to TEP, as TEP is stained with an acidic solution of Alcian Blue (Alldredge *et al.*, 1993). In later experiments, we compared the staining procedure for ABSP and TEP, and found that triplicate estimate of ABSP abundance was 44% higher than that of TEP, but the difference was not statistically significant (ANOVA, d.f. = 4, $P = 0.19$), indicating that most ABSP were likely to be TEP. The double-stained samples were prepared according to the filter-transfer-freeze method (Hewes and Holm-Hansen, 1983), except that ABSP were neither mounted in glycerol nor a glycerine gel. In short, filters were placed upside down on microscope slides with a drop (20 µl) of 0.2 µm pre-filtered Milli-Q water. After freezing at -18°C, filters were easily separated from the frozen samples. Subsequently, thawed samples were mounted with cover slides (24 × 32 mm) and analysed microscopically before the water evaporated. The maximum length (range 3.2–162.0 µm) of 66–100 ABSP (average 91) was measured in single microscopic tracks [average length 7.4 ± 5.4 µm (\pm SD), width 81 µm] at 1250× magnification. The number of attached bacteria was counted on each of the first 13–25 ABSP (average 22). ABSP partly inside the track were only scored when crossing the left delimitation of the track. Dependent on orientation, the abundance of ABSP sized 81–162 µm may be overestimated at maximum 2-fold. To correct this error, counts of ABSP > 81 µm were divided by $[P + 2(1 - P)]$, where $P = \arcsin(81/\text{average length})/(1/2\pi)$ is the probability that ABSP > 81 µm is oriented longitudinally crossing only one delimitation of the track. Abundances of ABSP and bacteria associated with ABSP were estimated without replicates, and quantitative interpretations are therefore confined to the average of 14 samples to minimize errors from stochastic variation. ABSP abundance in blank samples, i.e. 0.2 µm pre-filtered lake water, was insignificant, in agreement with Kiørboe and Hansen (1993) and Mari and Kiørboe (1996), and was therefore excluded from the sampling programme.

The size distribution of particles in aquatic systems is numerically dominated by smaller particles: they become scarcer with increasing size. This relationship has been described by the power law $dn/dl = k \cdot l^{-(\beta + 1)}$ [McCave, 1984; for TEP, see Passow and Alldredge (1994) and Mari and Kiørboe (1996)], where the value of β is positively correlated to the relative contribution of smaller particles. The size spectrum of ABSP was divided into six logarithmically increasing size intervals, i.e. the first interval being half the length of the second, etc. For each size interval, the concentration of ABSP (dn) was normalized to the width of the interval (dl) and depicted versus the arithmetic average length (l). The parameters k and β were estimated by linear regression after logarithmic transformation.

The bacterial colonization of ABSP was highly variable and only one-third of ABSP were colonized by bacteria. To reveal a general pattern, the measures of length and bacterial colonization of ABSP for each of the 14 sampling days were pooled. The average number of bacteria per ABSP was exponentially related to the average length (l) of the respective size classes. The average abundance of bacteria attached to ABSP was estimated from the sum of bacteria within each logarithmically increasing size class, calculated as ABSP abundance multiplied by the number of bacteria per ABSP for the corresponding average particle length (l).

Results

The phytoplankton were dominated by the cyanobacterium *Microcystis* spp. and concentrations of chlorophyll ranged between 30 and 75 $\mu\text{g l}^{-1}$ (Worm and Søndergaard, 1997).

The abundance of ABSP ranged from 0.84 to $8.7 \times 10^5 \text{ ml}^{-1}$ and averaged $3.64 \pm 2.49 \times 10^5 \text{ ml}^{-1}$ (\pm SD). The power law $dn/dl = k \cdot l^{-(\beta + 1)}$ described the size distribution of ABSP with values of r^2 above 0.91, and the parameters k and β averaged $1.92 \pm 1.93 \times 10^6$ and 1.17 ± 0.24 , respectively (Figure 1).

Thirty-five per cent of ABSP were colonized by bacteria. The β value of the size distribution of ABSP used to count the associated bacteria (312 ABSP from 14 samples) averaged 1.21 ± 0.26 and was not statistically different from the average β value of the entire period (t -test of theoretical mean: $P > 0.90$), indicating that ABSP used for bacterial counts reflected the overall relative size distribution (Figure 2). The bacterial colonization of ABSP increased exponentially from the smaller to the larger size classes according to the regression line: (bacteria per ABSP) = $0.040 \cdot l^{1.55}$ (Figure 2; $r^2 = 0.997$). From this relationship and the average length and abundance of ABSP analysed during the sampling period, the average abundance of ABSP–bacteria was $8.6 \times 10^5 \text{ ml}^{-1}$ (Table I).

Discussion

TEP are collected on 0.2 or 0.4 μm pore size filters and identified with an acidic (0.06% acetic acid) solution of Alcian Blue (0.02%) (Alldredge *et al.*, 1993),

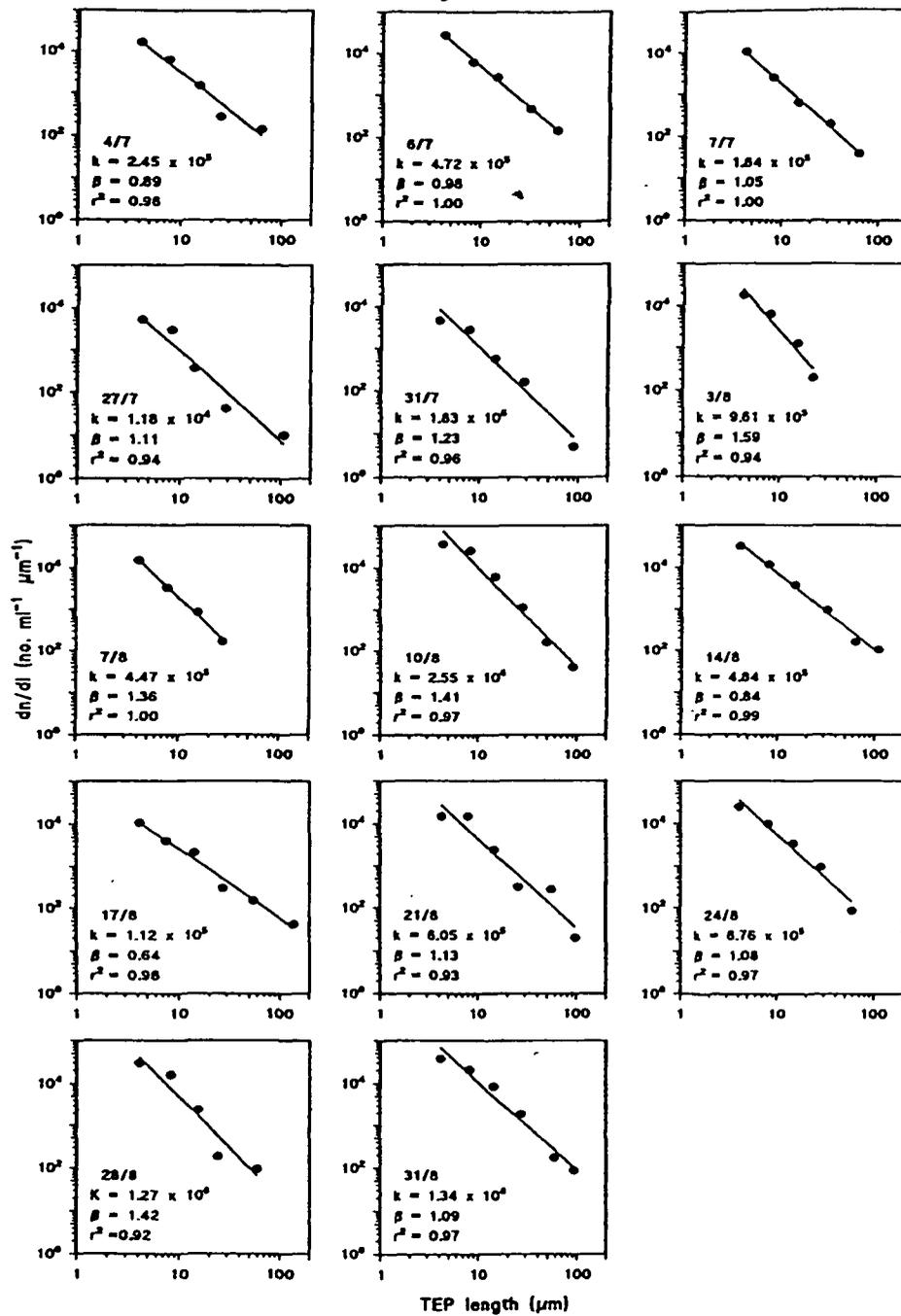


Fig. 1. Size distributions of ABSP in Frederiksborg Slotssø during summer 1995. dn/dl is the ABSP concentration normalized to the width of logarithmically increasing size intervals and l is the average maximum length of the respective ABSP. Regression line: $dn/dl = k \cdot l^{(\beta + 1)}$ (see the text for explanation).

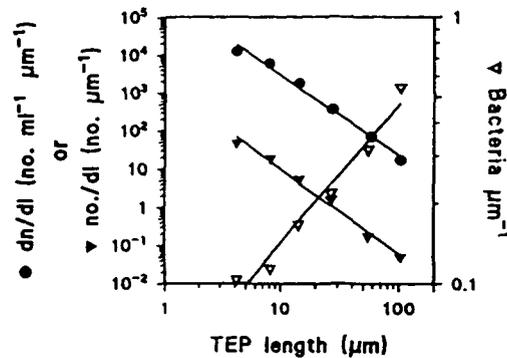


Fig. 2. The average size distribution of ABCP during the entire sampling period (solid circles) and the size distribution of ABCP used to count bacteria associated with ABCP (solid triangles). Parallel lines of normalized ABCP abundance (dn/dl and $no./dl$) versus particle length indicate that the relative size distributions were similar. dn/dl is ABCP concentration, $no./dl$ is ABCP number normalized to the width of logarithmically increasing size intervals and l is the average maximum length of the respective ABCP. The average number of bacteria per ABCP is depicted by open triangles. Regression line: bacteria per ABCP = $0.040 \cdot l^{-.55}$ ($r^2 = 0.997$).

whereas we used 3 μm pore size filters and stained with a neutral solution of Alcian Blue (0.05%) to identify ABCP. Despite the different staining procedure, we expect that most ABCP were identical to TEP because: (i) Alcian Blue complexes specifically with dissociated carboxyl and sulphate groups of acidic mucopolysaccharides (Parker and Diboll, 1966) and an increase in pH increases the number of binding sites already present on particle surfaces; (ii) 9.5% alcoholic solution of Alcian Blue also stains acidic polysaccharides (Murray *et al.*, 1994), indicating no change in specificity at neutrality. In support of these assumptions, triplicate counts of ABCP and TEP were not significantly different (ANOVA, d.f. = 4, $P = 0.19$), although ABCP on average were 44% more abundant than TEP. However, our estimate of ABCP abundance is conservative relative to previous findings of TEP because filters with ~ 10 -fold wider pore sizes were used to retain the fragile particles (Schuster and Herndl, 1995). In spite of this reservation, the concentration of transparent particles in the present study is among the highest reported and exceeds the level of marine TEP by at least one

Table 1. Average size distribution and bacterial colonization of ABCP specified for logarithmically increasing size intervals, Frederiksborg Slotssø, Denmark, July and August 1995

Size interval (μm)	Average length (μm) l	Bacteria per ABCP ($0.040 \cdot l^{-.55}$) A	No. of ABCP ($\times 10^3 \text{ ml}^{-1}$) B	No. of ABCP–bacteria ($\times 10^3 \text{ ml}^{-1}$) A \times B
3.2–5.8	4.16	0.36	131.9	47.5
5.8–10.8	8.04	1.01	123.6	124.8
10.8–20.9	14.77	2.6	68.6	178.4
20.9–41.0	27.67	6.87	26.8	184.1
41.0–81.4	53.06	18.86	10.5	198.0
81.4–162.0	90.26	42.96	3.0	128.9
Sum			364.4	861.7

order of magnitude. ABSP (of size 3.2–162 μm) averaged $3.6 \times 10^5 \text{ ml}^{-1}$ in Frederiksborg Slotssø during summer 1995. In comparison, TEP and clay particles ($>1 \mu\text{m}$) ranged from 0.5 to $4 \times 10^5 \text{ ml}^{-1}$ in the River Danube (Berger *et al.*, 1996) and the peak concentration of TEP $> 5 \mu\text{m}$ was 860 ml^{-1} during a diatom spring bloom in mesotrophic Lake Constance, Germany (Logan *et al.*, 1995). In marine systems, the abundance of TEP is reported to range from $<10^0$ to 10^4 ml^{-1} in the size spectrum of maximum length $>3 \mu\text{m}$ (Passow and Alldredge, 1994) or $>1 \mu\text{m}$ (Mari and Kiørboe, 1996). Reports on other classes of transparent particles include that DYP of size 2–15 μm ranged from 10^2 to 10^3 ml^{-1} in the Mediterranean (Mostajir *et al.*, 1995) and CSP between a few and several hundred micrometres ranged from 10^3 to 10^5 ml^{-1} in coastal water (Long and Azam, 1996).

The high concentration of transparent particles is presumably related to the eutrophic status of the studied lake, but such empirical information is not yet available for lakes. Dissolved and particulate organic matter, which are the building blocks of TEP, usually increase in concentration with the system productivity (Søndergaard and Middelboe, 1995). Accordingly, the abundance of TEP tended to increase along a productivity gradient in the Adriatic Sea (Schuster and Herndl, 1995) and the concentration of TEP was positively correlated to chlorophyll in the Santa Barbara Channel (Passow and Alldredge, 1995b). In addition, low salinity increases electrostatic repulsion between particles of equal charge (Fletcher, 1991). Other things being equal, the stickiness of freshwater particles is expected to be lower, which in turn reduces the probability that smaller particles aggregate into larger sized particles of less abundance (Kepkay, 1994). In agreement with this hypothesis, TEP coagulated more slowly in a freshwater lake than in coastal water (Logan *et al.*, 1995).

A systematic difference in particle stickiness should be reflected as higher β values in freshwater compared to more saline systems if aggregation controls the size distribution. However, a 10-fold difference in filter pore sizes is a more likely explanation for our β value of 1.2 being lower than average β values of 1.6 and 2.3 (Passow and Alldredge, 1994) and 2.3 (Mari and Kiørboe, 1996) estimated in two marine studies of TEP.

The average abundance of 8.6×10^5 bacteria attached to ABSP per millilitre (Table I) represents 7% of total bacterial abundance. In a parallel study, 20 μm pore size nets retained 8% of total bacterial abundance, operationally defined as bacteria attached to the cyanobacterium *Microcystis* spp. (Worm and Søndergaard, 1997). If most ABSP were forced through the 20 μm nets, because of high water velocity during filtration, we have a crude estimate of the bacterial distribution between particles and the surrounding water. Accordingly, free-living bacteria contributed 85% ($= 100 - 7 - 8$) of total bacterial abundance. The conclusion that the majority of bacteria were free living, despite the high level of particulate matter, seems valid. Although ABSP were at least one order of magnitude more abundant than in less productive marine systems, the contribution of ABSP-bacteria was similar. About 5% of total bacterial abundance is in general associated with TEP in marine systems (Mari and Kiørboe, 1996). The significance of ABSP as a microhabitat for attached bacteria in eutrophic freshwater therefore compares to that of TEP in marine systems.

In conclusion, transparent particles in the eutrophic Frederiksborg Slotssø in July and August were at least one order of magnitude more abundant than TEP in marine systems. Despite this marked difference, a similar small fraction (7%) of the total bacterial community colonized the particles.

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