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Microbial dynamics on diatom aggregates in Øresund, Denmark

Hans Peter Grossart[1,*], Susanna Hietanen[2], Helle Ploug[3,4]

[1]Institute of Freshwater Ecology and Inland Fisheries (IGB), Dept. of Stratified Lakes, Alte Fischerhütte 2, 16775 Neuglobsow, Germany

[2]Finnish Institute of Marine Research, PO Box 33, 00931 Helsinki, Finland

[3]Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark

[4]*Present address:* Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany

ABSTRACT: Size, chemical composition, bacteria, flagellate, and ciliate abundance, bacterial production and growth rates as well as community respiration rates were measured on natural diatom aggregates of different sizes collected by SCUBA divers on 5 subsequent days offshore from northern Zealand, Denmark. Aggregate size was highly variable (0.16 to 524 mm^3) throughout the sampling period, whereas aggregate (agg.) dry mass (70 to 390 µg $agg.^{-1}$) and organic carbon content (48 to 130 µg $agg.^{-1}$) varied less. The composition of particulate organic amino acids on aggregates was very different from that of total dissolved amino acids in the matrix water of the aggregates. Bacteria, flagellates, and ciliates were 10- to 10 000-fold enriched on aggregates compared to their abundance in the surrounding water. Enrichment factors of bacteria, flagellates, and ciliates decreased significantly with increasing aggregate size. Ciliates showed the highest and flagellates the lowest enrichment on aggregates. Absolute rate of bacterial production was significantly correlated with aggregate size, and it constantly increased on aggregates of similar size throughout the sampling period. Cell-specific production rates were consistently higher for aggregate-associated bacteria than for free-living bacteria, and 3 to 20% of all bacteria were produced on aggregates. High release of dissolved organic matter and bacteria into the surrounding water was indicated by the release of amino acids into the surrounding water and high calculated detachment rates of aggregate-associated bacteria. Marine snow, thus, should be regarded as comprising integral components rather than isolated microenvironments of the pelagic zone. Its nature has important consequences for organic matter cycling from small scales up to the global scale.

KEY WORDS: Aggregates · Bacteria · Heterotrophic nanoflagellates · Bacterial production · Respiration · Grazing · C:N ratio · Particulate combined amino acids

INTRODUCTION

Macroscopic organic aggregates (>0.5 mm in diameter, marine snow) comprise a significant fraction of the mass flux of organic matter in the ocean (Fowler & Knauer 1986, Alldredge & Silver 1988, Turley & Mackie 1994). Hence, marine snow is important for the export of energy, particulate organic matter, and nutrients from the euphotic zone to the sediments. Marine snow also forms important microenvironments for degradation and remineralization of particulate organic matter by heterotrophic bacteria and other microorganisms (Alldredge & Silver 1988, Grossart & Ploug 2001, Simon et al. 2002). These microenvironments are characterized by high concentrations of nutrients and organic matter (Shanks & Trent 1979), which lead to high numbers and activities of aggregate-associated bacteria, flagellates, and ciliates (Silver & Alldredge 1981, Caron et al. 1982, Alldredge & Gotschalk 1989).

*Email: hgrossart@igb-berlin.de

The major pathways of transformation of particulate organic matter by heterotrophic microorganisms are ecto-enzymatic hydrolysis, respiration, and production of new biomass. Recently, simultaneous measurements of respiration and production of aggregate-associated bacteria on laboratory-made aggregates have shown high apparent growth efficiencies (~0.5) throughout early bacterial colonization and decreasing apparent growth efficiencies on aging detritus aggregates (Grossart & Ploug 2001). On average, bacterial growth efficiencies on 1 to 3 d old detritus aggregates (Grossart & Ploug 2000) are almost the same as those of free-living bacteria growing on organic carbon freshly excreted by phytoplankton (Del Giorgio & Cole 1998). Increasing fractions of hard-to-degrade organic matter presumably cause decreasing growth efficiencies of heterotrophic bacteria on aggregates over time.

It is of major interest to better quantify and characterize the organic matter on aggregates of different sizes to better understand processes such as coagulation and vertical fluxes in the ocean (Honjo et al. 1984, Asper et al. 1992, Alldredge 1998). The extent to which microbial decomposition and grazing are also linked to the aggregate size spectrum has been less studied. It has been shown that there is a distinct relation between particle size and dry mass, particulate organic carbon and nitrogen content of natural marine snow (Alldredge 1998). Ploug & Grossart (2000) found similar relationships between these parameters and microbial metabolism on aggregates formed from freeze-thawed diatoms.

In the present study we collected individual diatom aggregates by SCUBA diving for size measurements and determination of chemical as well as microbial parameters. Our results show that various microorganisms on these aggregates are highly enriched and that their numbers, bacterial production, and community respiration are highly dependent on aggregate size. However, size-dependency of bacterial production rates and volume-specific dry mass were different on each sampling date, indicating specific changes in aggregate features over time.

MATERIALS AND METHODS

Natural diatom aggregates (50 to 100) were collected daily from 15 to 19 April 2000 by SCUBA within the euphotic zone (5 to 15 m depth) in acid-cleaned glass jars (120 ml, Wheaton) ca. 200 m offshore of Nakkehoved Lighthouse, northern Zealand, Denmark. Numbers of aggregates were counted visually by using a wire cube which had a volume of 1 l. Aggregates were collected in the morning (08:00 to 10:00 h) and remained in glass jars in a cooling box for <1 h until further analysis in the laboratory. The composition of algae on aggregates was determined after careful aggregate disruption in an Utermöhl microscope at 1000× magnification. Water temperature increased from 4 to 8°C throughout the sampling period whereas salinity remained constant at 10‰.

Enumeration of bacteria and protozoa. Bacteria on aggregates and in the surrounding water were counted immediately after 4′6′ diamidino-2-phenolindole (DAPI) staining by epifluorescence microscopy (Porter & Feig 1980). Attached bacteria were removed from aggregates by using ultrasonication in 2 mM Na-pyrophosphate (Velji & Albright 1986) prior to filtration onto 0.2 µm Nuclepore membranes. Flagellates and ciliates were counted on unfixed DAPI-stained and filtered samples. At least 3 aggregates of known sizes were examined each day for both bacterial and protozoan colonization.

Size measurements of aggregates. Single aggregates were kept in suspension above a net in a vertical flow system by an upward directed flow which was adjusted to oppose their sinking velocities (Ploug & Jørgensen 1999). Dimensions of all aggregates were measured in the flow system using a dissection microscope with a calibrated ocular micrometer. The volume was calculated as ellipsoids: $V = 4\,\pi abc/3$, where a, b, and c are the half-axes in each dimension (Spiegel 1968).

Community respiration in surrounding water and on aggregates. Community respiration was measured using Winkler titration. Seawater or individual aggregates were transferred into 11 ml glass vials which were filled with GF/F (Whatman) pre-filtered seawater when measuring respiration on aggregates (Ploug et al. 2002). The vials were fixed in containers on a roller table and rotated around their shortest axis (∅ 2.0 cm) in order to let the aggregates sink during the incubations; 5 replicate samples were used for zero-time oxygen measurements and 5 for measuring the bulk respiration rate during 20 h incubation in the dark at close to *in situ* temperature (7°C). Respiration was calculated from changes in oxygen concentration over time. In order to measure the time course of respiration rates, some of the aggregates collected on 16 April were kept in rotating vials in darkness for 3 d, after which the respiration was measured using a 20 h incubation.

Bacterial production. Bacterial production (BPP) was measured by incorporation of [^{14}C] leucine (^{14}C-leu; Kirchman et al. 1985, Simon & Azam 1989) and bacterial growth rate by incorporation of [^{3}H] thymidine (^{3}H-TdR; Fuhrman & Azam 1980) into the ice-cold trichloroacetic acid precipitate using the dual label approach (Chin-Leo & Kirchman 1988). The radiotracers (^{3}H-TdR [75 Ci mmol^{-1}] and ^{14}C-leu [312 mCi

mmol^{-1}], Amersham) were added to 3 samples and a formalin-killed control. All samples were incubated at close to *in situ* temperature (7°C) for 1 h in the dark. Individual aggregates were incubated in test tubes filled with 10 ml of sterile filtered seawater and kept in suspension by using a plankton wheel (2.5 rpm). Final concentrations of both radiotracers were 120 nM to ensure saturation of the respective uptake systems. Growth rates were calculated assuming an isotope dilution factor of 4 and a conversion factor of 2×10^{18} cells mol^{-1} ^{3}H-TdR (Grossart & Simon 1993). Bacterial production was determined from ^{14}C-leu incorporation using the intracellular isotope dilution factor of 2 (Simon & Azam 1989) and from ^{3}H-TdR using a specific carbon content of 0.3 pg C µm^{-3} and cell size of 0.01 and 0.5 µm^3 for free-living and attached bacteria, respectively.

To test whether BPP in the surrounding water was stimulated in the presence of aggregates, we measured BPP in particle-free seawater and seawater samples including single aggregates. For BPP measurements, all aggregates were removed after incubation with the radio label and prior to filtration of the free-living bacteria onto 0.2 µm cellulose nitrate filters (Sartorius). Hence, the BPP of free-living bacteria in the absence or presence of macroscopic aggregates could be measured.

Apparent net (minimum) growth efficiencies. Apparent net growth efficiencies were calculated by: BPP/(BPP + community respiration). Our calculations of apparent net growth efficiencies are minimum values since community respiration does not only include bacterial respiration but also respiration of all other organisms on the aggregate.

Hydrolytic enzyme activities. Aminopeptidase and β-glucosidase activities of attached and free-living bacteria were measured using l-leucine-methyl coumarinyl amide and methyl-umbelliferyl-β-D-glucoside as substrate analogs (Hoppe 1983). Five ml of sample (seawater or sterile-filtered seawater plus individual aggregates) were incubated in rotating vials at *in situ* temperature in the dark. Final concentrations of substrate analogs were 500 µM, which assured maximum hydrolysis as determined by saturation kinetics. Samples killed with paraform-aldehyde (4% final conc.) served as controls. Both fluorochroms were excited at 300 to 400 nm and their emission was measured at 410 to 610 nm in a fluorometer (TD 700, Turner Design). Activities of aminopeptidase and β-glucosidase of bacteria on aggregates and in the surrounding water were calculated after calibration with methyl-coumarinylamide and methyl-umbelliferyl, respectively.

Analysis of amino acids. Total dissolved amino acids (TDAA) in the surrounding water and in the matrix water as well as particulate combined amino acids (PCAA) on individual aggregates were measured by high performance liquid chromatography after precolumn derivatization with *o*-phthaldialdehyde. After pre-filtration of seawater samples or individual aggregates through 0.2 µm tuffrin filters (Gelman Acrodisc; low protein-binding capacity) hydrolysis of TDAA in both the surrounding water and the matrix water of the aggregates was done in double-distilled 6 N HCl for 20 h at 110°C. In contrast, for hydrolysis of PCAA the HCl was directly added to the aggregates. Amino acid oxidation due to possible high nitrate concentration was prevented by adding 20 µl of ascorbic acid (2 mg ml^{-1}) prior to hydrolysis.

CN analysis. The particulate organic carbon (POC) and nitrogen (PON) content of aggregates were determined by CHN analysis (EA1112, Thermo Finnigan) after determination of aggregate size and respiration rate. The aggregates were then transferred to tin cups, lyophilized overnight, and weighed on a microbalance before CN analysis.

RESULTS

Characterization of aggregates

Aggregate size was highly variable throughout the sampling period, with a maximum volume of 523 mm^3 on 16 April and a minimum volume of 0.16 mm^3 on 15 April. Mean volumes of aggregates strongly increased from 15 to 16 April, when the weather was calm and sunny. Thereafter volumes decreased until 19 April (Table 1). Aggregate abundance was fairly constant on all sampling days, ranging between 10 and 15 agg. l^{-1}. Throughout the whole sampling period, aggregates were almost exclusively composed of diatoms (*Skeletonema costatum*, *Chaetoceros* sp., and *Coscinodiscus* sp.) and were rather fragile. The average dry weight was less variable than aggregate volume, ranging between 250 µg agg.$^{-1}$ on 16 April and 110 µg agg.$^{-1}$ on 18 April (Table 1; note that no dry weights were measured on 15 April when aggregates were very small). Total aggregate carbon and nitrogen content on 16 and 18 April varied between 81 and 43 µg C agg.$^{-1}$ and 7.1 and 3.5 µg N agg.$^{-1}$, respectively. C:N ratios (12.4 and 12.3) remained almost the same (Table 1). Hence, aggregates collected on 18 April were more compact than those collected 3 d earlier.

The amount of PCAA per aggregate was much higher on 16 April (24.5 µg agg.$^{-1}$) than on 18 April (0.9 µg agg.$^{-1}$). The TDAA concentration in the matrix water of the aggregates strongly increased during this time (from 14 to 74 µM), suggesting an increase in proteolytic activity and degradation of the PCAA on the aggregates (Table 1). The mole percent (mol%) com-

Table 1. Average size, dry weight, carbon and nitrogen content, C:N ratio, and particulate combined (PCAA) and total dissolved amino acids (TDAA) on diatom aggregates (agg.) and in their matrix water (n = 3–9). nd: not determined

Date	Size (mm^3)	Dry wt (µg $agg.^{-1}$)	Carbon (µg $agg.^{-1}$)	Nitrogen (µg $agg.^{-1}$)	C:N ratio	PCAA (µg $agg.^{-1}$)	TDAA (µM)
15 Apr	0.6 ± 0.4	nd	nd	nd	nd	nd	nd
16 Apr	247.0 ± 199.0	250 ± 140	81 ± 24	7.1 ± 2.7	12.4 ± 4.2	24.5 ± 14.0	14.0 ± 11.0
17 Apr	24.6 ± 18.2	200 ± 70	nd	nd	nd	2.6 ± 1.2	3.6 ± 0.4
18 Apr	3.4 ± 2.4	110 ± 40	43 ± 6	3.5 ± 0.1	12.3 ± 1.5	0.9 ± 0.3	74.0 ± 43.0
19 Apr	3.4 ± 4.6	nd	nd	nd	nd	nd	nd

position of PCAA on aggregates and TDAA in the matrix water of the aggregates were different from each other. PCAA were characterized by an extremely high mol% of aspartate but a relatively low mol% of glutamate, serine, and arginine compared to TDAA (Fig. 1). There was a pronounced decrease in the mol% of aspartate and an increase in glycine/threonine, alanine, and isoleucine of PCAA on aggregates collected between 16 and 18 April. For TDAA in the matrix water of aggregates, however, the mol% of aspartate and glutamate increased whereas that of serine, glycine/threonine, and leucine decreased at the same time.

Microbial colonization

Bacterial abundance on aggregates varied between 0.1×10^9 and 37.0×10^9 bacteria (ml agg.)$^{-1}$. The numbers of heterotrophic flagellates and ciliates ranged between 1.1×10^6 and 9.0×10^6 flagellates (ml agg.)$^{-1}$ and 0.1×10^4 and 15.2×10^4 ciliates (ml agg.)$^{-1}$, respectively. The abundance of neither bacteria, flagellates nor ciliates showed a clear trend over time, but their abundance was clearly correlated to aggregate size, with highest volume-specific abundances for all organisms occurring on the smallest aggregates (Fig. 2). The abundance of free-living bacteria increased from 0.5 to 0.7×10^6 cells ml^{-1} between 15 and 19 April, whereas that of flagellates decreased between 17 and 19 April from 7.1 to 1.2×10^3 cells ml^{-1} and that of ciliates from 76 to 31 cells ml^{-1}. Enrichment factors of these organisms on aggregates of various sizes were calculated from their volume-specific abundance on aggregates relative to that in the surrounding water. Interestingly, enrichment factors for all organisms were highest on the smallest aggregates, with highest enrichment factors for ciliates and lowest for flagellates (Fig. 3). The size-specific abundance of flagellates showed the strongest decrease with increasing aggregate size, whereas that of ciliates decreased less.

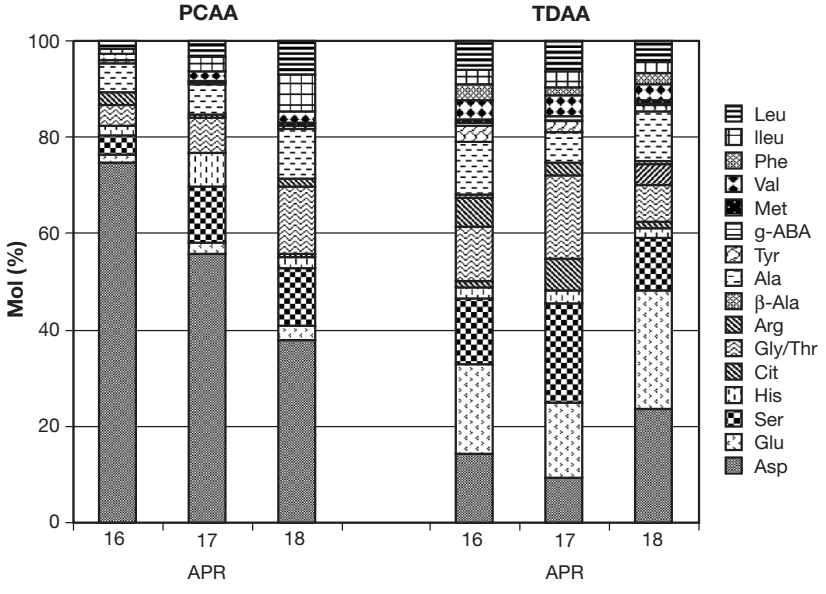

Fig. 1. Mole percentage composition of particulate combined (PCAA) and total dissolved amino acids (TDAA) in the matrix water of diatom aggregates collected in Øresund, Denmark, April 2000

The grazing rates of protists were calculated using the relationships between bacteria and protozoa abundance to estimate grazing rates of protozoa (Artolozaga et al. 2002). The average cell-specific grazing rate of protists was 2.8 ± 1.9 bacteria flagellate^{-1} h^{-1} and 27 bacteria ciliate^{-1} h^{-1} for attached bacteria; and 1.3 ± 0.1 bacteria flagellate^{-1} h^{-1} and 43 bacteria ciliate^{-1} h^{-1} for free-living bacteria. The calculated grazing rates varied between single aggregates (Fig. 4a). Bacteria and protozoa were more enriched on smaller aggregates, and therefore a larger fraction of the bacterial community on smaller aggregates (>50% d^{-1}) was grazed than on larger aggregates (10 to 30% d^{-1}, Fig. 4b). The average fraction of bacteria grazed on aggregates was 29 ± 15%

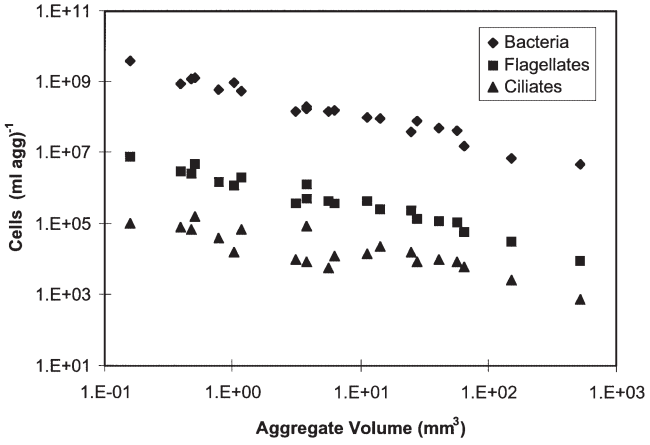

Fig. 2. Size-dependent colonization of diatom aggregates by bacteria, flagellates, and ciliates in Øresund, Denmark, April 2000

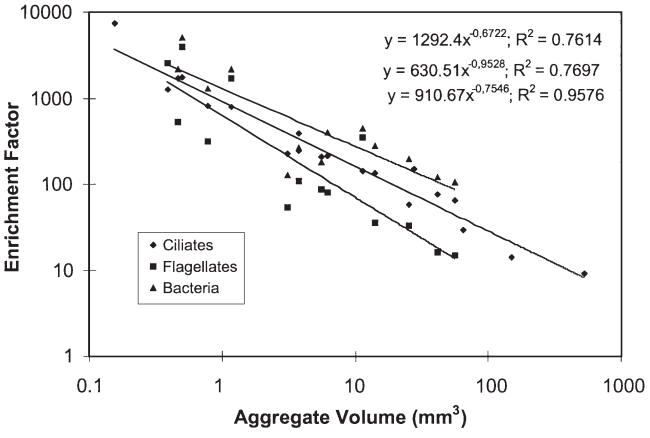

Fig. 3. Size-dependency of enrichment factors of bacteria, flagellates, and ciliates on diatom aggregates in Øresund, Denmark, April 2000. Enrichment factors of these organisms on aggregates of various sizes were calculated from their volume-specific abundance on aggregates relative to that in the surrounding water

d^{-1}, whereas the fraction of free-living bacteria grazed was 27 ± 18% d^{-1}. The average grazing pressure, thus, appeared to be similar on attached and free-living bacteria.

Metabolic activities

BPP, bacterial thymidine incorporation rate, and total respiration (algal and microbial dark respiration) on diatom aggregates and in the surrounding water varied greatly over time (Table 2). Bacterial production rates on aggregates were correlated with aggregate volume on each sampling date, and volume-specific rates greatly increased throughout the sampling period (Fig. 5). Higher volume-specific bacterial production rates occurred in parallel to higher cell-specific growth of aggregate-associated bacteria and decreasing aggregate size. Cell-specific bacterial production was on average 3.1 times higher on aggregates than in the surrounding water, and it was 6.8 times higher on the last sampling day (Table 2). Comparison of BPP calculated by ^{14}C leucine and ^{3}H thymidine incorporation was in the same order of magnitude when using cell volumes of 0.01 and 0.5 µm^3 for free-living and attached bacteria, respectively. However, the BPP of free-living bacteria calculated by ^{3}H thymidine incorporation on 18 April was 7.2 times lower than that calculated by ^{14}C leucine, indicating that cell size may have been larger on that date. In contrast, the BPP of attached bacteria calculated by ^{3}H thymidine was 2 to 19 times higher than that calculated by ^{14}C leucine, suggesting that the assumed volume of attached cells was overestimated to a varying degree. Overestimation was highest on 16 April (19 times) and lowest on 18 and 19 April (2 times).

Cell-specific cell multiplication was on average 5.7 times higher on aggregates than in the bulk water, and it was highest (9.9 times) on 17 April when community respiration was also high. Calculated apparent growth

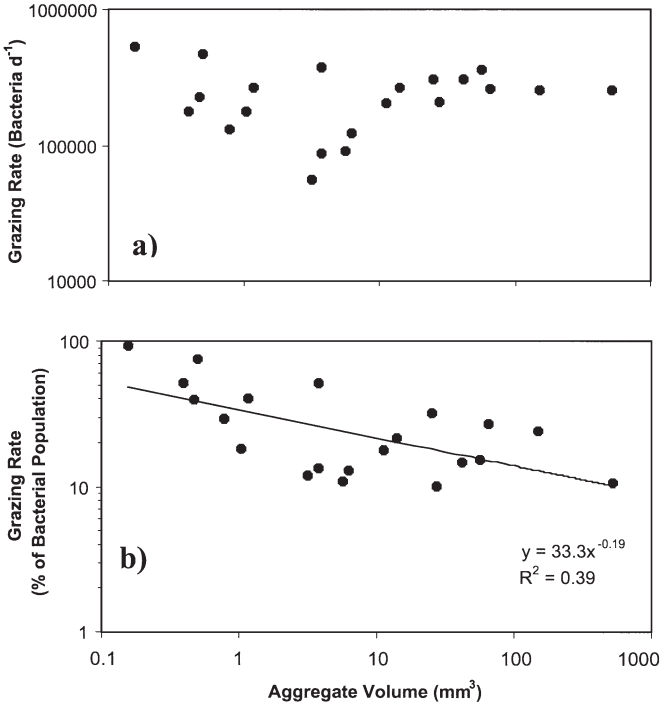

Fig. 4. Size-dependency of calculated protozoan grazing rates on diatom aggregates in Øresund, Denmark, April 2000. (a) Bacteria d^{-1}, (b) percent of total bacterial population

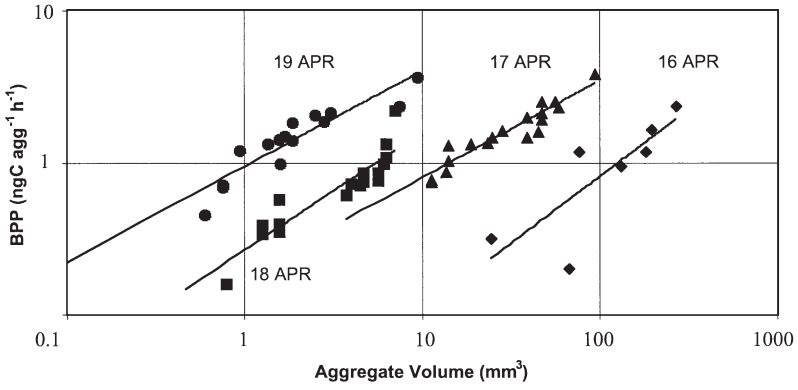

Fig. 5. Size-dependency of bacterial production (BPP) on diatom aggregates in Øresund, Denmark, April 2000. Correlation parameters are given in Table 3

efficiencies of bacteria on aggregates and in the surrounding water were on average 0.013 and 0.154, respectively. Low apparent growth efficiencies of bacteria associated with diatom aggregates were presumably explained by dark-respiration of the aggregated diatoms. On average, the ratio between community respiration and bacterial production on aggregates was 156, whereas it was only 7 in the surrounding water.

Bacterial abundance and activities (BPP, and bacterial thymidine incorporation rate) on aggregates as a percentage of the total (aggregates and surrounding water) varied up to 6-fold throughout the sampling period, whereas total aggregate volume varied by more than 100-fold at the same time (Fig. 6). Of the total respiration in Øresund surface water, 26 to 40% occurred on diatom aggregates even though these only accounted for a fairly small volume of the water column (10 to 1440 ppm).

Protease activities of aggregate-associated and free-living bacteria were 150 nmol agg.$^{-1}$ h^{-1} and 810 nmol l^{-1} h^{-1}, respectively, on 15 April and 260 nmol agg.$^{-1}$ h^{-1} and 790 nmol l^{-1} h^{-1}, respectively, on 16 April. Cell-specific activities of protease on these dates were 130 and 110 times higher on aggregates than for free-living bacteria. Activities of β-glucosidase of attached and free-living bacteria were 15 nmol agg.$^{-1}$ h^{-1} and 31 nmol l^{-1} h^{-1}, respectively, on 15 April and 2 nmol agg.$^{-1}$ h^{-1} and 36 nmol l^{-1} h^{-1}, respectively, on 16 April. Cell-specific activities of β-glucosidase were 360 and 180 times higher on aggregates than in the surrounding water. The ratio between protease and β-glucosidase activity on aggregates was 9.5 and 13.1 on 15 and 16 April, respectively, whereas it was 27 and 21.6 in the surrounding water, indicating increased hydrolysis of β-glycosidic polymers on aggregates compared to the surrounding water.

DISCUSSION

Our study shows that not only chemical parameters are related to particle size of natural diatom aggregates, but also biological parameters such as bacterial production (Table 3). However, size-dependency of both chemical and biological parameters changed between sampling dates presumably depending on *in situ* temperature, and the age and substrate quality of the aggregates (Table 1).

Chemical composition

Aggregate dry mass (corrected for salt concentration) was relatively high compared to that given by Alldredge (1998) for diatom aggregates in the Southern California Bight. Size-specific dry mass in the present study was much higher for smaller aggregates at the

Table 2. Average size (cumulated aggregate volume, Agg. vol), bacterial production (BPP), thymidine incorporation (Thy. inc.), and community respiration (Resp) on aggregates and in surrounding water

Date	Agg. vol (ppm)	Aggregate				Surrounding water			
		Cell-specific		Per liter		Cell-specific		Per liter	
		BPP (fg C h^{-1})	Thy. inc. (10^{-21} mol h^{-1})	BPP[a] (ng C l^{-1} h^{-1})	Resp[a] (ng C l^{-1} h^{-1})	BPP (fg C h^{-1})	Thy. Inc. (10^{-21} mol h^{-1})	BPP (ng C l^{-1} h^{-1})	Resp. (ng C l^{-1} h^{-1})
15 Apr	10	0.85	34	6.6	540	0.33	5.3	165	800
16 Apr	1440	0.75	57	11.3	1790	0.41	13.8	204	2990
17 Apr	322	1.13	61	17.7	1370	0.43	6.2	273	2290
18 Apr	302	1.27	133	7.6	950	0.68	32.0	483	1600
19 Apr	248	2.16	136	15.6	480	0.18	32.7	131	1340

[a]Parameters calculated using minimum abundance (10 agg. l^{-1}); thus all these calculated values are conservative

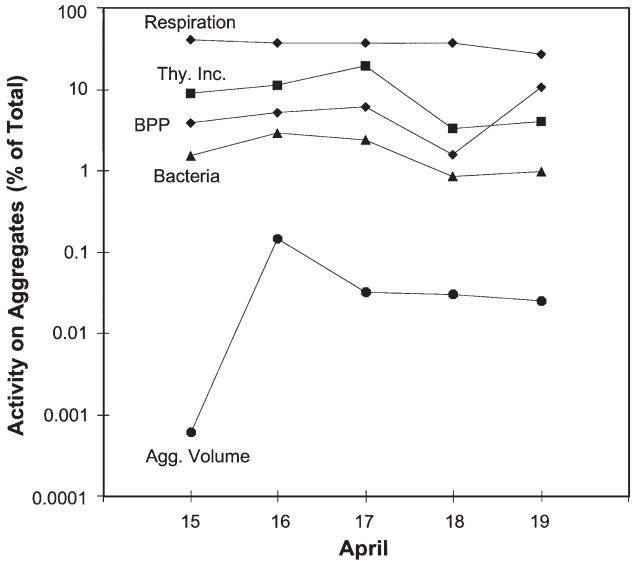

Fig. 6. Average aggregate volume (Agg. Volume), bacterial abundance (Bacteria), bacterial production (BPP), bacterial thymidine incorporation rate (Thy. Inc.), and respiration on diatom aggregates in Øresund, Denmark, April 2000, as percentage of total on aggregates and in surrounding water

end of the sampling period, indicating that these aggregates became more compact. Current velocities of ≥2 nautical miles h^{-1} are not rare in Øresund, whereas wave action is limited owing to the lack of large free stretches. Increased shear may, therefore, lead to more compact particles over time compared to those in other environments characterized by lower shear (Jackson 1994). Aggregates of all sampling dates were characterized by the dominance of *Skeletonema costatum*, *Chaetoceros* sp., and *Coscinodiscus* sp., indicating a similar source and origin.

Particulate organic carbon comprised between 32–39% and nitrogen 2.8–8.1% of total dry mass, similar to results of Alldredge (1998). Volume-specific carbon and nitrogen content increased between 16 and 18 April. In parallel, the percent of PCAA decreased from 9.8–8.2% dry mass. The decrease in PCAA was even more pronounced (12.2 to 8.4%) in terms of carbon. Preferential use of PCAA by aggregate-associated bacteria compared to POC and PON has previously been shown for laboratory-made aggregates (Grossart & Ploug 2001). Bacterial production on our diatom aggregates was significantly correlated to cell-specific production rates. In the present study, cell-specific BPP and growth rates were 1.8- to 10-fold higher on aggregates than in free-living cells. Parallel to decreasing amounts of PCAA, we found increasing concentrations of TDAA in the matrix water of the aggregates, indicating high protein hydrolysis, which compared well with our protease measurements. Substantial differences between PCAA and TDAA as well as aggregate quality at different sampling dates suggest preferential release of acid amino acids such as aspartate and glutamate.

Table 3. Bacterial production rate (BPP, ng C $agg.^{-1}$ h^{-1}) as a function of aggregate volume (mm^3)

Date	BPP	R^2	p	n
16 Apr	$0.014\ (vol)^{0.88}$	0.65	<0.05	7
17 Apr	$0.17\ (vol)^{0.64}$	0.90	<0.01	19
18 Apr	$0.26\ (vol)^{0.78}$	0.84	<0.01	17
19 Apr	$0.93\ (vol)^{0.63}$	0.84	<0.01	15

Colonization pattern

The abundances of aggregate-associated bacteria, flagellates, and ciliates were similar to those found in previous studies (Caron et al. 1982, 1986, Alldredge & Gotschalk 1990, Grossart & Simon 1993, Ploug et al. 1999, Artolozaga et al. 2000, Ploug & Grossart 2000). Relatively stable ratios for all microorganisms on aggregates of different sampling dates indicate a steady state relation between these organisms. The abundance, but also enrichment factors of all aggregate-associated microorganisms were highly size-dependent, with decreasing enrichment factors of ciliates, bacteria, and heterotrophic nanoflagellates with increasing aggregate size (Fig. 3). Higher enrichment factors of ciliates than of flagellates were also found by Caron et al. (1986) and Artolozoga et al. (1997). In an inter-ecosystem comparison, Sanders et al. (1992) found a strong correlation between specific community bacterivory and bacterial production as well as bacterial numbers. Considering the high densities of attached bacteria relative to that in the surrounding water, grazing rates of bacterivorous protists on these have previously been shown to be surprisingly low compared to those on free-living bacteria (Artolozaga et al. 2002). Low grazing rates of aggregate-associated protists, however, may partly be compensated by the higher cell-specific biomass and nutrient content of aggregate-associated microorganisms (Grossart & Simon 1998). Choanoflagellates on aggregates sinking through the water column may also increase their filtration rate of free-living bacteria. However, enrichment factors for flagellates decreased more with increasing aggregate size than for ciliates. The size-specific steady state of bacteria and protozoa abundance on aggregates depends on colonization rate, growth, predation, and detachment of attached bacte-

ria (Kiørboe 2000, Kiørboe et al. 2002). Variable ratios between aggregate-associated microorganisms may reflect changes in bacterial productivity and bacterivory due to changes in aggregate composition as indicated by changes in carbon, nitrogen, and PCAA content (Ploug & Grossart 2000, Grossart & Ploug 2001).

Despite the high production rates, bacterial abundance on aggregates did not change significantly over time. Baty et al. (2001) as well as Kiørboe et al. (2002) found high detachment rates for aggregate-associated bacteria. This is in good agreement with the hypothesis that aggregates act as 'baby machines' (Azam 1998), continuously releasing bacteria into the surrounding water.

Metabolic activities

The respiration rates on the diatom aggregates were ca. 4 times higher than those measured on marine snow of similar size in the Southern California Bight, which contained few phytoplankton cells (Ploug et al. 1999). Community respiration rates on aggregates of the present study were presumably mainly due to the phytoplankton. A decrease in carbon-specific respiration over time (from 0.09 to 0.05 d^{-1} between 16 and 18 April) indicates reduced viability of the aggregated phytoplankton. Aggregates that were kept in darkness from 16 to 19 April in rotating vials also showed a 47% decrease in respiration rate. Previous measurements showed that net bacterial growth efficiencies on detrital aggregates are up to 0.5 ± 0.1 (Grossart & Ploug 2000, 2001). Assuming similar net bacterial growth efficiencies on aggregates in the present study, bacterial respiration would have been in the order of 0.1 to 10 ng C $agg.^{-1}$ h^{-1}. Based on the estimated grazing rates (10 to 100% of the bacterial population) by protozoa, their respiration would at most be in the same range as that of bacteria (Fig. 4).

Cell-specific carbon production and cell multiplication of attached bacteria throughout the sampling period (0.85 to 2.16 fg C $cell^{-1}$ h^{-1} and 34 to 136 × 10^{-21} mol $cell^{-1}$ h^{-1}, respectively) suggest good growth conditions for heterotrophic bacteria. The ratio between protease and β-glucosidase, however, was higher in the surrounding water, suggesting higher availability of β-glycoside-linked polymers on diatom aggregates than in the bulk water. During a diatom bloom in the German Bight, photosynthetic activity and concentration of dissolved neutral sugars were closely related to each other (H. P. Grossart & C. Dürselen unpubl. data). In addition, bacterial production was significantly correlated to the concentration of neutral sugars. High photosynthetic release and high concentrations of organic and inorganic nutrients in the matrix water of the diatom aggregates may cause higher availability of β-glycoside-linked polymers for the attached bacterial community. Higher availability of β-glycoside-linked polymers may explain increased cell-specific bacterial production on diatom aggregates. Hence, diatom aggregates are microenvironments in which photosynthetic exopolymer release and bacterial production can be directly linked to each other.

Interactions between aggregates and surrounding water

Marine snow is an integral component of the pelagic zone. Sinking aggregates scavenge smaller particles, and attached biota may also take up dissolved organic matter and inorganic nutrients from the surrounding water during sinking. Sinking aggregates can thus comprise a significant fraction of the organic matter flux to sediments. The export flux, however, is reduced when dissolved organic matter and inorganic nutrients as well as organisms are released from aggregates into the surrounding water during microbial growth and remineralization processes. Yet, the relative importance of these processes is poorly quantified and understood.

In our study, total community respiration in the water column was greatly influenced by that on aggregates. Increased bacterial production and cell multiplication in the surrounding water occurred after the peak in aggregate volume. On average, 10% (range 3.3 to 20%) of all bacteria were produced on aggregates and 5% (range 1.5 to 10.6%) of total bacterial production was due to attached bacteria, although they comprised only 1% of the total bacterial community. High cell-specific bacterial production and even higher cell-specific hydrolysis rates of attached bacteria as well as increased concentrations of TDAA in the matrix water of the aggregates led to high bacterial growth and to high release of dissolved organic matter into the surrounding water (Smith et al. 1992, Grossart & Simon 1998). Stimulation of BPP in the surrounding water in the presence of aggregates could be directly measured by comparing BPP in the presence and absence of aggregates in the surrounding water sample. On 19 April, the BPP of free-living cells equaled 135 ng C l^{-1} h^{-1} when aggregates were absent, whereas this was 248 ng C l^{-1} h^{-1} in the presence of a single aggregate. The simultaneous measurement of both hydrolytic activities and BPP at the beginning of our study revealed that substrate hydrolysis and uptake by aggregate-associated bacteria were uncoupled and, thus, resulted in increased

release of dissolved organic matter (e.g. amino acids) into the surrounding water.

BPP increased on smaller aggregates and was much higher on the more compact aggregates on 19 April (Fig. 5). In contrast, BPP was lowest on the largest and more fluffy aggregates on 16 April, suggesting that also leakage of dissolved organic matter was high on these aggregates (Ploug et al. 2002). Interstitial solute transport can be further enhanced by feeding activities of protozoans such as filter-feeding ciliates (Glud & Fenchel 1999). Thus, diatom aggregates should be regarded as important sources of organic and inorganic nutrients not only for aggregate-associated but also for free-living microorganisms.

Phytoplankton may greatly benefit from increased nutrient concentrations in the matrix water. Grossart & Simon (1993) found that soluble reactive phosphate is enriched by more than 1000 times in the matrix water of lake snow, leading to improved growth conditions for phytoplankton at the peak of algal blooms when inorganic nutrients in the surrounding water are depleted. High algal biomass on aggregates may result in a high release of dissolved organic matter which stimulates microbial growth.

High concentrations of organic and inorganic nutrients as well as microbial activities on diatom aggregates attract not only microorganisms, but also invertebrate zooplankton (Kiørboe 2000) and fishes (Grossart et al. 1998). Close spatial coupling between photosynthetic polymer release and remineralization of organic nutrients by microbial loop organisms creates conditions favorable for increased bacterial growth, and consequently, bacterivory. However, enhanced cell-specific production rates and high detachment rates of aggregate-associated bacteria lead to a high flux of bacteria into the surrounding water. Even after grazing, the release of bacteria from diatom aggregates matched on average 8.9% of all bacteria produced in the surrounding water, and maximally 22.1% (17 April). High availability of organic polymers such as proteins and β-glycosids on diatom aggregates lead to rapid remineralization and turnover of particulate organic matter, but also to a high release of dissolved organic matter and microorganisms into the surrounding water.

The above mechanisms counteract the export of organic nutrients due to increased sedimentation of aggregated phytoplankton. Overall, diatom aggregates increase the heterogeneity of the organic matter field, which has important consequences for the activity and community structure of aggregate-associated as well as of free-living organisms in the sea. Therefore, relationships between attached and free-living organisms on a small-scale have profound implications for global organic matter fluxes and should be included in ecosystem models.

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LITERATURED CITED

Alldredge AL (1998) The carbon, nitrogen and mass content of marine snow as a function of aggregate size. Deep-Sea Res Part I Oceanogr Res Pap 45:529–541

Alldredge AL, Gotschalk CC (1989) Direct observations of the mass flocculation of diatom blooms: characteristics, settling velocities and formation of diatom aggregates. Deep-Sea Res 36:159–171

Alldredge AL, Gotschalk CC (1990) The relative contribution of marine snow of different origin to biological processes in coastal waters. Cont Shelf Res 20:41–58

Alldredge AL, Gotschalk CC, Silver, MW (1988) Characteristics, dynamics and significance of marine snow. Prog Oceanogr 20:41–82

Artolozaga IE, Santamaria E, Lopez A, Ayo B, Iriberri J (1997) Succesion of bacterivorous protists on laboratory-made marine snow. J Plankton Res 19:1429–1440

Artolozaga IE, Ayo B, Latatu A, Azua I, Unanue M, Iriberri J (2000) Spatial distribution of protists in the presence of macroaggregates in a marine system. FEMS Microbiol Ecol 33:191–196

Artolozaga IE,Valacarcel M, Ayo B, Latatu A, Iriberri J (2002) Grazing rates of bacterivorous protists inhabiting diverse marine planktonic microenvironments. Limnol Oceanogr 47:142–150

Asper VL, Honjo S, Orsi TH (1992) Distribution and transport of marine snow aggregates in the Panama Basin. Deep-Sea Res 39:939–952

Azam F (1998) Microbial control of oceanic carbon flux: the plot thickens. Science 280:694–696

Baty AM, Eastburn CC, Techkarnjanaruk S, Goodman AE, Geesey GG (2001) Spatial and temporal variations in chitinolytic gene expression and bacterial biomass production during chitin degradation. Appl Environ Microbiol 66: 3574–3585

Caron DA, Davis PG, Madi LP, Sieburth JMcN (1982) Heterotrophic bacteria and bacterivorous protozoa in oceanic macroaggregates. Science 218:795–799

Caron DA, Davis PG, Madin LM, Sieburth JMcN (1986) Enrichment of microbial populations in macroaggregates (marine snow) from surface waters of the North Atlantic. J Mar Res 44:543–565

Chin-Leo G, Kirchman DL (1988) Estimating bacterial production in marine waters from simultaneous incorporation of thymidine and leucine. Appl Environ Microbiol 54: 1934–1939

Del Giorgio PA, Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. Annu Rev Ecol Syst 29: 503–541

Fowler SW, Knauer GA (1986) Role of large particles in the transport of elements and organic compounds through the oceanic water column. Prog Oceanogr 16:147–194

Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia,

Antarctica, and California. Appl Environ Microbiol 39: 1085–1095
Glud RN, Fenchel T (1999) The importance of ciliates for interstitial solute transport in benthic communities. Mar Ecol Prog Ser 186:87–93
Grossart HP, Simon M (1993) Limnetic macroscopic organic aggregates (lake snow): occurrence, characteristics, and microbial dynamics in Lake Constance. Limnol Oceanogr 38:532–546
Grossart HP, Ploug H (2000) Bacterial production and growth efficiencies directly measured on single pelagic aggregates. Limnol Oceanogr 45:436–445
Grossart HP, Ploug H (2001) Microbial degradation of organic carbon and nitrogen on diatom aggregates. Limnol Oceanogr 46:267–277
Grossart HP, Simon M (1998) Bacterial colonization and microbial decomposition of limnetic organic aggregates (lake snow). Aquat Microb Ecol 15:127–140
Grossart HP, Berman T, Simon M, Pohlmann K (1998) Occurrence and microbial dynamics of macroscopic organic aggregates (lake snow) in Lake Kinneret, Israel, in fall. Aquat Microb Ecol 14:59–67
Honjo S, Doherty KW, Agrawal YC, Asper VL (1984) Direct optical assessment of large amorphous aggregates (marine snow) in the deeper ocean. Deep-Sea Res 31: 67–76
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
Jackson GA (1994) Particle trajectories in a rotating cylinder: implications for aggregation incubations. Deep-Sea Res 3: 429–437
Kiørboe T (2000) Colonization of marine snow aggregates by invertebrate zooplankton: abundance, scaling, and possible role. Limnol Oceanogr 45:479–484
Kiørboe T, Grossart HP, Ploug H, Tang K (2002) Bacterial colonization of sinking aggregates: mechanisms and rates. Appl Environ Microbiol 68(8):3996–4006
Kirchman DL, K'Nees E, Hodson RE (1985) Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl Environ Microbiol 49:599–607
Ploug H, Grossart HP (2000) Bacterial growth and grazing by protozoa on diatom aggregates: respiratory carbon turnover as a function of aggregate size and sinking velocity. Limnol Oceanogr 45:1467–1475
Ploug H, Jørgensen BB (1999) A net-jet flow system for mass transfer and microelectrode studies in sinking aggregates. Mar Ecol Prog Ser 176:279–290
Ploug H, Grossart HP, Azam F, Jørgensen BB (1999) Photosynthesis, respiration, and carbon turnover in sinking marine snow from surface waters of Southern California Bight: implications for the carbon cycle in the ocean. Mar Ecol Prog Ser 179:1–11
Ploug H, Hietanen S, Kuparinen J (2002) Diffusion and advection within and around sinking, porous diatom aggregates. Limnol Oceanogr 47:1129–1136
Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
Sanders R, Caron DA, Berninger UG (1992) Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar Ecol Prog Ser 86:1–14
Shanks AL, Trent JD (1979) Marine snow: microscale nutrient patches. Limnol Oceanogr 24:850–854
Silver MW, Alldredge AL (1981) Bathypelagic marine snow: deep-sea algal and detrital community. J Mar Res 39: 501–530
Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213
Simon M, Grossart HP, Schweitzer B, Ploug H (2002) Microbial ecology of organic aggregates in aquatic ecosystems. Aquat Microb Ecol 28:175–211
Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic activity on marine aggregates and implications for rapid particle dissolution. Nature 359:139–141
Spiegel MR (1968) Mathematical handbook of formulas and tables. Schaum's outline series. McGraw-Hill, New York
Turley CM, Mackie PJ (1994) Biogeochemical significance of attached and free-living bacteria and the flux of particles in the NE Atlantic Ocean. Mar Ecol Prog Ser 115:191–203
Velji MJ, Albright LJ (1986) Microscopic enumeration of attached marine bacteria of seawater, marine sediment, fecal matter and kelp blade samples following pyrophosphate and ultrasound treatments. Can J Microbiol 32: 121–126

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