The microbial community on aggregates in the Elbe Estuary, Germany

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ABSTRACT: In 1995, an extensive investigation was carried out in the Elbe Estuary in Germany between Cuxhaven and Geesthacht. Special attention was paid to microorganisms, including bacteria, amoebae, ciliates and flagellates, which were dispersed in the water column and associated firmly or loosely with different types of aggregates. The abundance, size and colonization by microorganisms of the aggregates varied in the limnetic, brackish and marine environments. There were differences in the locations of occurrence and abundance at each site, attributable to differences in the physical and chemical conditions. The composition of the aggregates mainly reflected the structure of the plankton community and also the benthic environment. In the upper estuary, aggregates were composed mainly of organic material, and most of the associated material consisted of remnants of the plankton. About 85% of the particles were colonized by bacteria, and 25% by protozoans. Abundances of dispersed bacteria varied between $0.6 \times 10^9$ and $25.5 \times 10^9$ bacteria l$^{-1}$, and dispersed protozoan abundance ranged between $241 \times 10^3$ and $8778 \times 10^3$ l$^{-1}$. Attached bacteria reached concentrations between $0.3 \times 10^6$ and $22.5 \times 10^6$ bacteria l$^{-1}$, while attached protozoans numbered from 98 to 22500 l$^{-1}$. Attached bacterial density accounted for about 75% of total bacterial density during the year. About 90% of the total bacterioplankton in the upper estuary were tightly attached to aggregates, only 40% were similarly attached in the lower estuary. During the whole year, aggregates in the lower parts of the estuary were dominated by mineral particles, and they were not as densely colonized as in the upper part. Dispersed and attached organisms in the Elbe Estuary showed an annual seasonal succession.

KEY WORDS: Elbe - Estuary - Aggregate - Colonization - Attached organisms - Particles - Protozoa - Bacteria

INTRODUCTION

Estuaries are regarded as complex ecosystems, influenced by physical and chemical factors both temporally and spatially. They are among the most productive ecosystems in the world (Mc Cluskey 1989), and the dynamic boundary between fresh and salt water is thought to be an important interface for biological reactions.

The last decade has seen a great deal of attention focussed on bacterial and protozoan plankton (Pomeroy 1974, Williams 1981, Azam et al. 1983). Tintinnid ciliates have attracted a relatively large amount of attention in estuaries (Hedin 1975, Harre 1981, Burkill 1982, Capriulo & Carpenter 1983, Henrot 1983, Sanders 1987, Sime-Ngando et al. 1995). They can be important grazers during the annual cycle (Burkill 1982, Capriulo & Ninivaggi 1982). However, they are usually outnumbered by nanoflagellates (flagellates ≤ 20 μm; NF) and large flagellates (flagellates ≥ 20 μm; LF). Because of methodological difficulties, there have been only a small number of detailed studies devoted to the flagellates of estuaries and other brackish water bodies (Smetacek 1981, Fenchel 1982, Anderson & Sørensen 1986, Hansen 1991).

Large numbers of aggregates, which are fragile, amorphous microscopic particles of different forms and sizes (Grossart & Simon 1983), are a conspicuous feature of the estuarine environment (Eisma 1992). Minerals, detritus and organisms, including bacteria,

In the Elbe Estuary, a few reports about dispersed and associated organisms are available: Böttcher et al. (1995), Fast (1993), Holst (1996), Humann (1996), Wolfstein (1996), Wolfstein & Kies (1995), Zimmermann (1975), Zimmermann & Kausch (1996) and Zimmermann et al. (in press). Most of the aggregated materials are in the turbid zone where the freshwater in the river mixes with seawater (Kies 1995). By providing a substrate for organisms, the aggregates may influence heterotrophic turnover of organic material. Their abundance may be a sign that there is a more complex food web in pelagic waters.

Caron et al. (1982) thought that aggregates are important loci of microbial activity in the water column, similar to the much larger oceanic ‘marine snow’ aggregates. However, the abundance and size of aggregates show considerable seasonal (Kies 1995, Zimmermann & Kausch 1996) and spatial variation (Kies 1995). Giving special consideration to these 2 kinds of variation, this study was conducted to elucidate the microbial colonization of aggregates in the Elbe Estuary.

### MATERIALS AND METHODS

**Sampling.** For an entire year, from January to December 1995, water samples were collected monthly from a boat moving with the tide up the estuary. They were taken each month, during a period of 2 or 3 d, from 8 stations in the Elbe Estuary from Geesthacht to Cuxhaven (Fig. 1) during low tide.

Samples were taken at a depth of 1 m below the surface using a horizontal tube constructed by Hydro Bios in Kiel, Germany. This device samples the aggregates in flowing water without causing turbulence or destruction of the aggregates.

**Plankton.** Samples were treated as described by Zimmermann & Kausch (1996) for enumeration of planktonic bacteria, amoebae, ciliates and flagellates.

**Aggregates.** Aggregates were selected from the horizontal sampling tube with a Pasteur pipette and were counted under a dissecting microscope in 5 bottles with openings 5 cm in diameter. They had been carefully filled with 10 ml of water. Several aggregates were always examined under a microscope at 10× and classified according to morphology and composition.
The size of the particles was measured directly using an ocular micrometer. The mean area of at least 20 particles per sample was calculated from the number of particles and the longest dimensions multiplied by the mean width of each particle.

The aggregates were kept no longer than 3 h in a cooling box before being examined on the ship or in the lab. Selected samples were stained with Alcian Blue, a specific stain for acidic mucopolysaccharides (Decho 1990). Amoebae, ciliates and flagellates were counted on each aggregate using the live counting technique (Zimmermann & Kausch 1996). Bacteria were dislodged by ultrasonic vibration, stained and counted on filters (Zimmermann & Kausch 1996).

Additional parameters. Suspended particulate matter (SPM) dry weight was determined after filtering of water through preweighed Whatman GF/C filters and drying for 24 h at 60°C (Marcus Hoberg, University of Hamburg pers. comm.).

Temperature, conductivity and oxygen concentrations were determined at each station with a portable measurement probe (WTW OXI-96, WTW LF-96).

Chlorophyll a determinations were carried out according to the method of Nusch (1980) (see also Zimmermann & Kausch 1996).

Water discharge rates were measured at the water depth gauge at Neu Darchau, river km 536.4, in Germany (Thomas Gaumert, ARGE Elbe in Hamburg, pers. comm.).

Evaluation of biotic and abiotic parameters. Spearman rank correlation coefficients (r) were computed among the following parameters using STATeasy: dispersed bacteria, bacteria attached to particles, number of aggregates, size of aggregates, discharge, temperature, conductivity, chlorophyll a, ciliates and flagellates.

RESULTS

During 1995, the Elbe Estuary received a mean monthly river discharge of 915 m³ s⁻¹. Mean monthly water discharge rates varied between 337 and 1774 m³ s⁻¹, and maximum values were determined during winter and spring with the highest value in February: 2163 m³ s⁻¹ (Thomas Gaumert pers. comm.). The salinity ranged from 0.5 to 35% and the conductivity ranged between 462 and 11860 μS cm⁻¹. The salinity in the Elbe Estuary was highest during the summer months, coinciding with periods of least rainfall and low water discharge rates. The upper border of the brackish water zone was previously near Glückstadt at river km 675 (Caspers 1959). From recent investigations, it is obvious that the brackish water border has moved 5 to 20 km upstream (Bergemann 1995, Riedel-Lorjej et al. 1995). From January to December, the water temperature ranged from 1.3 to 25.4°C. From +0.5 to +2.0°C higher temperatures were encountered mainly in the upper part of the estuary at river km 590 to 655. The oxygen concentrations ranged between 2.5 and 13 mg O₂ l⁻¹. They were nearly constant at all stations during winter, but during summer, minimal values were recorded in the freshwater part just downstream from Hamburg. The lowest value was 2.5 mg l⁻¹, determined in August at river km 625. From January through December, the chlorophyll a (chl a) concentration ranged from 1.0 to 184.7 μg l⁻¹. Chl a concentrations reached maxima as high as 89.24 μg l⁻¹ in late spring, and in late summer they reached 184.70 μg l⁻¹. In the middle of March, large diatoms became dominant in the phytoplankton, and another peak was reached in August due to the presence of cyanobacteria. The highest chl a concentrations, about 5 times greater than in the brackish part, were detected in the freshwater part of the estuary (Zimmermann unpubl. data).

SPM (Fig. 2) varied between 8.0 and 654.5 mg l⁻¹. Variations along the transect were low. Greatest values were reached at the freshwater-saltwater interface (Marcus Hoberg unpubl. data). In the Elbe Estuary, the number of aggregates (Fig. 3) varied between 20 and 4000 l⁻¹. The highest values, >100 aggregates l⁻¹, were encountered in the part farthest downstream, from river km 675 to 707 in the estuary. Seasonal peaks were observed during spring, in March and April, and another peak was reached in late summer and autumn, from July to October. Most aggregates were in the size range from 50 to 2500 μm (Fig. 4), but several reached 5000 μm. In contrast to abundance, the largest size classes were found in the upstream part of the estuary, mainly in the freshwater environment.

In the Elbe Estuary, the total bacterial concentration fluctuated remarkably, with low values occurring during winter. The dynamics in abundance of free (Fig. 5) and attached organisms (Fig. 6) showed the same trend, but the variations in unattached bacteria were not as pronounced. The abundance of unattached bacteria varied between 0.5 × 10⁶ and 25.5 × 10⁹ l⁻¹, while the density of attached bacteria varied from 0.3 × 10⁶ to 2.5 × 10⁸ per aggregate and accounted for about 75% of the total bacterial density during the year. The mean number of bacteria estimated to be attached to a single particle in the upper estuary was 11.5 × 10⁸, and at a seaward site in the estuary, the corresponding number was 0.85 × 10⁶. This indicates that about 90% of the total bacterioplankton in the upper estuary were tightly attached to aggregates, while only 40% were similarly attached in the lower estuary.

The abundance of protozoans was found to be in the range from 0.242 × 10⁶ to 8.78 × 10⁶ l⁻¹. Highest values were encountered in the freshwater part of the Elbe Estuary.
Regardless of salinity, substantial populations of naked amoebae were found at all sites throughout the year. The data show a clear seasonal trend, with the highest numbers being recorded in June and July. Most amoebae were probably attached to suspended aggregates.

Ciliate abundance ranged from $1 \times 10^3$ to $65 \times 10^3$ cells l$^{-1}$.

The seasonal patterns of flagellate abundance varied quite markedly between $241 \times 10^3$ and $8748 \times 10^6$ l$^{-1}$. The total number of combined heterotrophic and phototrophic flagellates fluctuated throughout the year. A large proportion, about 80 to 98%, of the flagellates were associated with aggregates.

Particles colonized by bacteria and protozoans were predominantly flocculent, consisting of a polysaccharide matrix, which is stained by Alcian Blue. The percentage of colonized particles varied between 74.5 and 98.9%, with highest values recorded during summer in the upper estuary. On average, 87% of the aggregates were colonized. The mean surface area of colonized particles varied between 0.80 μm$^2$ and 25000.00 μm$^2$. The number of attached organisms per unit of particle area decreased significantly with increasing particle size.

**DISCUSSION**

The combination of high water discharge and high suspended matter concentrations in rivers is responsible for the transfer of SPM from the terrestrial environ-
port colonization by bacteria. The particles can vary in 2 important ways: their total organic content and their size. Zimmermann et al. (in press) showed that the total organic content of particles varies considerably. For example, during winter when the water discharge rate is high, mineral particles are very abundant. Aggregates in the lower part of the estuary have an organic matter content of only about 10 to 30%. Probably due to the greater resuspension of sand grains and other inorganic particles, the number of attached bacteria on those aggregates remains low: $0.3 \times 10^6$ to $6 \times 10^6$ bacteria per aggregate. The greater abundances of bacteria found at the upstream estuarine sites and the variations in abundance throughout

Fig. 3. Seasonal changes in aggregate abundance in the Elbe Estuary
the year can be attributed, in part, to the great size of the aggregates in the water column. They are composed of a large amount of fresh material from algae or macrophytes. Both floristic elements are produced in the Elbe above Hamburg or in shallow and slow-flowing tributaries (Wolfstein & Kies 1995, Marcus Hoberg pers. comm., Zimmermann unpubl. data). The composition of the particulate organic material also changes as it is consumed by bacteria and other grazers, becoming less labile and supporting a less productive complement of bacteria (Crump & Baross 1996, Jürgens et al. 1997). Some turbid regions in some estuaries may support greater bacterial abundance than waters with low turbidity (Bent & Goulder 1981, Joint & Pomeroy 1982, Plummer et al. 1987, Painchaud & Therriault 1989). Downstream, SPM concentrations increasing to values 2 to 15 times greater than that in the upper part of the estuary is characteristic of the lower Elbe. The hydrological processes cause a nearly permanent suspension of material from the freshwater and the marine region. There are large numbers of bacteria in this region, where aggregates are small only when their organic content is

Table 1. Spearman's correlation matrix of Elbe Estuary (n = 85) parameters. The correlation coefficient (r) was computed among the following parameters: abundance of attached bacteria (bacteria aggregate⁻¹), abundance of free bacteria (bacteria l⁻¹), abundance of aggregates (aggregates l⁻¹), aggregates size (µm), chlorophyll a (µg l⁻¹), temperature (°C), conductivity (µS cm⁻¹), abundance of ciliates (cells l⁻¹), flagellates (cells⁻¹) and discharge (m³ s⁻¹). Significance level: * 5.0%, ** 1.0%, *** 0.1%

<table>
<thead>
<tr>
<th></th>
<th>Attached bacteria</th>
<th>Aggregate size</th>
<th>Free bacteria</th>
<th>Aggregate</th>
<th>Chl a</th>
<th>Temperature</th>
<th>Conductivity</th>
<th>Ciliates</th>
<th>Flagellates</th>
<th>Discharge</th>
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<td>Attached bacteria</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>Aggregate size</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Free bacteria</td>
<td>0.848***</td>
<td>0.652***</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aggregate</td>
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<td>-0.305***</td>
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<td>-</td>
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<td>Chl a</td>
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<td>0.405***</td>
<td>0.717***</td>
<td>-0.124</td>
<td>1</td>
<td>-</td>
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<tr>
<td>Temperature</td>
<td>0.477*</td>
<td>0.497***</td>
<td>0.690***</td>
<td>-0.295*</td>
<td>0.500***</td>
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<td>Conductivity</td>
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<td>-0.361***</td>
<td>-0.147*</td>
<td>0.254</td>
<td>-0.185***</td>
<td>0.019</td>
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<tr>
<td>Ciliates</td>
<td>0.366***</td>
<td>0.493***</td>
<td>0.488***</td>
<td>-0.404***</td>
<td>0.364*</td>
<td>0.411</td>
<td>-0.588**</td>
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<td>-</td>
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<tr>
<td>Flagellates</td>
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<td>0.493***</td>
<td>0.620***</td>
<td>-0.397**</td>
<td>0.506**</td>
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<td>0.663***</td>
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<td>Discharge</td>
<td>-0.303***</td>
<td>-0.186**</td>
<td>-0.436***</td>
<td>-0.128</td>
<td>-0.117</td>
<td>-0.274***</td>
<td>-0.222</td>
<td>0.280***</td>
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high. Most of the material from the freshwater zone decomposed on its way downstream.

The significant correlation between the abundance of attached bacteria and the size of the aggregates (Table 1) is positive. In most cases, large particles typically occur when the organic content is great (Zimmermann & Kausch 1995, Zimmermann unpubl.). It was reported by Hoppe (1984) that the characteristics of the particles and their adsorption capacities determine the degree of colonization. Many particles and interfaces may not provide the conditions necessary for the successful competition of attached bacteria with bacteria in the water. Most of the attached bacteria colonizing the aggregates in their late stages are not affected by the spatial and temporal changes, are larger in size (Zimmermann & Kausch 1996) and are more active than those dispersed in the pelagic water column (Hodson et al. 1981, Inberri et al. 1987, Unanue et al. 1992, Griffith et al. 1994). Spatial variations in their concentration were correlated in part with changes in the amounts of aggregates.

The conclusion that bacteria attached to particles account for increases in estuarine turbidity (Table 1) is contrary to the observation that smaller aggregates were more abundant outside the zone of maximum turbidity in the estuary (Crump & Baross 1996). The numbers of bacteria were greatest on flocculent, 'permanently suspended particles', particles that had not settled out after 6 h in a settling chamber (Zimmermann unpubl. data).

The number of bacteria did not correlate significantly with salinity (Table 1). It seems that colonization of aggregates by bacteria occurs independently of changes in salinity in the estuary. Nevertheless, there are direct effects from temperature (Table 1) and indirect effects related to light and temperature (Table 1). Temperature has been regarded as crucial for the oxygen regime in the Elbe Estuary because microbial processes depend greatly on temperature (Rheinheimer 1964, Flügge 1985). Aggregates which differed significantly in their organic composition were markedly affected by microbial degradation processes at water temperatures above 15°C. Generally, first indications of increasing oxygen deficiencies become detectable during late spring, in May, at the lower end of the freshwater region. With increasing temperature, the area of oxygen minima moves upstream and might produce critical conditions for the fish (Kerner et al. 1995) in August. The data reveal that during the cold season, from December to April, the oxygen concentrations remained constant along the whole transect and that an increased abundance of aggregates was not accompanied by a corresponding decrease in the oxygen concentration (Figs. 2 & 3). Bright illumination and high temperature provoke a spring proliferation of algae, especially diatoms, and the sticky diatoms are an important component of the first large aggregates that form during spring (Zimmermann & Kausch 1996).
From chlorophyll determinations, it was deduced that a large portion of algae are lost downstream in the estuary and transformed to algal detritus. It is known from the investigation by Fast (1993) that the chl a content of the Elbe water decreases drastically downstream from Hamburg long before the salinity gradient is reached. Algae attached to aggregates have a higher sinking velocity than algae suspended freely in the water and are subject to sedimentation and resuspension processes (Wolfstein & Kies 1995). It seems that oxygen demand in August downstream from Hamburg (Fig. 2) is increased by the activity of heterotrophic bacteria (Böttcher et al. 1995, Wolfstein & Kies 1995) which degrade organic material produced by the second algal maximum utilizing a large part of algal detritus. During periods of minimal oxygen concentrations, the chl a content is very low. In addition, reduced illumination in the lower part of the turbid estuary minimizes algal capacity to produce oxygen by photosynthesis.

There is a diverse fauna of pelagic and epibenthic bacterivores in the estuary. The dominant species include amoebae, ciliates, flagellates, larvae of Dreissena polymorpha, nematodes and rotifers. Due to the abundance of bacteria attached to particles, bacterial carbon in the estuary is packaged in particles of many sizes and may be available as food to all of these grazers. Bacteria may be consumed at many different trophic levels within the plankton, complicating and 'short-circuiting' the microbial food web (Lampitt et al. 1993, Zimmermann et al. in press).

Correlations between the abundance of the dominant grazers and the different bacterial parameters provided some clues to the structure of the detrital food web at different locations in the Elbe Estuary. Protozoans are capable of rapid growth in response to an increase in their food supply, so it was not unexpected to find a significant positive correlation between the abundances of flagellates and bacteria and those of ciliates and bacteria (Table 1). At individual locations in the Elbe Estuary, a balance between bacterial growth and grazing might have been the reason for the temporary uniformity of the total number of bacterial cells observed during this study. Many organisms are able to graze on dispersed or loosely attached bacteria (Laybourn-Parry et al. 1987, Rogerson et al. 1989, Polne-Fuller et al. 1990, Rogerson 1991, Arndt 1993, Jurens & Güde 1994), and others consume whole aggregates (Heinie & Flemer 1975, Heinie et al. 1977, Chervin 1978, Boak & Golder 1983, Zimmermann et al. in press, Zimmermann & Barkmann unpubl.). However, no correlation was found for the number of aggregates; only the size of the aggregates is an important factor.

At least 2 factors control the level of bacterial abundance in the water column of the Elbe Estuary. In the upstream part of the estuary, bacterial abundance is under strong grazing control, whereas in the seaward part, substrate limitation reduces bacterial growth.

Fig. 6. Bacterial abundance on aggregates in the Elbe Estuary
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