

TIDAL AND SEASONAL VARIATIONS IN DYNAMICS OF MICROAGGREGATES AND ASSOCIATED BACTERIAL COMMUNITIES IN THE GERMAN WADDEN SEA

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Introduction

In shallow turbid marine ecosystems like the German Wadden Sea suspended particulate organic and inorganic matter (SPM, POM) plays a very important role in the flux of energy and cycling of elements and in transport processes (EISMA, 1993; GÄTJE & REISE, 1998; DITTMANN, 1999). The great majority of SPM is composed of microaggregates <500 µm which undergo pronounced changes and restructuring during short-term current velocity changes such as tidal cycles (CHEN & EISMA, 1995; MILLIGAN, 1995; MCCANDLISS, *et al.* 2002). Microaggregates are heavily colonized by bacteria which decompose and solubilize the aggregate-associated POM. Recent studies have shown that microbial processes are more important in aggregation and disaggregation processes than previously assumed (SIMON *et al.*, 2002), but such studies have not been carried out in the Wadden Sea, in which hydrodynamic processes are assumed to mainly control aggregation and disaggregation. Despite the extensive biological studies carried out in the Wadden Sea (GÄTJE & REISE, 1998; DITTMANN 1999) surprisingly little is still known about the biogeochemical role of bacteria in the water column in this ecosystem (ADMIRAAL *et al.*, 1985; VAN DUYL & KOP, 1988). POREMBA *et al.* (1999a,b) showed that bacterial consumption of organic matter exceeds primary production in the water column and that bacterial abundance is only weakly correlated to chlorophyll. There is no information available on the relative significance of aggregate-associated versus free-living bacteria and on the composition of these bacterial communities.

The aim of this study was to investigate tidal and seasonal dynamics of SPM and abundance and size structure of microaggregates in relation to bacterial dynamics. Further, we asked the question how tidal and seasonal ecosystem dynamics are reflected in the composition of free-living, aggregate-associated and surface sediment-associated bacterial communities.

Methods

Samples were collected from the surface in the Spiekerroog tidal flat back barrier system of the German Wadden Sea at the Otzumer Balje on board RV Senckenberg in different seasons. Samples for the seasonal analysis of the composition of bacterial communities were taken near Neuharlingersiel. SPM, POM, aggregate abundance and size classes were determined as described by LUNAU *et al.* (this volume). These methods include a new correction for salt content without rinsing with deionized water avoiding any POC loss.

Bacterial numbers were counted on black 0.2 µm (free-living) or 5.0 µm (aggregate-associated) Nuclepore filters after DAPI staining and counterstaining with acridine orange (CRUMP *et al.*, 1998). The composition of the bacterial communities was analyzed by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments with a eubacterial primer set (MUYSER *et al.*, 1998). The community structure was further examined by cluster analysis as described in SELJE & SIMON (2003). The composition of the aggregate-associated bacterial community was also analyzed by fluorescence *in situ* hybridization

(AMANN *et al.*, 1995) with small subunit rRNA-targeted oligonucleotide probes of various specificity.

Results

Suspended matter and aggregates

SPM concentration, i. e. dry weight (DW), in February was more than twofold higher than in August but tidal dynamics were similar with peaks at the current velocity maxima and minima at slack water at high and low tide (Fig. 1). POM concentrations during both tidal cycles were rather similar and tidal dynamics with lower values at slack water much less pronounced than with DW. In August, POM constituted 43-65% of DW whereas in February it was only 17-29%, indicating that the organic phase and thus biological processes were more important in controlling SPM dynamics in August. Highest percentages always occurred at slack water, indicating that the inorganic fraction rapidly settled out.

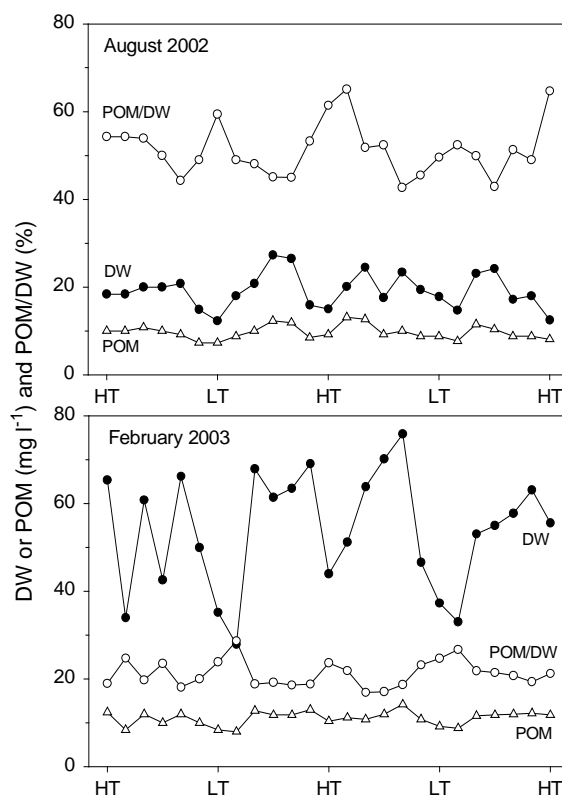


Fig. 1. Dry weight (DW), POM and POM/DW during two tidal cycles in August 2002 and February 2003 in the Spiekerroog tidal basin at the Otzumer Balje. HT: high tide, LT: low tide.

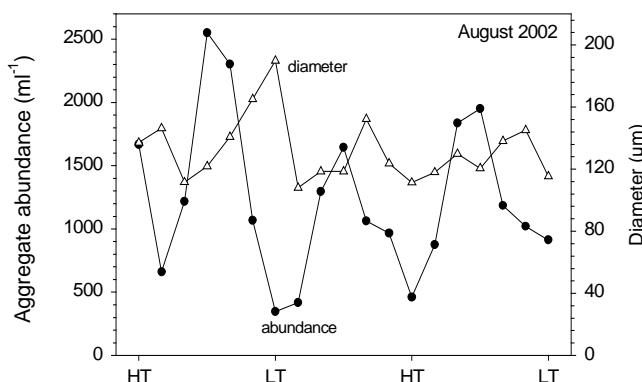


Fig. 2. Abundance and maximum diameter of aggregates over one and a half tidal cycles in August 2002 in the Spiekerroog tidal basin at the Otzumer Balje. HT: high tide, LT: low tide.

Aggregate abundance in August ranged between 346 and 2550 ml⁻¹ with highest values at the current velocity maximum and lowest values at slack tide (Fig. 2). The size of aggregates exhibited inverse patterns with maxima towards slack tide and minima towards the highest current speed. In February 2003, aggregate abundance was higher by more than one order of magnitude (ca. 1x10⁴ – ca. 4x10⁴), but the covariation with tidal dynamics was less pronounced than in August (Fig. 6 in Lunau *et al.*, this volume). The aggregates in February were smaller, the maximum diameter ranging between 55 and 110 µm. In contrast to August, aggregate size at this time covaried with aggregate abundance, indicating that aggregate dynamics were controlled differently during these two periods. This is also reflected by the completely different size class distribution of aggregates. In August, aggregates of 30-100 µm constituted ca. 40% and aggregates >200 µm 10->20%, whereas in February aggregates of 30-100 µm comprised >70% (Fig. 3).

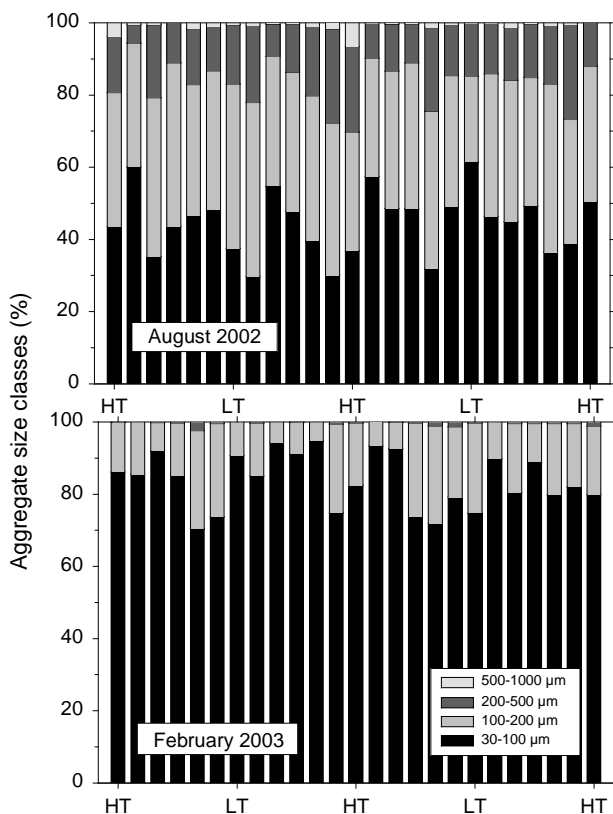


Fig. 3. Size classes of aggregates during two tidal cycles in August 2002 and February 2003 in the Spiekerroog tidal basin at the Otzumer Balje. HT: high tide, LT: low tide.

Abundance and composition of bacterial communities

In November, numbers of free-living and aggregate-associated bacteria were rather similar. Only the latter exhibited strong tidal variations with peaks at the current velocity maxima (Fig. 4) covarying with SPM dynamics (data not shown). In May, numbers of free-living bacteria were substantially higher than in November and than those of aggregate-associated bacteria but tidal dynamics were much less pronounced. Low numbers in May at high tide indicate advection of North Sea water.

Cytophaga-Flavobacteria (CF), α - and γ -*Proteobacteria* constituted the majority of the aggregate-associated bacterial community in both tidal cycles and comprised 20-40%, 8-45%, and 15-35% of DAPI cell counts, respectively (Fig. 5). α - and γ -*Proteobacteria* were relatively more important in May, and CF in November. β -*Proteobacteria*, which were assessed

only in May, constituted 10-22%. Sulfate-reducing bacteria were always detected and constituted ~5%. Because they typically live in the anoxic sediment their appearance on aggregates points to resuspension. Even though the relative proportion of the various bacterial groups exhibited variations during the tidal cycles they were not related to tidal dynamics. Relative proportions of the various aggregate-associated bacterial groups exhibited differences to those of free-living bacteria (data not shown).

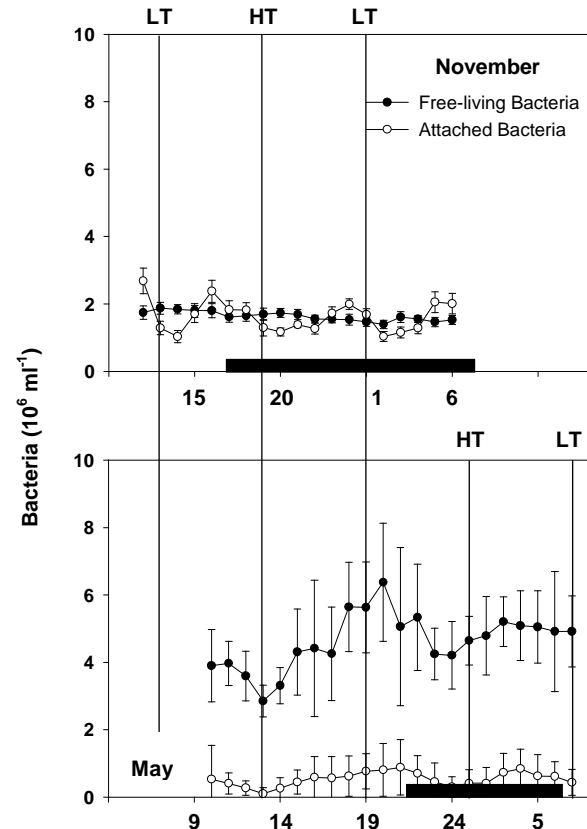


Fig. 4. Numbers of free-living and aggregate-associated (attached) bacteria during tidal cycles in November 1999 and May 2000 in the Spiekerroog tidal basin at the Otzumer Balje. The black bars indicate the dark period. HT: high tide, LT: low tide.

Our DGGE-analysis confirms that the structure, i. e. richness, of the aggregate-associated as well as that of the free-living bacterial community remained basically unchanged during the tidal cycles (Fig. 6), suggesting that only relative proportions of single populations, i. e. the evenness, shifted. The analysis also demonstrates that the composition of the free-living and sediment surface-associated bacterial communities exhibited pronounced differences. Differences were also obvious between the communities in November and May. The detection of several bands, i. e. phylotypes, in the free-living as well as in the aggregate-associated and in the latter as well as in the sediment surface-associated community indicates that exchange processes between the habitats of these communities and thus also between these communities occur.

In addition to the tidal cycles we also analyzed the composition of the three bacterial communities by DGGE seasonally from April 1999 to June 2000 to examine whether long-term changes within the communities exist and how interactions between them occur. The DGGE patterns showed that the distinct bacterial communities prevailed over the entire study period but also that the single communities underwent changes. A cluster analysis substantiated the relationships and temporal dynamics of the composition of these communities (Fig. 7). The sediment-associated

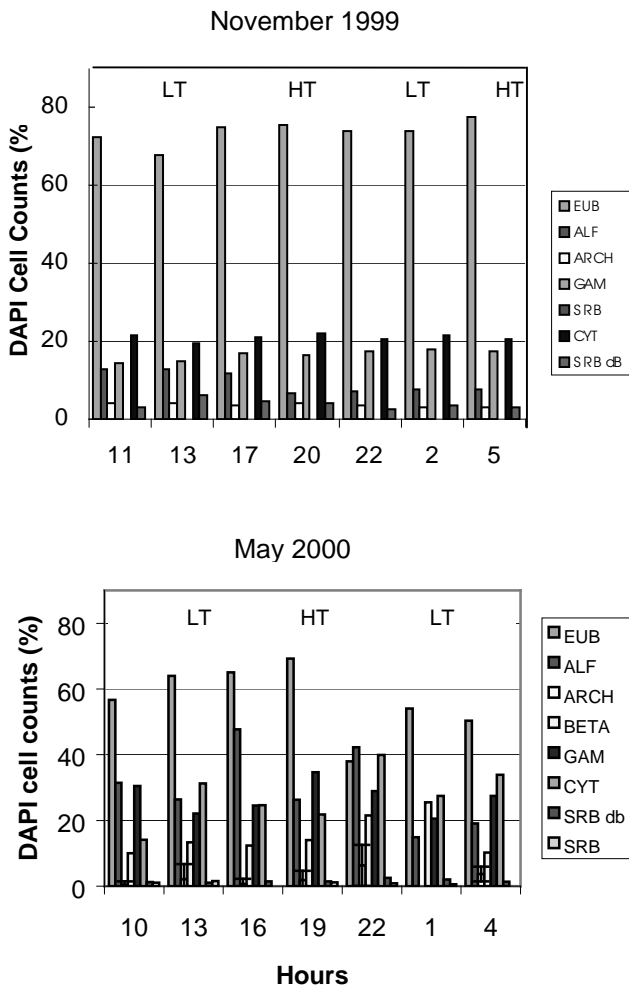


Fig. 5. Numbers of *Bacteria* (EUB), α - (ALF), β - (BETA), γ -*Proteobacteria* (GAM), *Cytophaga/Flavobacteria* (CYT), sulfate-reducing bacteria (SRB) and *Archaea* (ARCH) of the aggregate-associated fraction during tidal cycles in November 1999 and May 2000 in the Spiekeroog tidal basin at the Otzumer Balje. HT: high tide, LT: low tide.

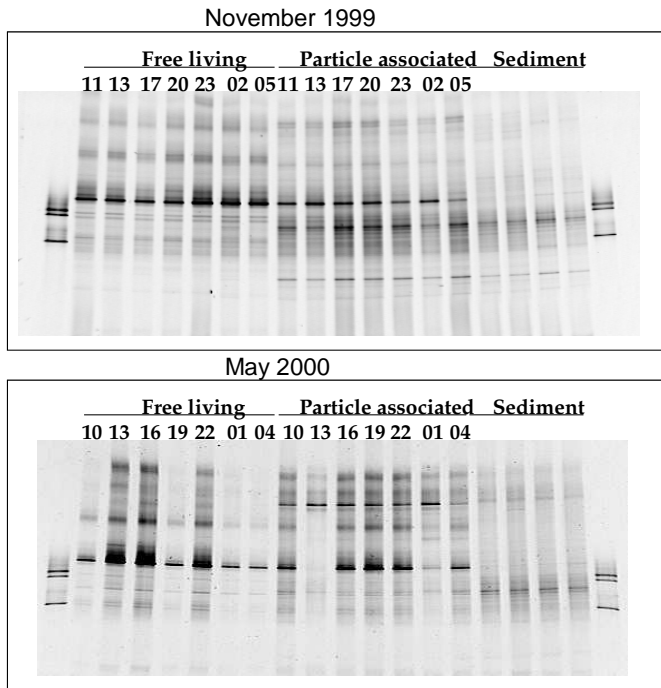


Fig. 6. DGGE patterns of the free-living, aggregate-associated and surface sediment-associated bacterial community during tidal cycles in November 1999 and May 2000 in the Spiekeroog tidal basin at the Otzumer Balje. Numbers are hours.

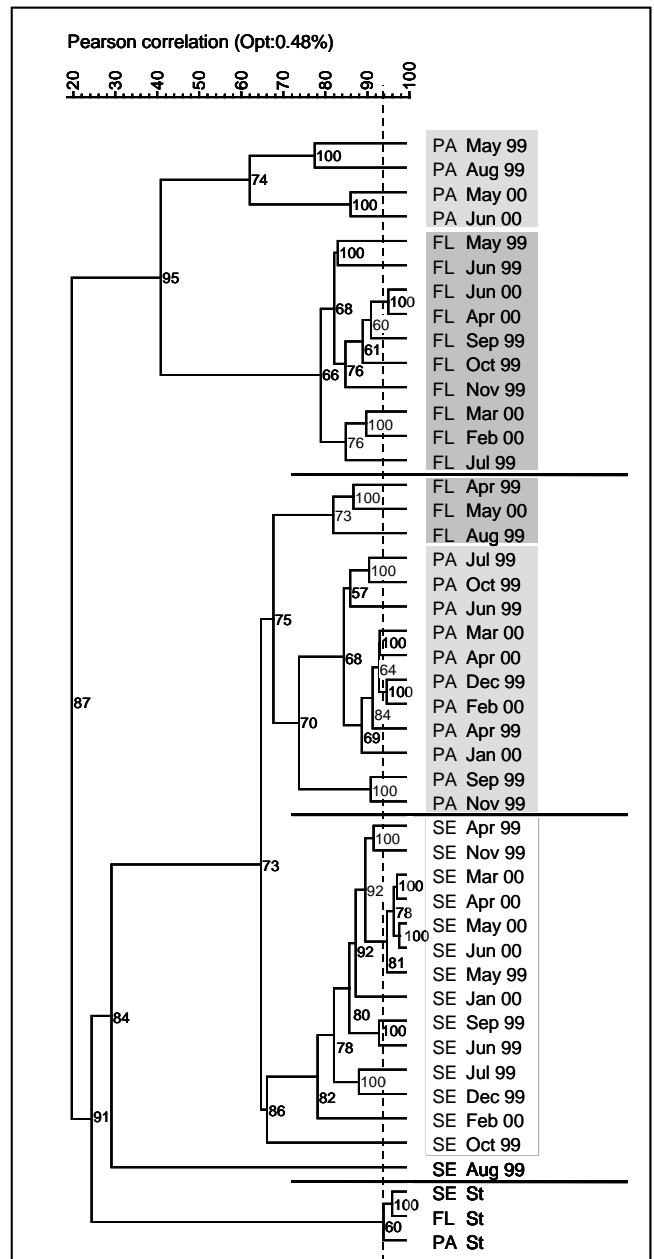


Fig. 7. Cluster analysis of DGGE patterns of the free-living (FL), aggregate-associated (PA) and surface sediment-associated bacterial communities (SE) between April 1999 and June 2000 in the Spiekeroog tidal basin near Neuharlingersiel. St: Standards.

community exhibited the greatest similarity with only minor temporal changes. The aggregate-associated community was clearly separated from the sediment-associated community. Some similarities and overlaps with the free-living bacterial community existed as the latter of April and August 1999 and of May 2000 clustered together with the aggregate-associated communities. One the other hand, the aggregate-associated communities of May 1999, May and August 2000 clustered together with the free-living bacteria communities of the other months. Distinct subcommunities of close similarity of the aggregate-associated fraction existed in summer and fall and from December 1999 to April 2000.

Discussion

Our results show clear differences between phases of low (November, February; winter) and high biological productivity (May, August; growing season) for the relative significance of organic matter in dynamics of suspended matter and microaggregate abundance and size distribution. In winter,

much higher concentrations of inorganic SPM and higher abundances of smaller microaggregates occurred, obviously a result of the lacking organic matter. It was shown that mucus and transparent exopolymer particles (TEP) produced by phytoplankton, benthic algae, bacteria and from dissolved organic matter (PASSOW, 2002), are sticky material leading to aggregation. Hence, at phases of low biological productivity, such as in winter, when this material is not continuously supplied, the sticking coefficient of available source particles is lower than during the growing season thus leading to generally smaller aggregates. Further, the sediment may be subjected to higher resuspension at similar shear, because the benthic biofilm produces less mucus as a stabilizing structure. Because smaller aggregates have a lower settling rate (EISMA, 1993), they accumulate in the water column. Relative differences in biological and physical processes for aggregate dynamics in shallow turbid systems were found in previous studies (e. g. McCANDLISS *et al.*, 2002) but the role of algae, bacteria and TEP has not yet been taken into account.

There is accumulating evidence from various studies that heterotrophic bacteria and specific populations are much more important in the formation, decomposition and disintegration of suspended aggregate than previously assumed, even though such information is not yet available from tidal flat ecosystems (for review see SIMON *et al.*, 2002, GROSSART *et al.* 2003). We have some preliminary evidence that this also applies to aggregates in the Wadden Sea (CZUB, 2000; F. LEVOLD, unpubl. results) but further work is in progress to elucidate the role of heterotrophic bacteria in aggregate dynamics in this ecosystem.

Our results show that distinct bacterial communities are present on aggregates, in the surrounding water and on the sediment surface. As shown by similar DGGE bands, some overlaps exist between the aggregate-associated and the free-living bacterial community in the bulk water, and between the former and that on the sediment surface. These overlaps may be due to exchange processes or resuspension, but may also indicate that some bacteria can prosper in more than one habitat. Changes in the community structure were small or non-existing during tidal cycles, which have a time scale in the same range as the generation time of bacteria. Pronounced changes, however, occurred seasonally but differently among the various communities, indicating that they adapted to the varying growth and environmental conditions. In order to identify the major players in the bacterial communities, selected DGGE bands were excised and sequenced (data not shown). The sequencing results indicate that each bacterial group detected by FISH is constituted by a limited number of phylotypes. To further elucidate the composition and dynamics of the bacterial communities we are designing specific primers and probes for FISH to detect and quantify selected bacterial groups or phylotypes (RINK *et al.*, this volume). This work will be combined with measurements of the bacterial turnover of organic matter such as substrate hydrolysis, uptake and growth. Thus we are working towards a better understanding of the role of bacteria in the formation and decomposition of aggregates in the Wadden Sea.

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