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Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight

II. Analysis of benthic bacterial populations *

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Abstract

Seasonal variations in bacterial populations (total number, biomass, biomass-spectrum, number of dividing cells) as well as in concentrations and decomposition rates of particulate organic material were followed in a sandy mud sediment of the Western Kiel Bight (Baltic Sea; FRG). The strong seasonal variations observed could be traced back to the effect of certain ecological situations and events in the sediment from which the input of the phytoplankton blooms in autumn and spring, respectively, the accumulation of organic material during winter, and the spring development of the benthic fauna turned out to be the most important. Bacterial carbon net production following the breakdown of the phytoplankton blooms ranged between 9 μg (autumn) and 16 μg (spring) per g of dry weight sediment per day. The consequences of shifts in the size composition of the bacterial populations as well as the importance of the measurement of enzymatic decomposition rates of particulate organic material in sediments are demonstrated and discussed in relation to the events mentioned above.

Introduction

The role of bacteria in marine coastal sediments is only poorly understood, although sediments play an important function in nutrient regeneration for marine ecosystems. Most of the microbiological work was concentrated on nutrient cycles in mostly anoxic sediments. Indirectly, the activity of the benthic bacteria was concluded from changes in concentrations or turnover rates of inorganic and organic chemical parameters. The bacterial populations themselves, however, were regarded as a "black box", mediating any kind of substrate turnover, which was traced back to bacterial metabolism since other organisms could not be responsible.

Direct observations of number, biomass and activity of bacterial populations in marine sediments are rare. Meadows and Anderson (1966) and Weise and Rheinheimer (1978) analysed marine sandy sediments using scanning electron and epifluorescence microscopy. The authors could demonstrate by impressive photos that bacteria colonize the crevices and depressions of sand grains in high numbers and large diversity. They are protected there against mechanical demages. From the microscopic analysis, the complexity of the particle surface as a microenvironment became obvious. It consists of an organic matrix of polysaccharides and detritus to which the bacteria are attached or embedded in.

Through the investigations of Dale (1974), the high number of bacteria in marine sediments was quantitatively documented. Subsequent studies (Griffiths *et al.*, 1978; Meyer-Reil *et al.*, 1978; Kepkay *et al.* 1979; Weise and Rheinheimer, 1979) confirmed these observations, analysing different types of sediment. Information on bacterial biomass is limited. However, the analysis of Meyer-Reil *et al.* (1980) and Moriarty (1980) have demonstrated that, correspondent to their high number, bacteria contribute significantly to the living carbon standing stock in sediments.

In agreement with the fragmentary knowledge about benthic bacteria, investigations of the seasonal variations of number, biomass and composition of the bacterial populations are practically lacking. The question, however, arises how bacterial populations react to ecological situations and events, which occur seasonally-dependent in boreal coastal ecosystems. Among these events, the input of the phytoplankton blooms into the sediment in autumn and spring, respectively, (Graf *et al.*, 1983 a), the accumulation of organic material during winter, and the

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development of the benthic fauna in late spring are probably the most important.

During an interdisciplinary joint research program at Kiel University (Sonderforschungsbereich 95), the pelagic-benthic coupling of processes in the Western Kiel Bight (Baltic Sea; FRG) was followed. The first paper of this series deals with the analysis of pelagic and benthic processes on a community level (Graf *et al.*, 1983 b). Closely related to this first report, this paper describes the response of the benthic bacterial populations to seasonal variations in the availability of organic material. Special emphasis was laid on the analysis of variations in the composition of the bacterial populations. Since particulate organic material represents the primary carbon source in sediments, the measurement of enzymatic decomposition rates of carbohydrate and protein seemed to be the most promising to be included in the investigation.

Materials and methods

Sampling

A series of sediment samples was withdrawn between September 9, 1981 and June 7, 1982, using a Reineck grab (surface area 20×28 cm) from an 18-m station located on the slope of the Kiel Bight channel system. This area, known as "Hausgarten", is restricted to research and has been subject to several investigations during recent years. At the station investigated, sandy mud prevails. For the analysis of parameters determined in this study and in Graf *et al.*, 1983 b, the 0 to 1-cm horizons from three grabs were combined on board ship and transported to the laboratory in insulated containers.

Redox potential, temperature

Redox potential was measured immediately after sampling with an Eh-electrode (Ingold, Pt-4800-M5) connected to a MV-meter (Knick). Temperature was determined with a portable TS-probe (Electronic Switchgear).

Organic matter

Organic material was analysed from dried (60 °C, 24 h) and ground sediment samples (mortar Pulverisette 1; Fritsch GmbH). Total organic matter content was reported as the difference between the dry weight of the sediment and the residue left after combustion at 550 °C. Protein analysis was based on the method of Lowry *et al.* (1951) using bovine serum albumin (Boehringer GmbH) as a standard. As discussed in detail by Hendrikson (1975), various organic material (for example humic acids) interfere with the protein determination, so that the expression "Folin-positive" material would be more appropriate. Carbohydrates were assayed according to Handa (1967) as modified by Hendrikson (1975) using soluble starch as a standard. Since the acid hydrolysis of the sediment had already resulted in a brown colour (the intensity of which was seasonal-dependent), these values (designed as acid-soluble material) were regarded as controls and subtracted from the sulfuric acid-phenol positive material (carbohydrates).

Decomposition of particulate organic material

The extracellular enzymatic decomposition rates of carbohydrates (α-amylase activity) and protein (proteolytic enzymes) were followed using Amylopectin Azure (Kim and ZoBell, 1974) and Hide Powder Azure (Little *et al.*, 1979; Meyer-Reil, 1981), respectively, as substrates (Calbiochem). These covalent-bound dye derivates are stable, water insoluble and sensitive against enzymatic reaction. Sediment samples were suspended in the six-fold amount of ice-cold homogenization buffer and homogenized in a mortar (cf. above). The homogenization buffer consisted of KH_2PO_4 (0.07 M) and Na_2HPO_4 (0.07 M), pH 6.0, with the addition of 2 ml of Triton X-100 and 1.5 g of polyvinylpyrrolidon (per 1 000 ml of buffer; cf. Bengtsson, 1982). Later experiments have shown that the homogenization procedure can be omitted. The experiments described, however, were based on homogenized samples. This means that the analysis of the decomposition rates of particulate organic material relates to the potential of the total sediment sample, including all organisms present. The enzymatic reaction was started by adding substrate at concentrations high enough to saturate the enzyme systems (200 mg of Amylopectin Azure and Hide Powder Azure, respectively, per 30 ml of homogenized sediment). The samples were incubated under shaking (200 rpm) at room temperature. At 0.5-h (α-amylase) and 3-h (proteolytic enzymes) intervals, 1.5 ml of the sample was removed, transferred into centrifuge tubes, and the enzymatic reaction was terminated by the addition of 0.5 ml of a stopper solution, consisting of one part formalin (4%) and one part H_3PO_4 (1 M). After centrifugation (5 000 rpm, 15 min), the release of the dye was measured in the supernatant (spectrophotometer Zeiss PM 2K) at 595 nm. Controls received the stopper solution prior to substrate addition. At least five time-dependent readings were made comprising three parallels. Preliminary experiments have shown that the enzymatic reaction was linear for at least 24 h.

Enzymatic decomposition rates (changes in absorbance per h per g of sediment) were calculated from the slope of the time-dependent activity curves by linear regression (r^2 was usually above 0.96 and always above 0.90). Using conversion factors of 100 (Amylopectin Azure) and 118 (Hide Powder Azure), respectively, activity rates were converted into mg of substrate decomposed per h per g of sediment. The conversion factors were obtained from decomposing a known amount of substrate with enzymes commercially available (α-amylase, type III-A; protease,

type V; Sigma Chemical Co.), and measuring the resulting absorbance. The measurement of the decomposition rates of particulate organic material used in this study represents a further development of the method described by Meyer-Reil (1981).

Bacterial parameters

One cm^3 sediment (3 parallels) was added to 10 ml of freshly prepared double distilled water supplemented with formalin (2%). Samples were sonicated (Sonifier B12; Branson Sonic Power) at 50 W for 1 min (ice bath) following a 30-s brake. This procedure was repeated three times. Scanning electron photographs demonstrated that this treatment liberates the overwhelming portion of the bacteria from the particles. After allowing the coarse particles to settle (30 s), subsamples were withdrawn from the supernatant and diluted 100 times with double distilled water supplemented with formalin (2%). Portions of the subsamples were added into the funnel of a filtration unit (Schleicher and Schüll) equipped with a Nuclepore filter (0.2-μm pore size, 25-mm diameter) prestained with Sudan Black (Zimmermann *et al.*, 1978). For a better distribution of the bacteria, a silver filter (Selas Flotronics) was positioned between the Nuclepore filter and the filter support (Zimmermann, 1977). The diluted subsamples were stained for 3 min with acridine orange (final concentration 1:10 000), and wedges of the filter were analysed with a drop of Cargille's immersion oil (type A) by epifluorescence microscopy (Zeiss Universal microscope, magnification × 1 600) using blue light excitation and an Osram HBO 200 burner (Zimmermann, 1977). Generally, bacteria were counted by means of a microscopic grid ($40 \times 40\ \mu m$) in a total of 40 microscopic fields distributed on three filters prepared from parallel samples. The microscopic fields were chosen at approximately even intervals between the periphery and the center of the filter to account for an uneven distribution of bacteria on the filter surface. The cell density was between 10 and 25 bacteria per grid. Only bodies with clear outline, bacterial shape and distinct fluorescence (orange or green) were counted as bacterial cells (Meyer-Reil, 1977). Conversions from bacterial number per cm^3 of wet sediment to number per g of dry weight sediment were carried out after determining the dry weight content of the individual samples counted.

On the same epifluorescence microscopy preparations, the number of dividing bacteria defined as cells with a clearly visible invagination, were analysed. At least 100 dividing bacteria were counted on filters prepared from three parallel samples (cf. above). Although the counting procedure was very time consuming, and the values obtained certainly represent an underestimation of the actual number of dividing cells, this parameter turned out to be valuable for the interpretation of the seasonal variations of the bacterial communities.

For biomass determinations, colour slides were prepared from characteristic microscopic fields (Kodak Ektachrome 400 film; exposure time 20–30 s) using a Zeiss CS-matic camera. The slides were projected onto the screen of a semi-automatic image analyser (MOP AM-02; Kontron). With a pencil, the inner outline of the sharply defined bacteria was copied and traced with the detection pen of the analyser. The data printed by the analyser comprise: maximum diameter, outline, area, and form factor. Assuming an ellipsoid, the volume of the individual bacteria can be calculated from area and maximum diameter as known variables. Biovolume (μm^3) was converted into biomass (mg) by presuming a bacterial specific gravity of 1. For conversion into bacterial carbon, a factor of 0.1 was used. At least 60 bacteria were analysed, and the mean biomass per cell (mg of carbon) was calculated. The biomass of the total population (mg of bacterial carbon per g of dry weight sediment) followed from multiplying the mean biomass per cell by the total number of bacteria in the corresponding sample.

For the analysis of the biomass spectrum of the individual populations, bacteria were grouped into three size classes according to their volume: < 0–$0.3\ \mu m^3$, 0.3–$0.6\ \mu m^3$, and $> 0.6\ \mu m^3$. Although as many size classes could have been established as bacteria were measured, these three size classes seemed to be the most suitable to demonstrate easy to survey seasonal variations in the composition of bacterial biomass. Subsequently, the total bacterial biomass associated with each size class was determined for the individual samples.

Bacteria ATP was calculated from carbon assuming a conversion factor of 1/250. For extrapolation, it has to be presumed furthermore that all bacteria contribute equally to the total bacterial ATP-pool, i.e. all bacteria counted were active. This assumption is certainly not justified. However, since the bacterial ATP data gained fit into the overall picture, the results were accepted, although the absolute ATP values remain questionable. Bacterial ATP was expressed as percentage of the total ATP content of the sediments as determined in Graf *et al.*, 1983 b.

Unless otherwise stated, chemicals were of p.a. grade, purchased from Merck Chemical Co.

Results

Redox potential, temperature

At the end of September, anoxic conditions (Eh −140 mV) were observed in the 0- to 1-cm horizon of the sediment. A storm at the beginning of October temporarily led to the introduction of oxygen into the sediment surface (Eh + 300 to + 420 mV). During November, suboxic conditions (Eh + 70 to + 200 mV) prevailed. Stable oxic conditions, however, were achieved in December and maintained throughout the winter with the exception of a decrease in redox potential in February and April. Temperature dropped gradually towards the winter (September 13.9 °C; January 2.0 °C) and increased slowly in spring (April 3.9 °C). An early drastic decrease in temperature

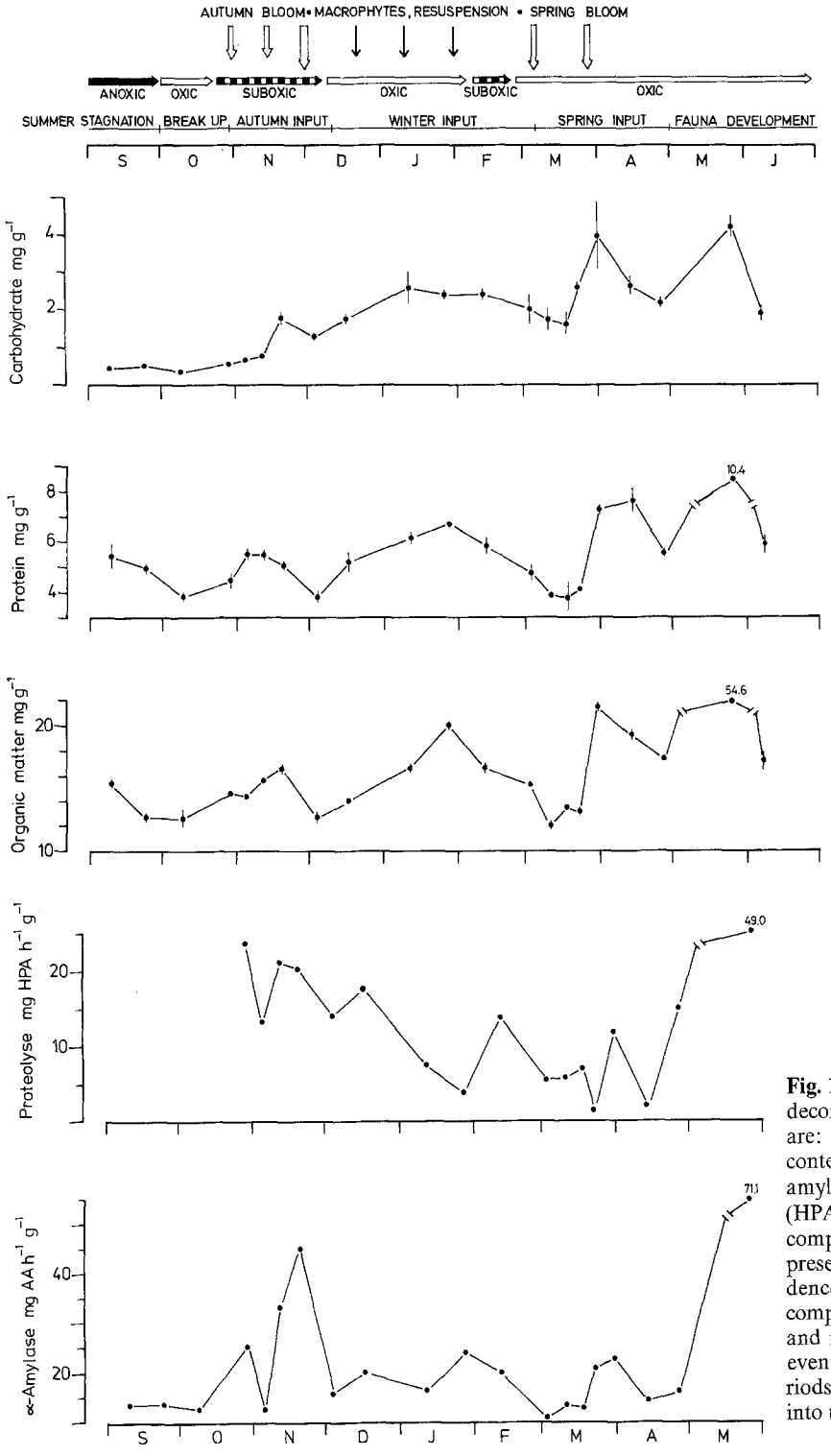

Fig. 1. Seasonal variations in concentrations and decomposition rates of organic material. Illustrated are: carbohydrates, protein, total organic matter content, activity of proteolytic enzymes and α-amylase expressed as mg of Hide Powder Azure (HPA) and Amylopectin Azure (AA), respectively, decomposed per h per g of dry weight sediment. Bars represent standard deviation of the mean (95% confidence level). For details of the measurement of the decomposition rates of organic material cf. "Materials and methods". The headline on top characterizes the events affecting the sediments. Arrows indicate the periods and the intensity of the input of organic material into the sediment

(from 6.2° to 3.2 °C) was observed in the first part of December. Details of the variations of redox potential and temperature are documented in Graf *et al.*, 1983 b. During the observation period, the water content (27.3% ± 1.5%) remained fairly constant. One cm^3 of wet sediment corresponded to 1.300 ± 0.064 g of dry weight sediment (± values represent standard deviation of the mean, 95% confidence level).

Organic matter

The variations of organic matter in the sediment surface were followed by total organic matter content (ignition lost after combustion), protein (serum albumin equivalents) and carbohydrate (starch equivalents). In general, variations of these parameters were comparable (Fig. 1), although the ratios between the individual parameters

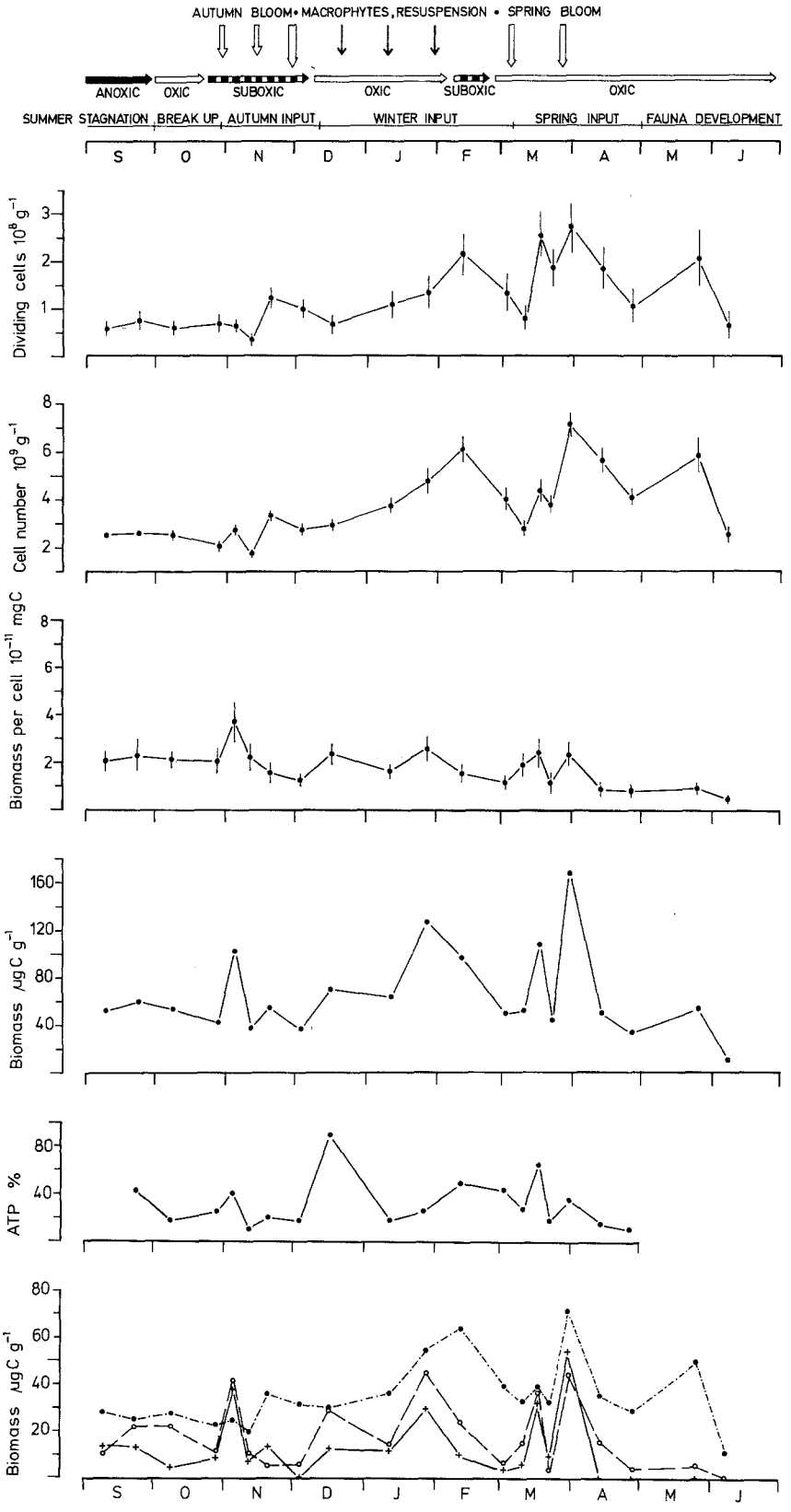

Fig. 2. Seasonal variations in microbiological parameters. Illustrated are: number of dividing cells, total number of cells, mean biomass per cell, total biomass, bacterial ATP (% of the total ATP), and biomass-spectrum. The biomass-spectrum comprises: small-size bacteria of a volume > 0–$0.3\ \mu m^3$ (closed circles), medium-size bacteria of a volume 0.3–$0.6\ \mu m^3$ (open circles), and large-size bacteria of a volume $> 0.6\ \mu m^3$ (crosses). Bars represent standard deviation of the mean (95% confidence level). For explanations of the headline on top see Fig. 1

varied strongly, reflecting quite different nutritional conditions for the benthic community. The variations of organic matter were characterized by the following pattern: peaks in autumn (mid November), high concentrations during winter (peaks in January), and maximum concentrations in early spring (end of March). Generally, concentrations of organic material were higher in winter and spring compared to autumn. Total organic matter varied between 12.1 and 21.5 mg per g of dry weight sediment, protein between 3.8 and 7.7 mg per g, and carbohydrates

between 0.4 and 4.0 mg per g. The high concentrations of organic material observed in the sample taken in late spring (end of May) represented a specific ecological situation characterized by high abundances of the polychaetes *Polydora* sp. and *Capitella capitata* (H. Rumohr, personal communication). Since the analysis was based on homogenized samples (cf. "Materials and methods"), the polychaetes were included and accounted for the high concentrations of organic material.

Decomposition of particulate organic material

Generally, the variation pattern of the decomposition rates of particulate organic material (activity of α-amylase and proteolytic enzymes; Fig. 1) reflected the variations of carbohydrates and protein. Peaks were recorded in autumn (end of October, November), winter (January, February) and early spring (end of March). Decomposition rates of particulate organic material, however, were higher in autumn compared to winter and spring. Again, the sample taken at the end of May represented an exception of the rule (cf. above).

Bacterial parameters

The seasonal variations in the composition of the bacterial populations were analysed by the following parameters: total number and biomass, size spectrum, and number of dividing cells. During the first period of observation (September, October) total number and biomass remained almost constant (Fig. 2). Two subsequent peaks, however, were recorded in November. Towards the winter, a continuous increase in cell number and biomass was observed with peaks in January and February, respectively. Following a decrease during February and the first part of March, cell number and biomass reached two distinct peaks in the middle and at the end of March, respectively. Towards spring, the bacterial population decreased in number and biomass. Generally, variations in biomass were much more pronounced compared to variations in cell number. In some samples (early November, mid March), cell number increased only slightly. Biomass, however, showed distinct peaks. Cell number and biomass were generally higher in winter and early spring compared to autumn. Number varied between 1.7×10^{9} and 7.2×10^{9} cells per g of dry weight sediment, biomass between 12×10^{-3} and 169×10^{-3} mg of bacterial carbon per g.

The number of dividing cells varied between 0.3×10^{8} and 2.7×10^{8} per g of dry weight sediment, representing between 2.0 and 5.9% of the total bacterial population (Fig. 2). Generally, the number of dividing cells was higher in winter and spring compared to autumn. During the first period of observation, the number of dividing cells was almost constant. The peaks recorded at the end of November corresponded to peaks in total bacterial number. The same applied to the high number of dividing cells observed in winter (February) and late winter/early spring (middle and end of March, respectively).

The mean biomass per cell varied between 0.5×10^{-11} and 3.7×10^{-11} mg of carbon (Fig. 2). During September and October, the mean biomass per cell remained almost constant. The highest biomass values were recorded at the beginning of November, leading to the first peak in total biomass. During the winter, relatively high mean biomass values were maintained. Subsequent peaks occurred in the middle and at the end of March. Towards late spring, the mean biomass per cell strongly decreased to a minimum of 0.5×10^{-11} mg of bacterial carbon at the beginning of June.

By size analysis, the contribution of different size classes of bacteria (cf. "Materials and methods") to the total biomass was followed (Fig. 2). Generally, small-size bacteria (volume > 0–$0.3\,\mu m^3$) represented the major part of the total bacterial biomass, followed by medium-size (volume 0.3–$0.6\,\mu m^3$) and large-size bacteria (volume $> 0.6\,\mu m^3$). The biomass of all three size classes strongly increased during winter (January, February) and again in early spring (end of March). Towards late spring, the biomass of the bacterial population was almost exclusively made up of small-size cells. Individual size classes of bacteria contributed differently to the peaks observed in total bacterial biomass (cf. "Discussion").

A rough estimate on the contribution of bacteria to the active fraction of the benthic community is possible by the analysis of the seasonal variations of bacterial ATP illustrated as percentage of the total ATP (Fig. 2). For the evaluation of the data, the reservations mentioned in "Materials and methods" have to be considered. However, since the seasonal variations of bacterial ATP fit into the overall picture, the data were included. Bacterial ATP made up between 11 and 88% of the ATP content of the benthic community (with the exception of macrofauna, which could not be considered because of the small size of the samples). Generally, peaks in the percentage of bacterial ATP coincided with peaks in bacterial number and biomass (beginning of November, mid March) except for the drastic increase observed in mid December (up to almost 90% of the total ATP). In late November and during April, the lowest values in bacterial ATP were recorded (11% of the total ATP).

Discussion

Periods relevant to the benthic community

Changes in the redox-potential and the different stages of enrichment of organic material in the sediment surface gave rise to the description of certain periods relevant to the benthic community (cf. Graf *et al.*, 1983 b). The first period of observation coincided with the termination of the "summer stagnation", a period in which anoxic conditions prevailed in the sediment as a consequence of stratification in the water column overlying the sediment.

This "break up" of summer stagnation was characterized by oxic conditions at the beginning and by suboxic conditions at the end of October. The enrichment of organic material during November could be traced back to the input of the autumn phytoplankton bloom ("autumn input") dominated by armoured dinoflagellates. During winter, a continuous slow increase of organic material was observed in the sediment surface ("winter input"). It may be speculated that part of this material was derived from macrophytes eroded by winter storms (cf. Webster *et al.*, 1975; Graf *et al.*, 1983 b). Material from terrestrial origin as well as resuspended sediment could have represented another part. The breakdown of the spring phytoplankton bloom (mainly diatoms) led to an enrichment of organic material in the sediment surface during late March to mid April ("spring input"). In late spring, the benthic fauna started to develop with high abundances of polychaetes. This period is called "fauna development". Seasonal variations in the enzymatic decomposition of particulate organic material and in benthic bacterial populations have to be interpreted as a reflection upon these different ecological situations and events in the sediment (cf. headline to Figs. 1, 2).

Enzymatic decomposition of particulate organic material

The overwhelming portion of the input of organic material into the sediment is particulate organic carbon, which has to be enzymatically decomposed, at least partly, prior to incorporation into cells. The pool of extracellular enzymes in sediments comprises those actively secreted by living bacteria (Corpe and Winters, 1972) as well as those liberated during the lysis of dead and decaying cells. Some of these enzymes may retain their activity by the formation of humic-enzyme complexes bound to clay particles (cf. model by Burns, 1980). If this hypothesis is valid, the continuous and wasteful production of bacterial enzymes is avoided. Since homogenized samples were assayed, the enzymatic activity rates determined in this study relate to the decomposition potential of the total benthic community with the exception of macrofauna.

Seasonal variations in the enzymatic decomposition of particulate organic material in relation to sedimentation events are only poorly understood. As could be shown in this study, the enrichment of organic material (carbohydrate, protein) in the sediment surface led to a corresponding increase in the enzymatic decomposition rates (activity of α-amylase, proteolytic enzymes). Generally, relationships between α-amylase activity and concentrations of carbohydrates were much more pronounced than relationships between proteolytic enzymes and protein. This may be explained by the specificity of carbohydrates and protein measurements (cf. "Materials and methods"). Enzymatic responses were higher in autumn compared to winter and spring. This is obviously a reflection of both the higher temperature and the higher benthic biomass in autumn. During the autumn and spring input, respectively, high enzymatic decomposition rates already occurred when concentrations of carbohydrates and protein started to accumulate in the sediment surface, indicating an induction of enzymatic activity by increasing concentrations of suitable substrate.

There is evidence from the data that during the anoxic period of summer stagnation, protein accumulated. However, parallel to the break up of summer stagnation (temporary introduction of oxygen into the sediment), concentrations of protein decreased, whereas concentrations and decomposition rates of carbohydrate remained almost unchanged on a low level. A tentative explanation for these observations is provided by laboratory experiments which have shown that the decomposition of protein is strongly reduced under anoxic conditions. Under the same circumstances, the decomposition of carbohydrate is much less affected (Meyer-Reil, unpublished data). Unfortunately, during the period of decreasing protein concentrations, data on the decomposition rates are lacking.

Peaks in enzymatic activity rates coincided or were related to peaks in bacterial parameters (mainly cell number; cf. below). This stresses the important role of bacteria in the decomposition of particulate organic material, a process by which high molecular weight material from the primary production becomes available for higher trophical levels.

Seasonal development of the benthic bacterial community

The total number of bacteria as determined by epifluorescence microscopy (between 17.3×10^8 and 71.7×10^8 cells per g of dry weight sediment) agree well with data reported in the literature for different types of sediment (Dale, 1974; Griffiths *et al.*, 1978; Meyer-Reil *et al.*, 1978, 1980; Weise and Rheinheimer, 1979). Bacterial carbon (between 12 and 169 μg per g of sediment) contributed significantly to the biomass standing stock in the sediments investigated (cf. data on ATP). Again, the range of the bacterial carbon data is in accordance with the literature, although the information is limited (cf. Meyer-Reil *et al.*, 1980; Moriarty, 1980).

Information on number, biomass and size spectrum of bacteria in the literature is almost exclusively based on single observations from which no general trend for seasonal variations could be detected (Cammen, 1982; Montagna, 1982). However, as shown in this study by a high time resolution in sampling, the development of the bacterial community is strongly influenced by seasonal variations in the nutrient supply closely connected to the specific ecological situations and sedimentation events mentioned above.

Changes from anoxic to oxic conditions (break up of summer stagnation) led to an internal shift in the composition of the bacterial biomass: large-size bacteria ($> 0.6\,\mu m^3$) tended to decrease. Parallel to this, bacterial ATP, as percentage of the total ATP, strongly decreased

from 43% (anoxic conditions) to 18% (oxic conditions). With decreasing Eh-values towards the beginning of November, however, bacterial ATP again increased (up to 40% of the total ATP), indicating an increasing importance of bacteria in benthic metabolism under suboxic conditions. Corresponding observations could be made following drastic changes in temperature. In mid December, an early strong decrease in temperature was observed (from 6.2° to 3.2 °C within less than two weeks). Bacterial ATP as percentage of the total ATP increased drastically (from 17 to 88%), indicating a strong reduction in the active biomass of the remaining benthic community and a dominance of bacterial biomass.

The accumulation of the more refractory organic material during the winter input was accompanied by a slow continuous increase in total bacterial number, number of dividing cells and biomass. In January/February bacterial parameters reached values that were even higher than those observed in autumn following the input of the phytoplankton bloom. This is surprising when the low temperature is taken into account. However, the limited number of grazers during winter and the relatively long time the bacterial population had available for its "undisturbed" development may explain the high bacterial standing stock in winter. In this respect, the development of the bacterial population differed basically from its development during autumn and spring, respectively (cf. below). Generally, it has to be considered that, with the sedimentation of particles onto the sediment (material from terrestrial origin, phytoplankton blooms), bacteria attached to particles enter the sediment. From the low density of bacteria on particles in the water column, a significant contribution of these bacteria to the autochthonous benthic bacterial population must be doubted.

Compared to the development of the bacterial population following changes in redox-potential, temperature and the input of organic material during winter, the response of the bacteria to the input of the phytoplankton blooms in autumn and spring, respectively, is much more complex. Generally, two separate, distinct peaks in bacterial parameters have to be distinguished. The first peak occurred when concentrations of organic material from the phytoplankton blooms started to accumulate in the sediment surface. Parallel to this, bacterial ATP significantly increased (up to 65% of the total ATP). This demonstrates that the bacterial population reacted almost immediately to the availability of decomposable organic material. The second peak in bacterial parameters was observed to coincide with the main input of organic material into the sediment following the final breakdown of the phytoplankton blooms.

Differences in the response of the bacterial populations to the input of organic material in autumn and spring, respectively, were certainly caused by differences in both the nutrient supply and the history of the bacterial populations. The autumn phytoplankton bloom was a mixed population dominated by armoured dinoflagellates, whereas at the end of the bloom, diatoms became more important. Part of the settled organic material may indeed be worked up by organisms in the water column. The bacteria faced with the input of this material were derived from an anoxic population (mainly fermentative bacteria, sulfate reducers) prevailing during the period of summer stagnation. Within this population, the input of freshly produced organic material caused a drastic shift. Bacteria primarily reacted with a strong increase in cell volume (biomass production). Not till the main input of organic material did the bacteria subsequently respond with cell division (increase in cell number). The spring phytoplankton bloom was mainly composed of diatoms, which at that time of year almost totally sink to the bottom due to the absence of zooplankton (v. Bodungen *et al.*, 1975). This material represents one third of the total yearly input into the sediment (Smetacek, 1980). Compared to autumn, the history of the bacterial population was quite different. Oxic conditions prevailed in the sediment during winter. Bacterial number, biomass and organic material were obviously declining due to the erosion of the sediment caused by winter storms. The input of freshly produced, almost unmodified organic material hit an impoverished bacterial community, which immediately reacted with both biomass production and an increase in cell number. Following a temporary decrease in bacterial parameters (cf. below), the main input of organic material into the sediment again stimulated biomass production and cell division: bacterial number and biomass reached their maximum values.

The further fate of the bacterial community in late spring was greatly dependent upon the development of the remaining benthic community. At the end of May, high abundances of the polychaetes *Polydora* sp. and *Capitella capitata* were observed in the sediment (H. Rumohr, personal communication). By their action, the sediment surface was firmly glued together. This specific ecological situation was reflected by an individual bacterial population consisting of almost exclusively small-size cells. Two reasons may be responsible for the pauperization of the benthic bacterial community: nutrient deficiency because of the limited transport through the consolidated sediment surface and, secondly, preferential grazing of medium- and small-size bacteria by the polychaetes. Since the bacteria actively grew (high number of dividing cells), the latter hypothesis is favoured. There is indeed evidence from the literature that grazing stimulates the metabolic activity of bacteria (Gerlach, 1978; Morrison and White, 1980). When the polychaetes had disappeared, bacterial parameters reached their lowest values. The population seemed to be indeed nutrient-limited.

Size spectrum of bacteria

As mentioned above, small-size cells ($< 0.3\,\mu m^3$) dominated the bacterial biomass. Generally, with increasing cell size, the associated biomass decreased. This is in

agreement with the observation of Schwinghamer (1981), who found a characteristic distribution of benthic biomass ("Sheldon" spectrum) with two main peaks in the largest (> 2 mm; corresponding to macrofauna) and in the smallest size classes (< 2 μm; corresponding to bacteria), respectively. The author has already pointed out that variations from the typical pattern might be interpreted as the effects of exogenous disturbance. This can be related to the distribution of bacterial biomass as well. The input of the phytoplankton blooms in autumn and spring, respectively, into the sediment caused a drastic shift in the composition of bacterial biomass. Deviating from its "normal" distribution, the biomass was dominated by medium (0.3–0.6 μm^3) and large-size (> 0.6 μm^3) bacteria. However, shortly after the exogenous "disturbance" (approximately one week), the normal pattern of distribution of the bacterial biomass was re-established: medium and large-size bacteria decreased, and small-size bacteria again dominated the biomass. Increasing predation pressure, especially on medium and large-size bacteria, may be the most important factor for the restitution of the normal distribution in connection with a stimulation of the cell-division of small-size bacteria. Analysing systems that differ from a pattern regarded as "typical" may offer a promising approach in understanding the dynamics of benthic bacterial populations.

Bacterial production

The high time resolution in sampling during autumn and spring permits an estimation of the bacterial net production in the sediment surface. As response to the input of the autumn phytoplankton bloom, bacterial production amounted to 9 μg of carbon per g of dry weight sediment per day. The corresponding values in spring were 8 and 16 μg of carbon per g per day, respectively, based upon the two peaks in bacterial biomass. However, for reasons discussed above, almost the same amount of bacterial carbon disappeared from the system within less than one week. It is interesting to note that these bacterial net production estimates correspond to the lower range of production measurements carried out in nearshore western Atlantic Ocean sediments using the thymidine uptake method (Fallon *et al.*, 1983). With the same technique, Moriarty and Pollard (1982) found approximately one order of magnitude lower bacterial production values for surface sediments associated with seagrass beds in Moreton Bay, Queensland, Australia. Sandy beaches of the Kiel Fjord and the Kiel Bight revealed, under summer conditions, a bacterial net production of 5 μg of carbon per g per day based on the uptake of glucose (Meyer-Reil *et al.*, 1980). This two to three times higher production corresponds to the higher bacterial standing stock carbon in shallow water sediments of the Kiel Bight (this study), indicating a similar bacterial production to biomass ratio in beaches and shallow water sediments, respectively, of the Kiel Bight.

Conclusion

This study presents a first insight into the dynamics of the decomposition of particulate organic material and the development of the bacterial populations in the shallow water marine sediments of the Kiel Bight. Based on a high time resolution in sampling, the strong seasonal variations in enzymatic decomposition rates of particulate organic material and bacterial parameters could be traced back to certain ecological situations and events in the sediment. Changes from oxic to anoxic conditions as well as drastic decreases in temperature strongly favoured the dominance of bacteria in benthic metabolism. Only during winter, in the absence of grazers, did bacteria exhibit an "undisturbed" development despite the low temperature. The bacteria almost immediately reacted to the input of freshly produced organic material into the sediment following the breakdown of the phytoplankton blooms in autumn and spring, respectively. The input of the autumn phytoplankton bloom primarily stimulated biomass production and the subsequent increase in cell number. In early spring, both bacterial biomass production and cell division were stimulated simultaneously. In late spring, the fate of the bacterial population turned out to be greatly dependent upon the development of the benthic fauna. Increasing grazing pressure led to an impoverished bacterial community consisting of almost exclusively small-size cells. Deviations from the "normal" size distribution pattern of bacteria have to be interpreted as indications of effects of exogenous disturbance factors. Generally, concentrations of particulate organic material (carbohydrate, protein) were closely related to enzymatic decomposition rates (activity of α-amylase, proteolytic enzymes). Peaks in bacterial parameters (mainly cell number) coincided with peaks in enzymatic activity rates, stressing the importance of bacteria in the decomposition of particulate organic material. Future research will be concentrated on investigations of shifts in the metabolic activity of the benthic bacterial populations as well as on interactions between bacteria and the faunal components of the benthic communities.

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