

Competition between benthic cyanobacteria and diatoms as influenced by different grain sizes and temperatures

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ABSTRACT: An experimental laboratory set-up was used to study the influence of different grain size compositions and temperatures on the growth of benthic cyanobacteria and diatoms, and on the competition between these 2 groups. Monospecific cultures of 3 species of cyanobacteria (*Merismopedia punctata*, *Microcoleus chthonoplastes*, *Oscillatoria limosa*), and of 2 species of benthic diatoms (*Phaeodactylum tricornerutum* and *Nitzschia* sp.) were used. The organisms were cultured in 100 ml flasks filled with medium and 3 different kinds of sediment: (1) Sand (fine sand, 63 to 200 µm), (2) Mud-I (mixed fine sand and mud <63 µm in the ratio 80:20 wt %), (3) Mud-II (mixed fine sand and mud in the ratio 50:50 wt %). Experimental temperatures were 10, 15 and 25°C. At 10°C and 15°C, both diatom species achieved the highest biomass on the sediments of the finest grain size (50 wt % < 63 µm) while cyanobacteria achieved low biomass levels. Coarsening of sediments at the same temperature levels revealed a gradually lower biomass of the diatoms. Particularly on sand, the diatoms never reached the same concentrations of chlorophyll *a* as on mud. The cyanobacteria, on the other hand, had the highest biomass on sand at 15°C. In the competition experiments the benthic diatom species *Nitzschia* sp. dominated all types of sediments at 10°C and 15°C. The experiments at 25°C were dominated by the filamentous cyanobacterium *M. chthonoplastes*. This indicates the importance of abiotic conditions for the distribution and abundance of benthic phototrophic micro-organisms.

KEY WORDS: Competition · Cyanobacteria · Diatoms · Temperature · Sand · Mud

INTRODUCTION

Coastal shallow areas are among the most productive ecosystems in the world. Main primary producers in these systems are both planktonic and benthic cyanobacteria and diatoms. There is growing interest concerning the response of phototrophic communities to the increasing eutrophication in coastal areas. This has promoted an increasing knowledge of the ecology of phytoplankton organisms (Sommer 1994) and their benthic analogs (Krumbein et al. 1994). However,

there is still a shortage of information about the ecology of microphytobenthic communities. One reason may be that there are still problems with the handling of benthic micro-organisms in batch and chemostat cultures. However, some publications suggested new culture designs (Fairchild & Lowe 1984, Pringle & Bowers 1984, Krumbein et al. 1991, Sundbäck & Snoeijs 1991).

Several authors stress the importance of nutrient limitation controlling the presence or absence of phytoplankton organisms (Tilman 1977, Sommer 1994). Similarly, it may be of interest to understand the factors controlling the composition and productivity of micro-

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benthic primary producers. Admiraal (1977) and Colijn & van Buurt (1975) reported that benthic diatoms are the most important components of mud flats of the Ems-Dollart estuary. Studying mud flats of the Dutch Wadden Sea, Admiraal (1977) and Admiraal et al. (1982) found that nutrients (P, N, Si) were not growth-rate limiting. These authors assumed that there was no direct relationship between the nutrient concentration and the distribution of benthic diatoms. Meyer-Reil (1994) considered the high allochthonous and autochthonous input of organic matter into the Wadden Sea and reached similar conclusions. However, the existence of nutrient limitation had so far only been shown experimentally for microalgae inhabiting sediments (Sundbäck & Snoeijs 1991, Nilsson 1995) or growing on hard substrates (Hillebrand & Sommer 1997). Till recently, mud flats have been the main study sites of benthic diatoms. It seems important to extend research to sandy deposits, which generally are more nutrient depleted than mud flats (Krumbein et al. 1994). On intertidal nutrient-poor sandflats, phototrophic micro-organisms with the ability for aerobic nitrogen fixation are most favoured. Cyanobacteria were found to be resistant to nutrient stress (Villbrandt et al. 1990, Villbrandt 1992) Schulz (1937) and Hoffmann (1942) described the occurrence of cyanobacteria in high-level sandy tidal flats. The term 'Farbstreifen-Sandwatt' derives from these studies. Gerdes et al. (1985, 1987) and Krumbein (1987) conducted various studies on the 'Farbstreifen-Sandwatt' biotopes. Cyanobacteria, on the other hand, may also occur on mud flats, although information is scarce. Colijn & van Buurt (1975) mentioned in a subordinate remark 'only during the summer months do blue-greens occasionally occur'. Thus, little is still known about factors that may control the distribution and ecological response of both groups of microbenthic phototrophic micro-organisms, cyanobacteria and diatoms, in the depositional environments of the Wadden Sea area.

This paper presents results of studies on the growth and competition of benthic cyanobacteria and diatoms using different sedimentary grain sizes at different temperatures. The aim of the studies was to contribute to a better understanding of factors controlling the distribution of benthic phototrophic micro-organisms. A further purpose was to test the hypothesis that diatoms preferentially colonize on mud-sized, and cyanobacteria on sand-sized, sediments.

MATERIAL AND METHODS

Test organism. Benthic cyanobacteria: The following species were used for experimental treatment: *Merismopedia punctata* (Strain 86), *Microcoleus chtho-*

noplastes (Strain 11) and *Oscillatoria limosa* (Strain 23). All these species occur on Mellum Island tidal flats (southern North Sea coast). *M. punctata* (Meyen) is a coccoid cyanobacterium that colonizes sandy tidal flats even in the lower intertidal zone. *M. chthonoplastes* (Gomont) and *Oscillatoria limosa* (Gomont) are filamentous species that mainly colonize the high eulittoral zones of sandy tidal flats. Both species form mats (fibrous coatings on sedimentary surfaces; Krumbein 1983). *O. limosa* is a pioneer organism due to its nitrogen-fixing capacity (Stal & Krumbein 1987). Paerl et al. (1996) assume that *Microcoleus* sp. is a nitrogen fixer and have shown that it can produce anaerobic microsites within bundles and layers of filaments. *M. chthonoplastes* does not have heterocysts, but some authors indicate that this species is able to fix nitrogen under anoxic conditions (Krumbein & Stal 1991). The cyanobacteria used for this study were isolated by the working group of W. E. Krumbein, University of Oldenburg. All 3 species grew in liquid cultures in the medium ASN III (Rippka et al. 1979, Stal & Krumbein 1985, Palinska et al. 1996).

Benthic diatoms: The species *Nitzschia* sp. and *Phaeodactylum tricornutum* were used for the experiments. Nitzschiaceae are very characteristic diatoms of the Wadden Sea. Specimens of *Nitzschia* sp. were isolated from a mud flat near Dangast, Jade Bay (southern North Sea coast) by the working group of W. E. Krumbein. The agar plating technique was used (Medium 5, Schlösser 1994) to obtain monospecific cultures. *P. tricornutum* was supplied by the SAG-algae collection at the University of Göttingen. This species occurs in planktonic as well as in benthic communities (Round et al. 1990). All cultures used were non-axenic. All organisms were cultured in liquid medium prior to the experiments; thus no pre-adaptation to grain sizes occurred.

Culture techniques. All experiments were performed with 3 replicates in an enriched sea water medium, based on Medium 5 (Schlösser 1994). Filtered North Sea water (salinity 30‰) was supplied with the following additions: 1.98 $\mu\text{mol l}^{-1}$ KNO_3 , 0.1 $\mu\text{mol l}^{-1}$ K_2HPO_4 and 0.08 $\mu\text{mol l}^{-1}$ $\text{MgSO}_4 \times \text{H}_2\text{O}$. To this solution 5 ml micronutrients (see ASN III, Rippka et al. 1979) and 30 ml soil extract per litre were added. The pH value was 8. It is generally agreed that cyanobacteria prefer ASN III or BG 11 (Rippka et al. 1979, Stal & Krumbein 1985). However, to maintain comparable conditions Medium 5 was chosen for both cyanobacteria and diatoms. The successful growth of cyanobacteria on Medium 5 was detected in pilot tests (Watermann 1996).

The organisms were transferred to 3 different kinds of sediment (1) Sand (63 to 200 μm), (2) Mud I (mixed fine sand and Mud <63 μm in the ratio 80:20 wt %),

Table 1. Properties of sediments mixed for the experiments. The 3 mixtures (Sand, Mud I and Mud II) consisted of different proportions of different size classes expressed in phi values (negative logarithm of the bound dimensions of grain size classes to the base 2). Proportional sand and mud fractions in wt %

| Classification | Medium sand | | Fine sand | | | Mud | | |
|----------------|-------------|---------|-----------|---------|---------|---------|---------|---------|
| | Phi-values | 1.0–1.5 | 1.5–2.0 | 2.0–2.5 | 2.5–3.0 | 3.0–3.5 | 3.5–4.0 | 4.0–4.5 |
| Sand | | 0 | 11 | 22 | 61 | 6 | 0 | 0 |
| Mud I | | 0 | 0 | 2 | 3 | 43 | 32 | 20 |
| Mud II | | 0 | 0 | 3 | 5 | 23 | 19 | 50 |

and (3) Mud II (mixed fine sand and mud in the ratio 50:50 wt %) (Table 1). The sediments were dried, sieved and autoclaved. The sand was collected in the 'Farbstreifen-Sandwatt' of Mellum and used in its natural composition. The mud was collected from a mud flat in the Jade Bay. It was sieved in order to produce single fractions. These were weighed and mixed into the mud types I and II (Table 1).

The non-axenic monospecific cultures of cyanobacteria and diatoms were cultivated in 100 ml Erlenmeyer flasks containing a 1 cm layer of sediment with 40 ml of medium added. The liquid phase above the sediment was initially 2 cm. The monospecific cultures grew in a semi-continuous system. They were diluted every 5 d by suspending the cells in the medium and removing 40% of the suspension and the sediment (Admiraal et al. 1982). From one part of the removed sediment the chlorophyll *a* content was measured; the other part was diluted and the cell numbers were determined. After resettling, medium and substrate were replaced. All work was done under a clean bench.

The competition experiments were conducted in Erlenmeyer flasks and inoculated with 5 species. Equal volume of a well-suspended culture of each species was pipetted into the experimental flasks. Therefore the proportion of species was equal for each replicate. Sampling was done as in the monospecific cultures, leading to 40% renewal of sediment and medium every fifth day. Cell numbers were determined in the competition experiments by cells per ml. Later, cell counts were transformed to biovolume by assigning nearest geometric shapes to the cells and measurement of 20 cells or trichomes in the microscope (Hillebrand et al. 1999).

Laboratory conditions. The cultures were grown in incubators at 10, 15 and 25°C, respectively. Extra cool white fluorescent tubes (Philips TLD 18W/86) provided a quantum irradiance of 90 $\mu\text{E m}^{-2} \text{s}^{-1}$. The experiments were performed under a light period of 16 h d^{-1} . The monospecific cultures were grown either 20 or 25 d, the competition experiments lasted for 30 d.

Biomass estimates. The chlorophyll *a* content of the cultures was measured using the method of Lorenzen

(1967). For each replicate, chlorophyll *a* was determined in triplicate. The pigments were extracted from sediment in 90% acetone and measured spectrophotometrically at wavelengths of 665 and 750 nm before and after acidification. The chlorophyll *a* contents were used as an alternative to counting, with errors assumed to be high in sediment samples. In the competition experiments besides the chlorophyll *a* content the numbers of

cells or trichomes were determined. Before counting cell numbers, 10 ml of the suspension from the cultures and the diluted sediment were treated in a sonication bath for 2 min (35 kHz). A defined amount dependent on the sediment type was filled in counting chambers and the cells fixed with Lugol's iodine solution. The counting was done using an inverse microscope (Utermöhl 1958). Four hundred cells were counted (Lund et al. 1958) to reach a 95% confidence interval of $\pm 10\%$. Chlorophyll *a* contents were expressed as μg per dry weight of sediment.

Statistics. Two-factor ANOVA was conducted with the chlorophyll *a* values at Day 20 for all monospecific cultures (Software: Statistica 5.1), with sediment type and temperatures as factors. To assess significant impact of treatments, a Tukey-HSD test was performed for all monospecific cultures and for total biovolume of all species in the competition experiments for every significant ANOVA-factor (Software: Statistica 5.1).

RESULTS

Effects of temperature and grain size on the growth of monospecific cultures

The 3 species of benthic cyanobacteria grew very slowly at 10°C (Fig. 1a to c) on all the sediment types used (Table 1). The set-up at 10°C revealed the lowest values of all chlorophyll *a* concentrations measured. The cyanobacteria showed a clear increase in chlorophyll *a* contents at temperatures of 15 and 25°C (Fig. 1a to c). The chlorophyll *a* content of *Microcoleus chthonoplastes* was less than 1 $\mu\text{g g}^{-1}$ at the end of the experiment at 10°C (Fig. 1b). The sediment coarseness indicated no significant growth differences with respect to low chlorophyll *a* contents.

Chlorophyll *a* contents at 15°C of all tested species of cyanobacteria increased as compared to 10°C (Fig. 1a to c). Between the sediment types used, the growth revealed differences. Both filamentous species showed higher biomass on sand compared to mud. In a relatively short incubation time, *Meris-*

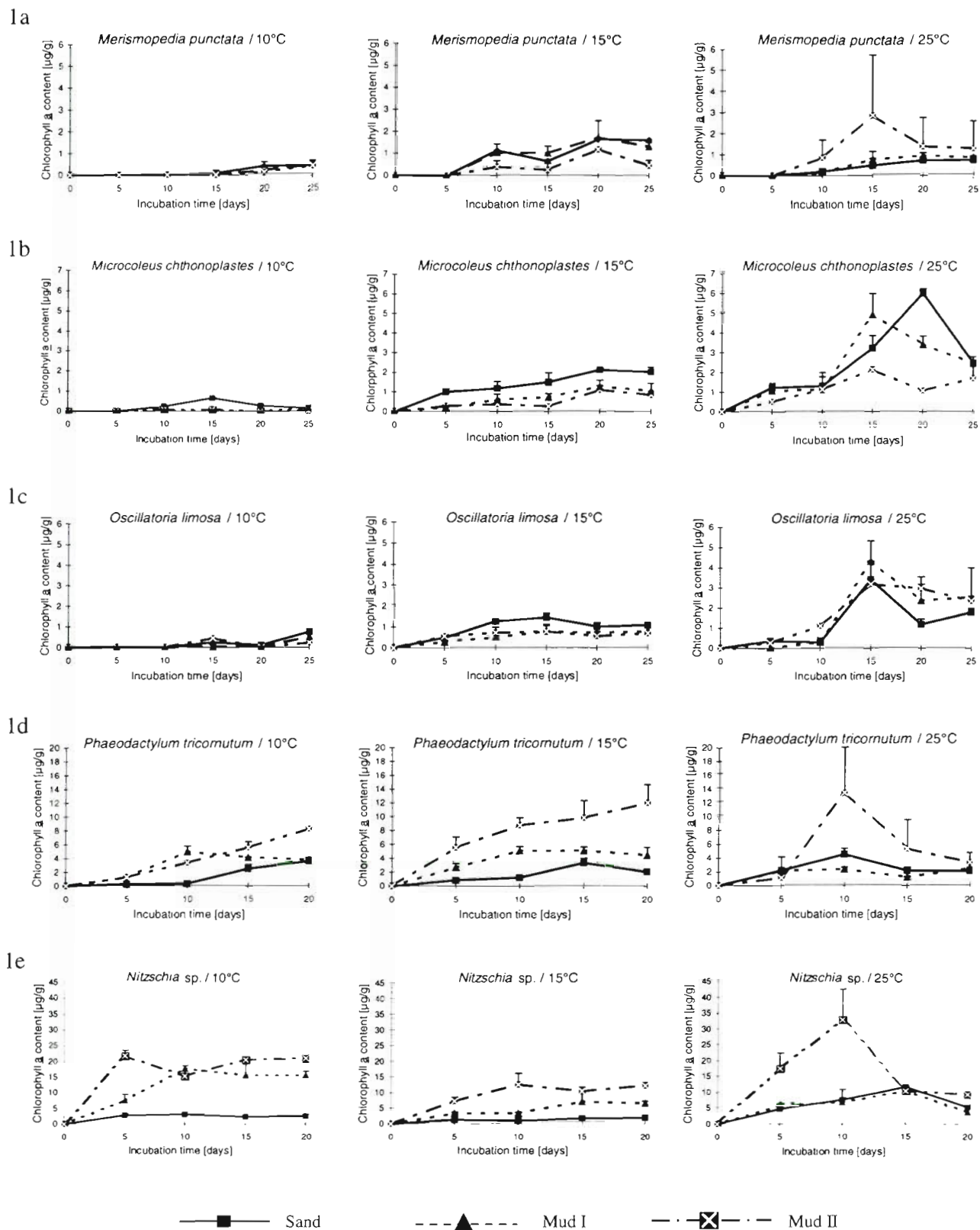


Fig. 1 The influence of different grain sizes (see Table 1) and temperatures (10, 15 and 25°C) on growth expressed as chlorophyll a of benthic cyanobacteria and diatoms species (mean ± SD)

merismopedia punctata and *Oscillatoria limosa* achieved chlorophyll *a* increases. This may confirm the pioneer state of *O. limosa* (Stal & Krumbein 1987). *M. chthonoplastes* reached the highest concentration after 20 d (Fig. 1 a to c).

At 25°C all 3 species achieved their highest chlorophyll *a* concentrations. Differences between the sediment types still existing at 15°C disappeared (Fig. 1a to c), indicating that at 25°C, grain size may not longer control the growth behaviour of the cyanobacteria tested; the substrate factor is apparently overruled by temperature effects.

At 10°C the 2 diatom species both grew fairly well on the mud types tested (Fig. 1d to e). Mainly for *Nitzschia* sp., the difference between growth on sand and on mud was significant (Fig. 1e). *Phaeodactylum tricorutum* lives under benthic as well pelagic conditions, revealed less clear differences. In general, the cultures of this species achieved a lower total chlorophyll *a* content, and the differences between the sediments were lower compared to *Nitzschia* sp. Nevertheless, for this species as well there was a clear chlorophyll *a* increase visible for micro-organisms inhabiting mud sediments.

The results at 15°C were comparable to those of the 10°C treatments for the 2 diatom species. All sediment types revealed differences. In all measurements at 15°C, growth of organisms was lower if cultured on the sandy substrate (Fig. 1d to e).

At the beginning of the 25°C experiment, the diatom species grew well on the fraction of the finest grain size (Fig. 1d to e). But after 10 d, the biomass decreased, although the total chlorophyll *a* content on Mud II was the highest relative to all values obtained. At the end of the incubation time, the content was almost the same for all treatments. Thus, a comparison of the 3 sediment types showed that the differences in the chlorophyll *a* concentrations almost disappeared at 25°C.

After 20 d, the monospecific cultures showed significantly different biomass values with respect to temperature and sediment type (2-factor ANOVA, Table 2). The cyanobacteria were generally favoured by higher temperatures (Fig. 2), especially distinct for *Oscillatoria limosa* and *Microcoleus chthonoplastes* (significant Tukey HSD-test for all 3 species, $p < 0.05$). The coarseness of the sediment favoured *M. chthonoplastes* at every temperature, but had no significant effect on *M. punctata* and *O. limosa*, although for the latter the low temperature cul-

ture developed better on sand than on mud (significant interaction term, Table 2). Both treatments had substantial impact on the 2 diatom species (Fig. 2) which performed better at 10 or 15°C than at 25°C, and better on the Mud II sediment compared to Mud I and Sand. Significant differences were confirmed for *Nitzschia* sp. for all 3 sediment types and for the lowest temperature (Tukey HSD-test, $p < 0.05$), whereas 15 and 25°C were not significantly different. *Phaeodactylum tricorutum* showed significantly higher growth on Mud I compared to Sand and Mud II at 15°C.

Effects of competition on the growth of selected microbenthic primary producers (mixed cultures)

The total chlorophyll *a* content of the sediments is represented in Fig. 3, and the biovolume proportions of each individual species to the total biovolume are documented in Figs. 4 to 6. Total chlorophyll *a* content was significantly dependent on sediment type and temperature (Table 3). At 10°C, the culture was dominated by *Nitzschia* sp. (Fig. 4). *Phaeodactylum tricorutum* survived in low cell numbers. All cells of the 2 species of filamentous cyanobacteria disappeared after 10 d. Only low cell numbers of the coccoid cyanobacterium *Merismopedia punctata* were still alive. The total chlorophyll *a* content of the sediments indicates highest values for Mud II, the muddiest sediments used. This is due to the dominance of *Nitzschia* sp., which developed higher chlorophyll *a* concentrations, as can be seen in the unialgal cultures (Fig. 1e). The pattern

Table 2. Results for the experiments with unialgal cultures as analyzed with 2-factor ANOVA with sediment type and temperature as independent factors and chlorophyll *a* concentration on Day 20 as the dependent variable. The table lists species name, variable and the mean squares and degrees of freedom of the measured effects. *F*-ratio is given with significance level (ns: not significant, ** $p < 0.01$, *** $p < 0.001$)

| Species | Variable | MS effect | df | MS error | df | <i>F</i> -ratio |
|-----------------------------------|-------------|-----------|----|----------|----|-----------------|
| <i>Merismopedia punctata</i> | Sediment | 0.0004 | 2 | 0.115 | 18 | 0.004 ns |
| | Temperature | 3.617 | 2 | 0.115 | 18 | 31.553 *** |
| | Interaction | 0.330 | 4 | 0.115 | 18 | 2.880 ns |
| <i>Oscillatoria limosa</i> | Sediment | 0.384 | 2 | 0.206 | 18 | 1.868 ns |
| | Temperature | 10.659 | 2 | 0.206 | 18 | 51.837 *** |
| <i>Microcoleus chthonoplastes</i> | Interaction | 1.119 | 4 | 0.206 | 18 | 5.441 ** |
| | Sediment | 9.580 | 2 | 0.068 | 18 | 140.45 *** |
| | Temperature | 26.328 | 2 | 0.068 | 18 | 386.04 *** |
| <i>Nitzschia</i> sp. | Interaction | 4.961 | 4 | 0.068 | 18 | 72.749 *** |
| | Sediment | 244.529 | 2 | 1.130 | 18 | 216.46 *** |
| | Temperature | 122.233 | 2 | 1.130 | 18 | 108.20 *** |
| <i>Phaeodactylum tricorutum</i> | Interaction | 49.570 | 4 | 1.130 | 18 | 43.881 *** |
| | Sediment | 70.870 | 2 | 2.001 | 18 | 35.442 *** |
| | Temperature | 28.419 | 2 | 2.001 | 18 | 14.203 *** |
| | Interaction | 15.954 | 4 | 2.001 | 18 | 7.973 *** |

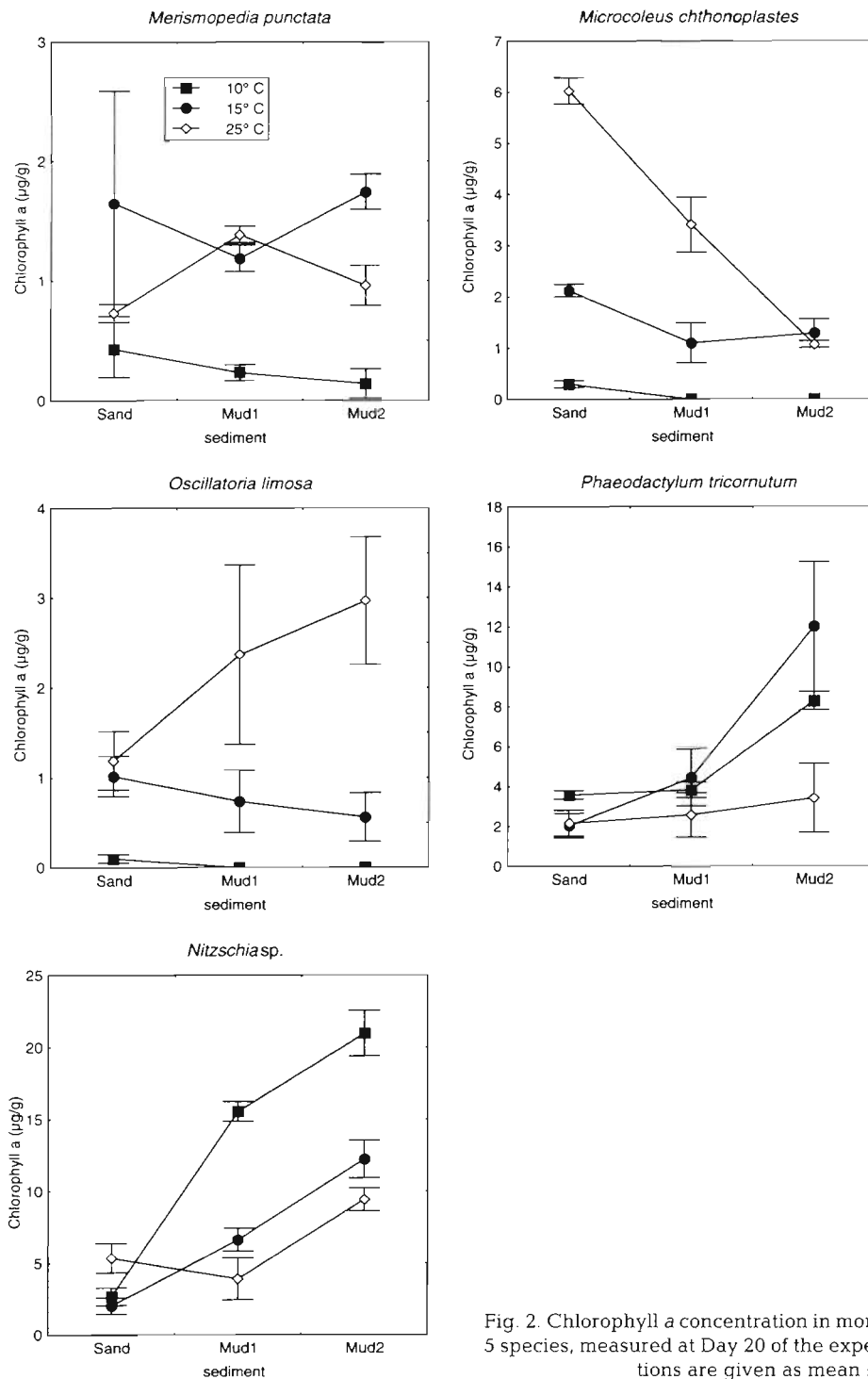


Fig. 2. Chlorophyll a concentration in monospecific cultures of 5 species, measured at Day 20 of the experiments. Concentrations are given as mean \pm SE

of species development was similar between the unialgal cultures of both diatom species and their performance in the competition experiments at this and the other temperatures.

At 15°C, the cultures were still dominated by *Nitzschia sp.* The growth rate was highest on Mud II,

and lowest on Sand (Fig. 3). Concerning the cyanobacteria, only *Oscillatoria limosa* was never observed following Day 5, although it was inoculated. *Microcoleus chthonoplastes* slightly increased on the sandy substrate at the end of the experimental time, however, at a much lower density than *Nitzschia sp.* (Fig. 5).

Table 3. Results of the competition experiments as analyzed with 2-factor ANOVA on total chl a concentration on Day 20 as dependent on the factors sediment type and temperature. For details see Table 2

| Variable | MS effect | df | MS error | df | F-ratio |
|------------------------|-----------|----|----------|----|----------|
| Sediment | 7.87 E14 | 2 | 7.68 E13 | 18 | 10.25** |
| Temperature | 2.07 E15 | 2 | 7.68 E13 | 18 | 26.92*** |
| Sed × temp interaction | 9.86 E14 | 4 | 7.68 E13 | 18 | 12.84*** |

At 25°C, chlorophyll a contents were relatively low (Fig. 3) compared to the measurements at lower temperatures. The culture was dominated by the filamentous cyanobacterium *Microcoleus chthonoplastes* (Fig. 6). The diatom species *Phaeodactylum tricornutum* was not observed. *Nitzschia* sp. was still observed after 20 d on the mud fractions (Fig. 6) but disappeared after 30 d.

There was a positive correlation between the low chlorophyll a content in the competition experiment and the low content in the experiments with mono-specific cyanobacterial cultures. As a result of increased temperature, indications of substrate-specific growth disappeared (Figs. 4 to 6). This had already been observed for the mono-cyanobacterial cultures of *Microcoleus chthonoplastes* at the same temperature.

DISCUSSION AND CONCLUSIONS

One major aim of the present study was to test the hypothesis that benthic diatoms prefer muddier sediments (Colijn & van Buurt 1975, Admiraal 1977), and cyanobacteria prefer coarser sediments for colonization (Krumbein et al. 1994). The results confirm that grain sizes play

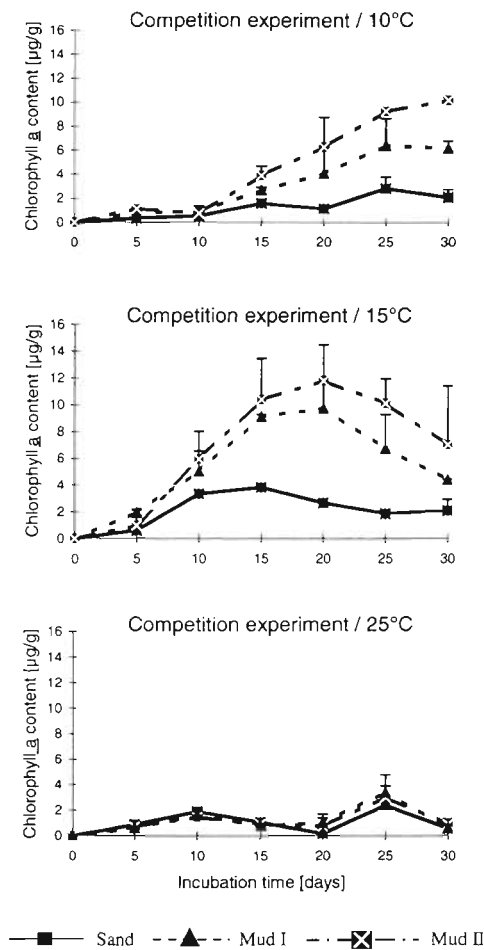


Fig. 3. Total chlorophyll a concentration (mean ± SD) of the competition experiment at 10, 15 and 25°C for different sediment types

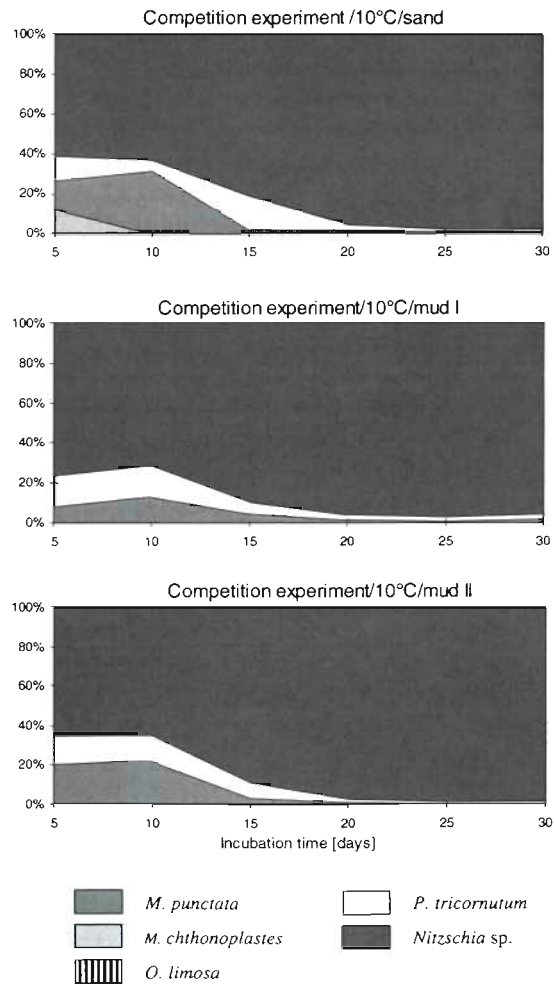


Fig. 4. Proportion of species in terms of biovolume in competition experiments at 10°C for different sediment types

an important role for the distribution and abundance of microbenthic primary producers. Besides grain size temperature is also an important factor. Apparently, this is particularly important for species of the epipelon and was a very important factor determining the distribution of benthic cyanobacteria and diatoms. The importance of these abiotic conditions for sediment-inhabiting species becomes evident from the significant interaction term of ANOVA (Table 2) The growth-enhancing effect of low temperature for *Nitzschia* sp. becomes less obvious if the sediment type is unfavourable. On the other hand, favourable temperature conditions and favourable sediment types show synergistic effects on the growth of *Microcoleus chthonoplastes*. It became obvious that the chlorophyll *a* concentration of the sediment in the monospecific cyanobacteria cultures (Fig. 2) was less than in comparable diatom cultures. In the competition experiments as well, lower total chlorophyll *a* was measured

where cyanobacteria were dominant (Fig. 3).

Since the 3 cyanobacteria species did not grow well at 10°C, even in monospecific cultures, the results of the experiments with all 5 species together may not indicate competition (negative influence). Furthermore the higher chlorophyll *a* content of the *Nitzschia* monocultures compared to the competition experiment at 10°C indicated the influence of factors other than competition. However, results of the experiments at 15 and 25°C do indicate competition between cyanobacteria and diatoms, because all species were able to grow on the 3 kinds of sediment at 15 and 25°C in the monospecific cultures (Fig. 1a to e). This was also confirmed by the observation that in the monospecific cultures both cyanobacteria and diatoms build visually detectable biofims. A biofilm is defined as being composed of cells and extracellular polymeric substances [EPS] immobilised at a substratum and additionally

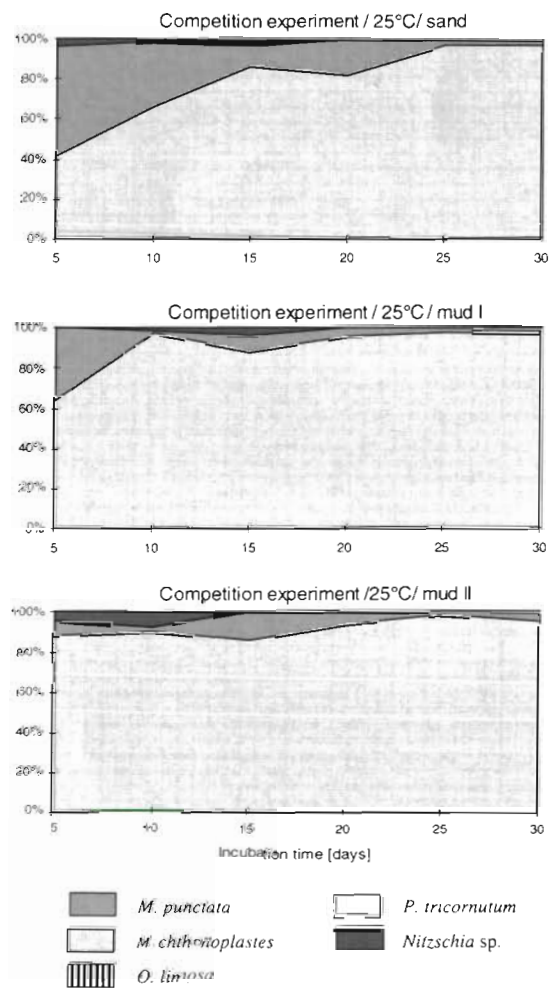
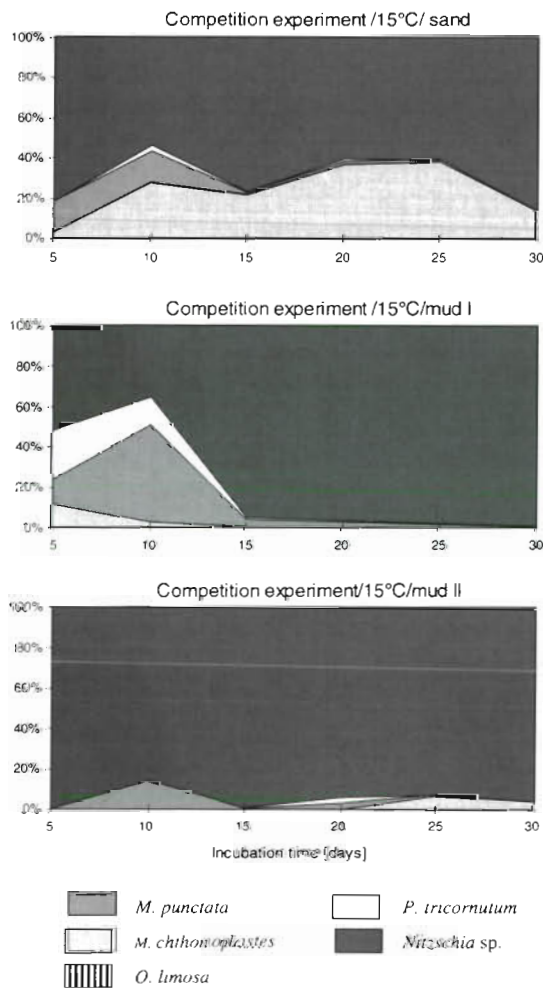


Fig. 5. Proportion of species in terms of biovolume in competition experiments at 15°C for different sediment types

Fig. 6. Proportion of species in terms of biovolume in competition experiments at 25°C for different sediment types

(Characklis & Marshall 1990). In the competition experiment, however, *Nitzschia* sp. was more successful at 15°C (80 to 90% of total biovolume), whereas *Microcoleus chthonoplastes* dominated the biofilm at 25°C (97 to 98% of total biovolume).

The results of the competition experiments corresponded to the patterns seen in the monospecific cultures, with cyanobacteria favoured by high temperature and coarser sediment and diatoms dominating at low temperatures and on mud. The preference of epipelagic diatoms for smaller grain size may be due to their mobile lifestyle. With larger grain size, the increased interstitial space of the sediment may be too large to support raphe-based gliding. Both cyanobacteria and diatoms have high migration rates. Round (1971) reported migration rates for 'slow' diatom species of 1.0 to 5.2 $\mu\text{m s}^{-1}$ and for 'fast' ones of 120 to 140 $\mu\text{m s}^{-1}$. Studying migration rates of the cyanobacterium *M. chthonoplastes*, Whale & Walsby (1984) measured a net migration rate of 0.08 $\mu\text{m s}^{-1}$, and Carius (1990) one of 0.04 $\mu\text{m s}^{-1}$. The flaky texture of muddy sediments may play a decisive role for the motility of phototrophic micro-organisms. Paterson (1994) emphasized that epipelagic diatoms usually are motile, while epipsammic diatoms live attached to the substratum. Filamentous cyanobacteria such as those used in this paper may be hindered by the texture and the small grain sizes of mud in both attachment and migration to the sediment surface. Cyanobacteria are photo- (Häder 1987) and chemotactic organisms (Richardson & Castenholz 1987). Geotactic responses are not known for cyanobacteria (Whale & Walsby 1984). Diatoms show photo-, chemo- and geotaxis (Admiraal 1984).

Another factor correlated with grain sizes is light penetration into sediments. Depending on the grain size and organic content, light irradiance in sandy sediments decreases to 1 percent at a depth of 2 to 3 mm (Rasmussen et al. 1983). On the other hand, in fine estuarine mud the same light absorption can be observed in a considerably thinner surface layer of 0.14 mm (Admiraal 1984). Thus, light irradiance rapidly disappears with depth in mud. Since light is the major limiting factor for primary production, benthic photosynthesis in muddy sediments is limited to a very thin photic zone (<2 mm; Pinckney 1994). Hence, due to their high migration rates, geotaxis and high cell division rates, the diatom species studied in this paper have an advantage for occupying the muddy sediment habitats, particularly at lower temperatures.

Our experiments clearly showed the influence of grain size and temperature on microbenthic species composition. However, it should be kept in mind that the outcome of competition between these species might be influenced by other factors as well. Grain size is correlated to nutrient content of the pore water

in situ, whereas in our experiments all sediments were washed and nutrients were supplied only with liquid media. Washing and autoclaving the sediment also altered other sediment properties (e.g. physicochemical gradients) which may be important in the field. Furthermore, natural sediments are influenced by water movement, which was excluded in our experiments. Finally, competitive dominance in the natural habitat is also determined by biotic interactions, e.g. herbivory.

The role of temperature was also evidenced by the results of the competition studies. At 10°C, the results were unequivocal. Growth rates of the cyanobacteria were low and those of diatoms high. Only after 10 d was *Microcoleus chthonoplastes* detected in the sand cultures while the diatoms dominated all 3 kinds of sediments. These results correspond to observations in the field (Watermann unpubl.), and may contribute to explaining the high biomass of diatoms observed in spring and autumn (Underwood & Paterson 1993). For diatoms it seems possible that they utilize high temperatures for active photosynthesis, but require a lower temperature (at night) to complete cell division at high rates (Admiraal 1977). This condition was not given in the experiment at 25°C in which the temperature was constantly high.

Microcoleus chthonoplastes dominated all 3 kinds of sediments used in the 25°C experiments, indicating increased growth rates. Competitive dominance of cyanobacteria at higher temperatures has previously been found in phytoplankton (Tilman et al. 1986) and microphytobenthic studies (Sundbäck & Snoeijs 1991). The dominance of *M. chthonoplastes* was independent of the substrate type at highest temperatures. In the competition experiments the temperature effects overrode the effects of the sediment type, although there was a significant interaction between both factors in monospecific cultures (Table 2). The *in vitro* results corresponded with field observations presented by Colijn & van Buurt (1975), who observed a bloom of cyanobacteria on mud in summer. Normally every 12 h the intertidal sediments of the Wadden Sea become flooded and cooled by the tides. Especially *M. chthonoplastes* is prone to survive at high temperatures and salinity in extreme summer conditions.

Several cultures growing at high temperatures showed a decrease of total biomass at the end of the experiments. Since our cultures were not axenic, it can be assumed that under high temperatures nutrient limitation was achieved more quickly and induced bacterial degradation of biomass.

Microcoleus chthonoplastes may replace the diatoms at high temperatures by sealing of the sediment surface. The filamentous cyanobacteria *M. chthonoplastes* and *Oscillatoria limosa* are true microbial mat

builders. Microbial mats are defined as fibrous coatings of sediments and rocky surfaces, which by their morphology, physiology, and arrangement in space and time interact with the physical and chemical environment to produce a laminated pattern (Krumbein 1983). The characteristic patterns of laminated biomass intertwined with siliciclastic grains is a typical phenomenon of sandy deposits, which however is usually lacking on mud. Here, the filamentous cyanobacteria only build surficial biofilms (Paterson 1994).

Due to their EPS production and motility, the diatoms also produce a biofilm that coats the mud. Such a biofilm is relatively resistant to disturbances (Madsen et al. 1993), and stabilizes the substrate (Paterson 1989, Krumbein et al. 1994). Compared to the grain size of sand, the size of diatoms is relatively small so that diatoms usually form biofilms around single grains. This stabilizes sedimentary surfaces less effectively against erosion than the filamentous network of cyanobacteria. In the competition experiments at 15°C, *Microcoleus chthonoplastes* appears on sand towards the end of the experiment. This is not surprising since the mono-specific cyanobacterial cultures of *M. chthonoplastes* at 15°C also show higher growth rates on sand. This suggests that *M. chthonoplastes* has an advantage on sand, probably due to its production of EPS in the form of homomers. In contrast, the diatoms produce a heteropolymer (Underwood & Paterson 1993). The mats dominated by cells and EPS of *M. chthonoplastes* effectively stabilize the sand.

In conclusion, these experiments strengthen the importance of abiotic conditions for sediment-inhabiting phototrophic micro-organisms. On the other hand, nutrients have not been tested in these experiments. In other experiments with epilithic microflora (Hillebrand & Sommer 1997) or with nutrient enrichment of sediments and the water column (Sundbäck & Snoeijs 1991) it was shown that nutrient supply can control the biomass and species composition of benthic autotrophs. The dependence of the growth of phototrophic micro-organisms on grain size, temperature and nutrient content has to be tested independently, in order to be sure that results of nutrient experiments are not caused by the abiotic conditions used.

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LITERATURE CITED

- Admiraal W (1977) Influence of light and temperature on the growth rate of estuarine benthic diatoms in culture. *Mar Biol* 39:1–9
- Admiraal W (1984) The ecology of estuarine sediment-inhabiting diatoms. *Prog Phycol Res* 3:269–322
- Admiraal W, Peletier H, Zomer H (1982) Observations and experiments on the population dynamics of epipelagic diatoms from an estuarine mud flat. *Estuar Coast Shelf Sci* 14:471–487
- Carius RT (1990) Interaktionen zwischen den mattenbildenden Cyanobakterien *O. limosa* und *M. chthonoplastes* und Versuche zur Mikrobienmattengenesse. Masters thesis, Universität Oldenburg
- Characklis WG, Marshall KC (1990) Biofilms. In: Cohen Y, Rosenberg E (eds) *Microbial mats*. American Society of Microbiology, Washington. John Wiley & Sons, Chichester
- Colijn F, van Buurt G (1975) Influence of light and temperature on the photosynthetic rate of marine benthic diatoms. *Mar Biol* 31:209–214
- Fairchild GW, Lowe RL (1984) Artificial substrates which release nutrients: effects on periphyton and invertebrate succession. *Hydrobiol* 114:29–37
- Gerdes G, Krumbein WE, Reineck HE (1985) Verbreitung und ökogeologische Bedeutung mariner mikrobieller Matten im Gezeitenbereich der Nordsee. *Facies* 12:75–94
- Gerdes G, Krumbein WE, Reineck HE (1987) Mellum Portrait einer Insel. Verlag Waldemar Kramer, Frankfurt am Main
- Häder DP (1987) Photosensory behavior in prokaryotes. *Microbiol Rev* 51:1–21
- Hillebrand H, Sommer U (1997) Response of epilithic microphytobenthos of the Western Baltic Sea to *in situ* experiments with nutrient enrichment. *Mar Ecol Prog Ser* 160:35–46
- Hillebrand H, Dürselen CD, Kirschtel D, Pollinger U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. *J Phycol* 35:403–424
- Hoffmann C (1942) Beiträge zur Vegetation des Farbstreifen-Sandwattes. *Kieler Meeresforsch Sonderheft* 4:85–108
- Krumbein WE (1983) Stromatolites — the challenge of a term in space and time. *Precambrian Res* 20:493–531
- Krumbein WE (1987) Das Farbstreifen-Sandwatt. In: Gerdes G, Krumbein WE, Reineck HE (eds) *Mellum, Portrait einer Insel*. Verlag Waldemar Kramer, Frankfurt am Main, p 170–188
- Krumbein WE, Stal LJ (1991) The geophysiology of marine cyanobacterial mats and biofilms. *Kieler Meeresforsch Sonderheft* 8:137–145
- Krumbein WE, Carius RT, Stal LJ (1991) On the interaction of the two mat-forming cyanobacteria *Microcoleus chthonoplastes* and *Oscillatoria limosa* and laboratory experiments on bundle formation and *de novo* genesis of microbial mats. *Kieler Meeresforsch Sonderheft* 8:146–151
- Krumbein WE, Paterson DM, Stal LJ (1994) Biostabilization of sediments. Bibliotheks- und Informationssystem der Carl von Ossietzky Universität Oldenburg (BIS)
- Lorenzen CJ (1967) Determination of chlorophyll and pheopigments spectro-photometric equations. *Limnol Oceanogr* 12:333–342
- Lund JW, Kipling C, Le Cren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiol* 11:143–170
- Madsen KN, Nilsson P, Sundbäck K (1993) The influence of benthic microalgae on the stability of a subtidal sediment. *J Exp Mar Biol Ecol* 170:159–177
- Meyer-Reil LA (1994) Mikrobielle Besiedlung und Produktion. In: Meyer-Reil LA, Köster M (eds) *Mikrobiologie des Meeresbodens*. Gustav Fischer Verlag, Jena, p 38–81
- Nilsson C (1995) Microphytobenthos communities with emphasis on algal-nutrient relations. Dissertation, University of Göteborg

- Paerl HW, Fitzpatrick M, Bebout BM (1996) Seasonal nitrogen fixation dynamics in a marine microbial mat: potential roles of cyanobacteria and microheterotrophs. *Limnol Oceanogr* 30:419–427
- Palinska KA, Liesack W, Rhiel E, Krumbein WE (1996) Phenotype variability of identical genotypes: the need for a combined approach in cyanobacterial taxonomy demonstrated on Merismopedia-like isolates. *Arch Microbiol* 166:224–233
- Paterson DM (1989) Short-term changes in the erodibility of intertidal cohesive sediments related to the migratory behavior of epipelagic diatoms. *Limnol Oceanogr* 34:223–234
- Paterson DM (1994) Spatial variability in sediment erodibility on the island of Texel. In: Krumbein WE, Paterson DM, Stal LJ (eds) *Biostabilization of sediments. Bibliotheks- und Informationssystem der Carl von Ossietzky Universität Oldenburg (BIS)-Verlag*, p 107–120
- Pinckney J (1994) Development of an irradiance-based eco-physiological model for intertidal benthic microalgal production. In: Krumbein WE, Paterson DM, Stal LJ (eds) *Biostabilization of sediments. Bibliotheks- und Informationssystem der Carl von Ossietzky Universität Oldenburg (BIS)-Verlag*, p 55–83
- Pringle CM, Bowers JA (1984) An *in situ* substratum fertilization technique: diatom colonization on nutrient-enriched, sand substrata. *Can J Fish Aquat Sci* 41:1247–1251
- Rasmussen MN, Henriksen K, Jensen A (1983) Possible causes of temporal fluctuations in primary production of the microphytobenthos in the Danish Wadden Sea. *Mar Biol* 73:109–114
- Richardson LL, Castenholz RW (1987) Diel vertical movements of the cyanobacterium *Oscillatoria terebriformis* in a sulfide-rich hot spring microbial mat. *Appl Environ Microbiol* 53:2142–2150
- Rippka R, Deruelles J, Waterbury JB, Hermann M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* 111:1–61
- Round FE (1971) Benthic marine diatoms. *Oceanogr Mar Annu Rev* 9:83–139
- Round FE, Crawford RM, Mann DG (1990) *The diatoms*. Cambridge University Press
- Schlösser UG (1994) Seawater media. *Bot Acta* 107:176–186
- Schulz E (1937) Das Farbstreifensandwatt und seine Fauna, eine ökologisch-biozoologische Untersuchung an der Nordsee. *Kieler Meeresforsch* 1:359–378
- Sommer U (1994) Are marine diatoms favoured by high Si:N ratios? *Mar Ecol Prog Ser* 115:304–315
- Stal LJ, Krumbein WE (1985) Isolation and characterization of cyanobacteria from a marine microbial mat. *Bot Mar* 28:351–365
- Stal LJ, Krumbein WE (1987) Temporal separation of nitrogen fixation and photosynthesis in the filamentous, non-heterocystous cyanobacterium *Oscillatoria* sp. *Arch Microbiol* 149:76–80
- Sundbäck K, Snoeijs P (1991) Effects of nutrient enrichment on microalgal community composition in a coastal shallow-water sediment system: an experimental study. *Bot Mar* 34:341–358
- Tilman D (1977) Resource competition between planktonic algae: an experimental and theoretical approach. *Ecology* 58:338–348
- Tilman D, Kiesling R, Sterner R, Kilham SS, Johnson FA (1986) Green, bluegreen and diatom algae: taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. *Arch Hydrobiol* 106:473–485
- Underwood GJC, Paterson DM (1993) Seasonal changes in diatom biomass, sediment stability and biogenic stabilization in the Severn estuary. *J Mar Biol Assoc UK* 73:871–887
- Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. *Mitt Int Ver Limnol* 9:1–38
- Villbrandt M (1992) Interaction of nitrogen fixation and photosynthesis in marine cyanobacterial mats (Mellum, Southern North Sea). Dissertation, University of Oldenburg
- Villbrandt M, Stal LJ, Krumbein WE (1990) Interactions between nitrogen fixation and oxygenic photosynthesis in a marine cyanobacterial mat. *FEMS Microbiol Ecol* 74:59–72
- Watermann F (1996) Competition between benthic diatoms and cyanobacteria as influenced by different grain sizes and temperatures. Master thesis, University of Oldenburg
- Whale GF, Walsby AE (1984) Motility of the cyanobacterium *Microcoleus chthonoplastes* in mud. *Br Phycol J* 19:117–123

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