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# Seasonal and interannual patterns of intertidal microphytobenthos in combination with laboratory and areal production estimates

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ABSTRACT: From April 1994 to December 1997, we studied the microphytobenthic assemblages in surface (0 to 0.5 cm) and subsurface (0.5 to 2 cm) sediments at spring low tide along a transect of 5 stations in an estuarine sandflat of the Seto Inland Sea, Japan. At the innermost sampling station, microphytobenthos biomass (chl *a*) was also investigated in a vertical profile to 10 cm depth from December 1994 to April 1996. The chl *a* contents at the 2 uppermost layers were well correlated with each other, with a mean decrease of 34% from the surface to subsurface layer. Chl *a* tended to decrease rapidly through the vertical profile and was reduced to 3.2 ± 1.4% SD in the 9 to 10 cm layer. There was a progressive decrease in the chl *a* content every year in fall and the occurrence of major peaks in early spring and/or summer. This was accompanied by a significant increase in microphytobenthos biomass from 1994 to 1995 and from 1995 to both 1996 and 1997. The microphytobenthos biomass in surface sediments (mean of 5 stations) ranged between 27.7 (October 1994) and 120 mg chl *a* $m^{-2}$ (July 1997), or between 3.9 (November 1994) and 20.3 µg chl *a* $g^{-1}$ dry wt (July 1996). Annual mean (1995 to 1997) biomass was 72.3 ± 27.1 mg chl *a* $m^{-2}$ and 11.0 ± 4.3 µg chl *a* $g^{-1}$ dry wt. These values rank in the mid-upper range of microphytobenthic biomass for intertidal sediments. In addition to the field investigations, we conducted laboratory experiments on a dominant diatom species, *Navicula* sp. The photosynthetic rate of *Navicula* sp. was saturated at a light intensity of 165 µE $m^{-2}$ $s^{-1}$ at 21°C. No photoinibition was found at higher light intensities up to 400 µE $m^{-2}$ $s^{-1}$. The relationship between temperature and photosynthetic rate was positive and linear within a temperature range between 10 and 35°C at 55 µE $m^{-2}$ $s^{-1}$. Areal 'potential' primary production of microphytobenthos was between 0.32 (December 1994) and 3.0 g C $m^{-2}$ $d^{-1}$ (July 1997), with an annual mean of 1.2 g C $m^{-2}$ $d^{-1}$. Uni- (summer) or bi-modal (spring and summer) peaks of microphytobenthos biomass and primary production highlighted a marked interannual variability. Marked seasonal patterns were also recognizable, with primary production of microphytobenthos significantly higher both in spring and summer than in winter and fall.

KEY WORDS: Microphytobenthos · Biomass · Chl *a* · *Navicula* sp. · Primary production · Seasonality · Interannual variability · Tidal flat · Seto Inland Sea



## INTRODUCTION

During the last decade, major interest has arisen on the role of intertidal microphytobenthos as a primary carbon source for estuarine food webs and as an important component in the cycling of nutrients in tidal estuaries (Sullivan & Moncreiff 1990, Heip et al. 1995, MacIntyre et al. 1996, Guarini et al. 1998, Middelburg et al. 2000).

Due to the high variability of benthic microalgae distribution in these ecosystems (Colijn & De Jonge 1984, Lukatelick & McComb 1986, Delgado 1989, Burford et al. 1994, Guarini et al. 1998), it is important that studies on the occurrence and development of microphytobenthic assemblages should be based on detailed and/or extended field observations, also depending on the temporal scale to be investigated. Several reports have focused on the short-term (hours, days) variability of

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microphytobenthos biomass and primary production in relation to their rhythmic vertical migration (Pinckney & Zingmark 1991, Barranguet et al. 1998, Kromkamp et al. 1998), influenced by tidal and light cycles (Pinckney & Zingmark 1991, Dring & Lüning 1994, Smith & Underwood 1998), and tidal currents and waves (Stevenson 1983, Kingston 1999). On a longer term, earlier studies suggested that the annual primary production of intertidal microphytobenthos can be estimated on the basis of relatively few chl *a* samples distributed over the year (Cadée & Hegeman 1977, Colijn & De Jonge 1984). Colijn & De Jonge (1984) however acknowledged that this method only yields a limited insight into the year-round variability of the primary-produced carbon available for estuarine processes (e.g. grazing, burial). More recently, simulation models have been used to calculate the annual primary production of intertidal microphytobenthos based on either monthly (Cammen 1991, Pinkney & Zingmark 1993) or shorter (fortnightly and daily, Serôdio & Catarino 2000) timescale measurements. However, field observations on a longer (interannual) scale are very limited (Cadée & Hegeman 1974, Riaux-Gobin 1985, Peletier 1996). Little information is thus available on the extent of seasonal and interannual fluctuations of intertidal microphytobenthos biomass and primary production in these highly variable ecosystems.

This work is part of a long-term multidisciplinary project which aims to quantify the cycling of biophilic elements (C, N, P, Si) in a tidal estuary of the Seto Inland Sea, Japan, and to assess the role of primary producers (microphytobenthos) and consumers (macrofauna) in this cycling. As an initial phase of this project, we evaluated the short-term (24 h) and seasonal (2 yr) variability of the water chemistry along the estuary (Montani et al. 1998, Magni & Montani 2000, Magni et al. 2002). In parallel, we investigated the spatial/temporal distribution of macrofaunal communities on the intertidal and the subtidal zones of this estuary (Magni 1998, Magni & Montani 1998) and quantified their contribution on the processes of nutrient regeneration within the estuary (Magni et al. 2000b). The present study is an extension of a bi-weekly survey which lasted 13 mo (July 1993 to July 1994) and was carried out on the same flat, and investigated the development of microphytobenthic assemblages at an upper and a lower intertidal station differing in elevation and grain-size composition (Magni & Montani 1997). Here, we will focus on the seasonal and interannual variability of microphytobenthic assemblages in the lower part of the intertidal zone based on extended field observations over a 4 yr period. Additionally, in the laboratory we conducted incubation experiments on the photosynthetic rates of a dominant microphytobenthic species (*Navicula* sp.) collected from the study area. Using an indirect approach, we will apply these rates to the microphytobenthic biomass found in the field and photosynthetically competent to evaluate the seasonal and/or interannual patterns of both biomass and primary production of microphytobenthos over a period spanning from April 1994 to December 1997.

## MATERIALS AND METHODS

**Field surveys.** ***Study area and sampling procedure:*** We carried out the field investigations on an intertidal flat in the Seto Inland Sea, SW Japan (Fig. 1). The flat is located in a mixed-semidiurnal type estuary with a mean tidal range of ca. 2 m (Montani et al. 1998, Magni et al. 2002). Complete emersion of the sampling site occurs twice a month during a spring low tide at ca. +50 cm the local (Takamatsu Port) mean sea level (Magni & Montani 1998). We fixed a transect line of 5 stations (Stns B1 to B5) set at 25 m regular interval (slope of ca. 30 cm toward the seashore) between the LWL (low water level) and the ELWL (extreme low water level) (Fig. 1). The sediments are sandy and relatively homogeneous with a mud fraction (<0.063 mm) comprising <3% of the total weight. Sampling activities were always conducted during a spring low tide of every month to ensure protocol uniformity throughout the 4 yr study period and to minimize within-day variability in the distribution of microphytobenthos (Pinckney & Zingmark 1991, Barranguet et al. 1998, Kromkamp et al. 1998). On each sampling occasion, we randomly collected sediment samples at 7 to 8 locations of each station using an acrylic core tube (3 cm in diameter) which was gently pushed by hand into the sediment. The sediment was carefully extruded and the surface (0 to 0.5 cm) and subsurface (0.5 to 2 cm) layers sliced off. Sediment samples from the same layer were pooled and brought to the laboratory within 2 h for further treatment and analysis. Additionally, at Stn B5, we collected sediment samples through the sediment column (surface: 0 to 0.5; subsurface: 0.5 to 2 cm; and each following cm to 10 cm depth).

During sampling activities, we also monitored salinity, temperature and dissolved oxygen (DO) concentration of ebbing water at a shallow creek (20 to 50 cm in depth) formed during low tide (Stn H1, Fig. 1), where nutrients and suspended particulate matter were also investigated between 1994 and 1996 (Magni & Montani 2000). Hydrological measurements were made using a portable salinometer (YSI model 30) and DO meter (UK 2000), respectively. Data of monthly rainfall and solar radiation were obtained from the Takamatsu Meteorological Agency Station, located near the flat under investigation.

We conducted the field surveys on a monthly basis from April 1994 to December 1997, except in May and September 1995, when sampling occurred fortnightly (total of 47 sampling occasions). For May and September 1995, data from 2 sampling dates for each station are averaged and reported as monthly values. Measurements through the vertical profile at Stn B5 were made monthly from December 1994 to April 1996 (total of 17 sampling occasions).

***Sediment treatment and analysis:*** In the laboratory, chlorophyll *a* (chl *a*) and phytopigment degradation products (i.e. total phaeopigments) were extracted from duplicate subsamples of wet sediment (ca. 1 g) using 90% acetone. After 24 h of darkness at 4°C, the samples were sonicated for 5 min, centrifuged at 3000 rpm ($1000 \times g$) for 10 min, and extracts were spectrophotometrically analyzed for chl *a* and pheaopigment content. Chl *a* and phaeopigments values were obtained before and after acidification with 1 N HCl, respectively, according to Lorenzen's (1967) method, as described in Parsons et al. (1984), where the volume of water is substituted by the dry weight (DW) of the sediment expressed in gram. Values were thus expressed, corrected for porosity as measured by the water content, as µg chl *a* $g^{-1}$ DW. This was obtained after drying duplicate sediment subsamples (ca. 1 g) at 105°C for 20 h. From the same samples, we also measured the acid-volatile sulfide content and the nutrient concentrations ($NH_4^+$-N, [$NO_3^-$+$NO_2^-$]-N, $PO_4^{3-}$-P and Si [OH]$_4$-Si) in the porewater, for which relevant results will be reported elsewhere (P. Magni & S. Montani unpubl.).

During the 4 yr study, a total of 470 sediment samples, including the surface and subsurface layer, were analyzed in duplicate. At Stn B5, 170 sediment samples were taken through the vertical profile.

**Laboratory experiments.** ***Microalgal isolation and growth rate:*** Surface sediment samples for microalgal isolation were collected at Stn B4 on 3 different occasions during our routine field survey (May 23, June 24 and July 22, 1997). In the laboratory, ca. 1 g of sediment sample was placed in a Petri dish along with 10 ml of enriched medium containing 120 mg $l^{-1}$ $NaNO_3$, 5 mg $l^{-1}$ $K_2HPO_4$ and 33 mg $l^{-1}$ $NaSiO_3 9H_2O$. This was incubated for 3 d at 21°C with a 14:10 h light:dark photoperiod at 55 µE $m^{-2}$ $s^{-1}$. Observations were made using a stereomicroscope to evaluate the composition of these benthic microalgal assemblages. On all the different occasions, an individual diatom species, *Navicula* sp., was found to be dominant in our study area and was subsequently selected for microalgal isolation. After 3 d of incubation, *Navicula* sp. was isolated using a modified Pasteur micropipette and transferred into a test tube (previously autoclaved at 120°C). Test tubes were incubated for 2 wk and inspected for bacterial contamination and/or the presence of different microalgal species. After which, axenic monoclonal cultures of *Navicula* sp. were selected. To investigate the growth rate of *Navicula* sp., a known volume of the monoclonal culture was added to several glass vials and incubated at 21°C in 5 ml of enriched medium with a 14:10 h light:dark photoperiod at 55 µE $m^{-2}$ $s^{-1}$. Triplicate samples were filtered daily on a GF/F (Whatman) filter. Each filter

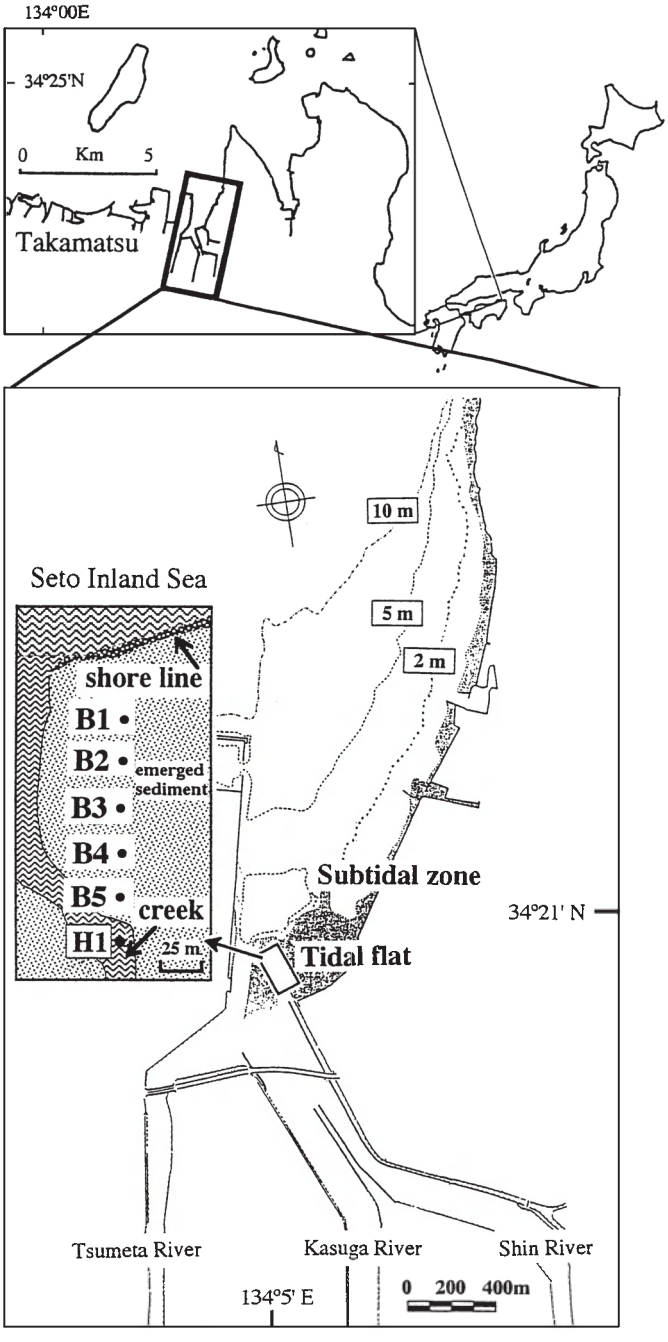


Fig. 1. Study area and location of sampling stations. Stns B1 to B5: emerged stations for sediment samples; Stn H1: sampling site of hydrological measurements in ebbing water

placed into the original vial and the chl *a* content extracted by N,N-dimethylformamide (DMF) and determined as according to Lorenzen's (1967) method. The extinction coefficient was 665 nm and correction for turbidity was made at 750 nm, using both DMF and acetone (field survey) as an extractant (Magni et al. 2000a).

***Production rates of Navicula sp.:*** A volume of newly isolated *Navicula* sp. culture was transferred into a 100 ml Erlenmeyer flask and incubated for 4 d. Following this, the flask was filled with enriched medium and tightly closed with a rubber cap ensuring that no air remained. An oxygen probe (YSI model 5300) was fitted to the cap. On the internal side of the flask wall, a magnetic stirrer was provided. The flask was illuminated using a combination of artificial and microscope light (Olympus, Model LGPS). The oxygen evolution in the flask and that in an air-saturated vessel were recorded continuously for 1 to 2 h. The flask was then covered to generate a dark condition and the oxygen evolution was again recorded continuously for 1 to 2 h. The difference between light and dark oxygen evolution was used to calculate the production rate, after converting $O_2$ measurements to C values according to Redfield et al. (1963) stoichiometric ratios. Several incubations were conducted keeping either temperature or light intensity constant. In treatments at 21°C, 7 different light intensities were used, varying from 0.7 to 396 µE $m^{-2}$ $s^{-1}$; in treatments where light intensity was constant at 55 µE $m^{-2}$ $s^{-1}$, temperatures were 10, 15, 25, 30 and 35°C.

Chl *a* content (microphytobenthos biomass) was determined at the end of the incubation. The culture was filtered on a GF/F (Whatman) filter, which was then transferred into the original flask, and 20 ml of DMF were added for pigment extraction. Samples were at –20°C until analysis, which was carried out within 10 d. Chl *a* content was analyzed after centrifugation at 2000 rpm (800 × *g*) for 10 min according to Parsons et al. (1984).

***Cell number/chl a relationship:*** Ten ml of distilled water were added into the flask which was sonicated to suspend the diatoms attached to the flask wall. The resuspension was transferred into 3 tubes which were centrifuged at 2000 rpm (800 × *g*) for 10 min. After this, the supernatant was removed and 1 ml of microalgae was diluted in 100 ml. Cell count was conducted in triplicate.

***Elemental composition of Navicula sp.:*** For the determination of the elemental composition (C, N, P and Si) of *Navicula* sp., *Navicula* sp. was placed on a number of pre-ignited GF/F filters and incubated in enriched medium in a Petri disk. After 4 d during the exponential growth phase, the filter was cut in 2 equal parts. One half of the filter was used for the chl *a* determination (as according to Parsons et al. 1984), and the other half for the determination of elemental composition of *Navicula* sp. Carbon (C) and nitrogen (N) content were determined using a CHN analyzer (Yanako, model MT-3), and phosphorus (P) and silicon (Si) content were determined using $K_2S_2O_8$ and $Na_2CO_3$ digestion method, respectively (Parsons et al. 1984).

**An indirect approach for areal production estimates.** As an attempt to evaluate the temporal scaling and variability of areal primary production of microphytobenthos in our study area, we derived an indirect and integrated approach based on our regular and extended field measurements of microphytobenthos biomass, in combination with irradiance- and temperature-dependent production estimates of the predominant microalgal form found in the field, *Navicula* sp. (see above, 'Laboratory experiments').

For each month during the 4 yr of the survey, we calculated the irradiance intensity reaching the surface sediments in 3 different situations, while also taking into account the temporal variation of sediment submersion and the extent of the screening effect of the water column during daytime. The first situation was that on the sampling day, the other days were selected at 2 different tidal cycles within 1 wk before and after the sampling day. The other 2 situations were considered in relation to the tidally dependent variability of emersion/submersion period of sediments versus water column depth during daytime (e.g. complete sediment emersion occurs only during a spring tide, i.e. the sampling day). For each of the 3 situations, the daily irradiance (MJ $m^{-2}$ $d^{-1}$) intensity reaching the surface sediments, converted into µE $m^{-2}$ $s^{-1}$ (1 MJ $m^{-2}$ $d^{-1}$ = 4.52 E $m^{-2}$ $d^{-1}$), was calculated in 2 opposite tidal states occurring within that day: one as a daytime high-tide situation and maximum screening effect of the water column, and the second one as a daytime low-tide situation. Accordingly, the actual irradiance reaching the surface sediments was calculated using the following exponential equation:

$$y = 95.2 \times 10^{0.143x}$$

where $y$ is the percentage of irradiance reduction through the water column, i.e. 100% of light intensity at 0 m water depth during sediment emersion occurring at +50 cm (Magni & Montani 1998), and $x$ is the depth of the water column (maximum depth 2 m, Montani et al. 1998).

The irradiance attenuation through the surface sediments was calculated by direct measurements from core samples of sediments collected at the sampling stations. To determine the coefficient of irradiance attenuation through the sediments, we used a light sensor LI-COR LI-200SA. Firstly, in the laboratory, the light sensor, which was placed beneath a cylindrical

plate that was open in the middle, was exposed to full light passing through a wrapping film. The value recorded (1249 µE $m^{-2}$ $s^{-1}$) was used as 100% light penetration. Subsequently, the uppermost 2 and 5 mm layers of the sediments were carefully extruded, the cores sliced off and alternately placed on the wrapping film. The mean values of light intensity recorded by the sensor below the 2 and 5 mm slices (1.3 and 0 µE $m^{-2}$ $s^{-1}$, respectively) were used to calculate the curve of light reduction with depth. This was described by the following exponential equation:

$$y = 100 \times 10^{-1.49x}$$

where $y$ is the percentage of light reduction through the sediments (i.e. 100% of light intensity at 0 mm depth) and $x$ is the sediment depth. This equation was used to calculate the photosynthetic active layer. Based on these calculations, light penetration exponentially decreased to ca. 77 and 97% at the 0.4 and 1 mm depths, respectively. Accordingly, factors of light attenuation through both the water column (i.e. $f_w$, varying depending on the tidal state/amplitude and being maximum, $f_w$ = 1, during sediment emersion) and the surface sediments (i.e. $f_S$, being $f_S$ = 0.5032 and 0.0323 in the uppermost 0 to 0.2 and 0.8 to 1 mm of sediments, respectively) were used in our subsequent calculations of the actual irradiance intensity available for photosynthesis.

In addition, the microalgal biomass (chl *a*) within the photosynthetic active layer was assumed not to be constant, as commonly adopted (Pinckney & Zingmark 1993, Barranguet et al. 1998), but was calculated as decreasing with depth. An exponential equation was used, based on an averaged 52% reduction of chl *a* content from the surface (0 to 0.5 cm) to the subsurface (0.5 to 2 cm) layer, as obtained from HPLC measurements (Magni et al. 2000a). The decrease with depth was expressed by the following equation:

$$y = 100 \times 10^{-0.439x}$$

where $y$ is the percentage of chl *a* content reduction in the uppermost 2 cm of the sediments and $x$ is the depth of the sediments. The microphytobenthos biomass measured in surface sediments (0 to 0.5 cm) during our routine survey (mean chl *a* content of the 5 stations) was then re-assessed in steps of 0.2 mm for the first mm (where the light intensity exponentially decreased to 3.2% at the 0.8 to 1 mm layer) and each mm for the next ones down to 5 mm depth. As such, only a minimal fraction of the total biomass measured in the uppermost 5 mm of sediments was considered as photosynthetic-competent, e.g. 10% of microphytobenthos biomass was calculated in the uppermost 0.4 mm as receiving ca. 77% of the total irradiance available for photosynthesis.

We subsequently calculated a photosynthetic rate factor (i.e. $f_T$), described by the regression line $f_T$ = 2.60 × temperature + 48.1, $r^2$ = 0.979 and ranging between $f_T$ = 0.602 (at 4.7°C) and $f_T$ = 1.208 (at 29.8°C), based on the results of our laboratory experiments on irradiance (I)- and temperature (T)-dependent photosynthetic rates (µg C µg chl $a^{-1}$ $h^{-1}$) of *Navicula* sp. This factor was then applied to the actual irradiance intensity available at each re-assessed photosynthetic competent layer to obtain an I- and T-dependent photosynthetic rate, and multiplied by the relevant fraction of microphytobenthos biomass calculated for each sub-layer.

The production of microphytobenthos was finally calculated for each photosynthetic sub-layer and summed as an all-layer production. These calculations were conducted for the 3 different situations and the 2 opposite, high and low, tidal states. Within-month variations of these 6 derived production estimates were always <15%. For each month, the mean was used to express the primary production of microphytobenthos on an areal basis as g C $m^{-2}$ $d^{-1}$.

## RESULTS

### Field surveys

#### Environmental variables

Monthly mean air temperature at the sampling site varied from 4.7°C (February 1996) to 29.8°C (August 1995) (Fig. 2a), with an annual mean of 16.1 ± 8.1°C. Monthly mean solar radiation varied from 7.4 MJ $m^{-2}$ (December 1994 and January 1995) to 22.5 MJ $m^{-2}$ (July 1994), with an annual mean of 13.8 ± 4.1 MJ $m^{-2}$. Solar radiation noticeably showed a temporary decrease every year in June (Fig. 2a). The rainfall was lowest in December 1995 (10 mm $mo^{-1}$) and peaked on several occasions, such as in September 1994, May and July 1995 (maximum of 335 mm $mo^{-1}$), June 1996, and July 1997 (Fig 2b). In ebbing water (Stn H1), salinity widely varied between 1.3 psu (September 1996) and 32.1 psu (November 1995), sharply decreasing during rainfall events and being highest during periods of little rainfall (Fig. 2b). Annual mean salinity at Stn H1 was 17.5 ± 10.6 psu. Also, water temperature at Stn H1 varied largely from 3.6°C (December 1995) to 32.2°C (July 1994) (Fig. 2c), with an annual mean of 17.1 ± 8.6°C SD. Dissolved oxygen (DO) concentration showed a seasonal pattern, with a tendency to oversaturation between early spring and summer (maximum of 15.1 mg $l^{-1}$ or 173% of air saturation in April 1996) (Fig. 2c). Annual mean of DO concentration was 8.4 ± 2.6 mg $l^{-1}$ or 94.4 ± 32.3% of air saturation.

### Microphytobenthos biomass (chl *a*) in sediments

In surface sediments (0 to 0.5 cm), the chl *a* content at individual stations ranged between 1.6 µg $g^{-1}$ (Stn B4, June 1994) and 25.3 µg $g^{-1}$ (Stn B1, May 1995) (Fig. 3a). Spatial differences in chl *a* content between individual stations were limited to an annual mean significantly higher at Stns B3 (9.9 ± 4.6 µg $g^{-1}$) and B5 (12.0 ± 4.6 µg $g^{-1}$) than at Stn B4 (8.8 ± 4.1 µg $g^{-1}$) (ANOVA: single factor, $p < 0.001$ and $p < 0.05$, respectively, plots not shown). The chl *a* content at the subsurface layer (0.5 to 2 cm) was constantly lower than that at the surface, displaying similar spatial and temporal variability (Fig. 3b), with a mean decrease of 34% ($y$ [subsurface] = 0.49$x$ [surface] + 1.8, $r^2 = 0.64$, n = 235, ANOVA: $p < 0.001$, plots not shown). The investigations through the vertical profile of sediments at Stn B5 indicated a further and progressive decrease of chl *a* content with depth in all different seasons and irrespective of its standing stock (Fig. 4a). At this individual station, chl *a* varied from 1.3 µg $g^{-1}$ (8 to 9 cm, August 1995) to 20.0 µg $g^{-1}$ (0 to 0.5 cm, February 1996). At the surface, the chl *a* content accounted for 22.8 ± 5.7 SD of the total content in the uppermost 10 cm of the sediment, while it was lowest at the 9 to 10 cm layer with a mean of 3.2 ± 1.4% (Fig. 4b).

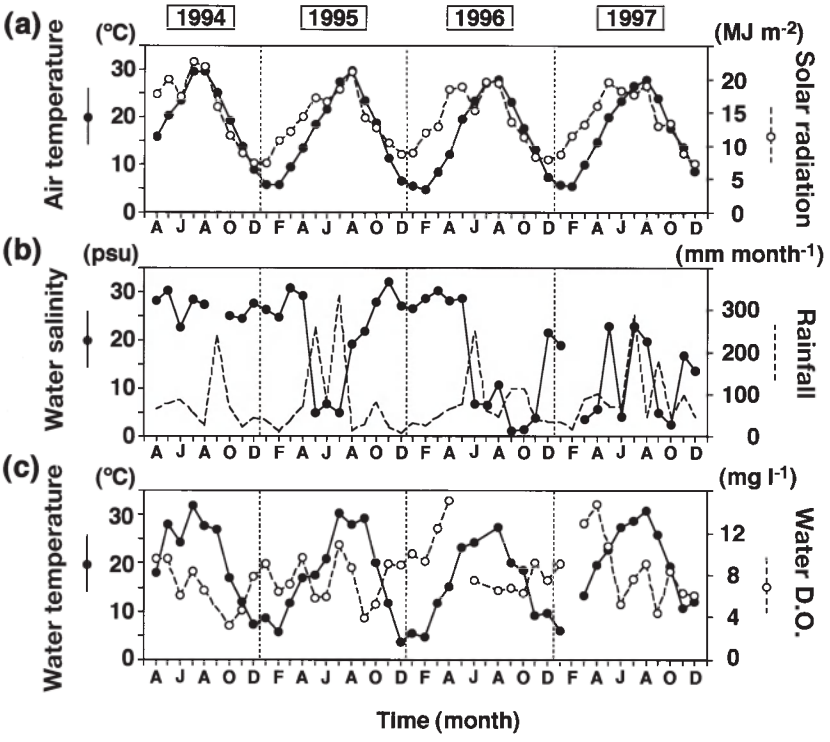


Fig. 2. Seasonal and interannual variability of environmental conditions at the study site. (a) Monthly mean of air temperature (left scale) and solar radiation (right scale). (b) Water salinity at Stn H1 (left scale) and monthly rainfall (right scale). (c) Water temperature (left scale) and dissolved oxygen concentrations (right scale) at Stn H1. Interrupted lines: data not available between 2 mo

Measurements of chl *a* content were also expressed on an areal basis (mg chl *a* $m^{-2}$) by accounting for the bulk-density of the sediment particle as 2.5 g $cm^{-3}$

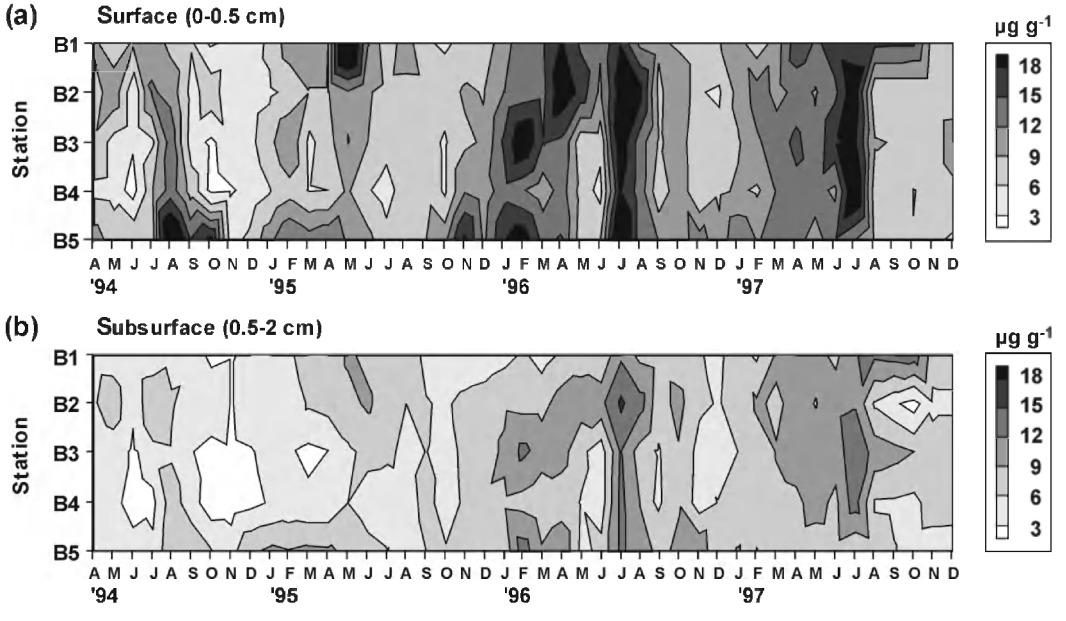


Fig. 3. Spatial and temporal distribution of microphytobenthos biomass (chl *a*) at the 5 sampling stations (Stns B1 to B5)

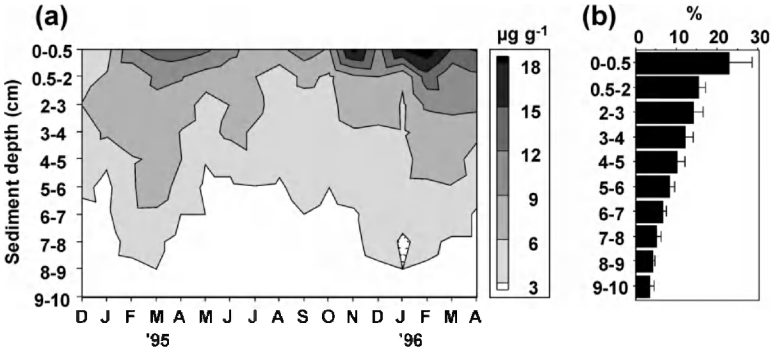


Fig. 4. Vertical distribution (0 to 10 cm depth) of microphytobenthic biomass (chl *a*) at Stn B5. (a) Chl *a* content. (b) Mean ± SD of the relative percentage of chl *a* content at each layer during the period of investigations

and taking into consideration the spatial and temporal variations of the porewater content. Accordingly, we calculated a factor $f$ from the ratio between the sediment particle bulk-density and the total volume, where the maximum value of $f = 1$ is for a hypothetical sediment with 0% of porewater. Thus, this ratio changed depending on the different porewater content in each sample (not corrected for salinity) and varied from 0.20 (i.e. water content 61.2%, Stn B5, October 1994) to 0.66 (i.e. water content 17.1%, Stn B4, June 1994). The conversion equation was:

$$\text{mg chl } a \text{ m}^{-2} = 5000 \text{ cm}^3 \text{ m}^{-2} \times 2.5 \text{ g cm}^{-3} \times f \times \text{µg chl } a \text{ g}^{-1} \text{ DW sediment}$$

The square regression line of all chl *a* plots was: $y = 5.43x + 10.7$ ($r^2 = 0.78$, n = 235, $p < 0.001$), where $x$ and $y$ are the chl *a* content expressed as µg g$^{-1}$ and mg m$^{-2}$, respectively, indicating a good correlation of chl *a* estimates on a weight and an areal basis.

Fig. 5 shows the monthly estimates of microphytobenthos biomass in surface sediments, expressed in both ways, as a mean (±SD) of chl *a* values from the 5 stations. On a dry weight basis, the chl *a* content varied between 3.9 ± 1.0 µg g$^{-1}$ (November 1994) and 20.3 ± 2.6 µg g$^{-1}$ (July 1996), with an annual mean (1995 to 1997 period) of 11.0 ± 4.3 µg g$^{-1}$ (Table 1). On an areal basis, it varied between 27.7 ± 14.7 mg m$^{-2}$ (October 1994) and 120.2 ± 24.3 mg m$^{-2}$ (July 1997), with an annual mean of 72.3 ± 27.1 mg m$^{-2}$ (Table 1). In both cases, significant differences of chl *a* content between 2 subsequent sampling periods were restricted to a few and were irrespective of the season (Fig. 5). Yet the chl *a* content showed a clear seasonal pattern with a decrease every year between late summer and early autumn, and the lowest values in October during 1994 and 1995 (27.2 ± 14.7 and 47.0 ± 14.3 mg m$^{-2}$, respectively) or in November during 1996 and 1997 (53.4 ± 22.5 and 46.9 ± 1.9 mg m$^{-2}$, respectively). In addition, for 4 consecutive years, after a progressive increase in winter (i.e. 76.2 ± 25.6 and 111 ± 25.2 mg m$^{-2}$ in February 1995 and 1996, respectively, and 76 .8 ± 21.3 mg m$^{-2}$ in January 1997), the chl *a* content tended to decrease noticeably between May and June. This coincided with the temporary decrease of solar radation (Fig. 2a). The highest peaks of chl *a* occurred in April 1996 (116 ± 20.7 mg m$^{-2}$) and July 1997 (120 ± 24.3 mg m$^{-2}$). On a yearly basis, we found a significant trend of increasing microphytobenthos biomass from 1994 to 1997 (Table 1).

## Laboratory experiments

### Growth and production rate of *Navicula* sp.

Fig. 6 shows the results of the incubation experiments on the growth of *Navicula* sp. The chl *a* content of *Navi-*

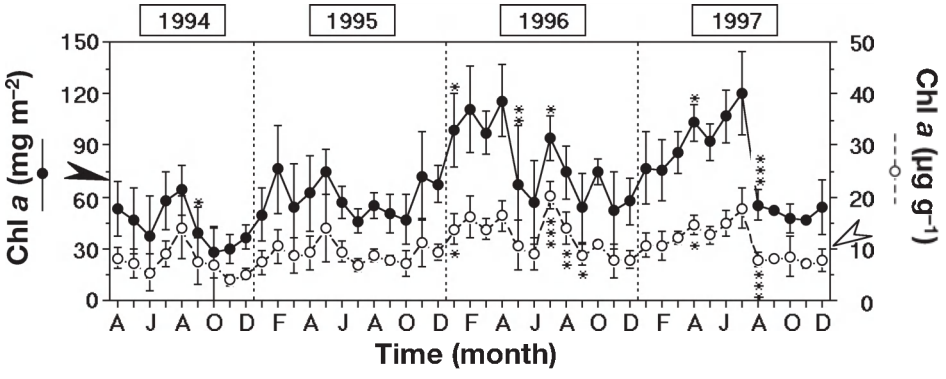


Fig. 5. Seasonal and interannual variability of microphytobenthic biomass (chl *a*), expressed as a mean ± SD of the 5 sampling stations (Stns B1 to B5). Left scale: areal basis (mg chl *a* m$^{-2}$); right scale: weight basis (µg chl *a* g$^{-1}$ DW sediment). Asterisks (upper ones for the left scale and lower ones for the right scale) indicate significant difference between each sampling month and the previous one (ANOVA, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$)

Table 1. Yearly means (± SD) and ranges (minimum and maximum values) of microphytobenthos biomass (as chl *a*) in surface sediments (0–0.5 cm). *April to December period; **1995 to 1997 period. [a] and [b]: significant differences between yearly estimates. DW: dry wt

| Year | | µg chl *a* $g^{-1}$ DW[a] | mg chl *a* $m^{-2}$,[b] |
|---|---|---|---|
| **1994*** | Mean ± SD | 7.5 ± 4.6 | 43.5 ± 18.6 |
| (n = 45) | (min–max) | (1.6–24.1) | (12.9–84.1) |
| **1995** | Mean ± SD | 9.2 ± 3.4 | 59.2 ± 18.6 |
| (n = 70) | (min–max) | (4.7–39.7) | (36.2–117.1) |
| **1996** | Mean ± SD | 12.6 ± 4.7 | 79.9 ± 29.0 |
| (n = 60) | (min–max) | (4.8–24.5) | (25.9–39.8) |
| **1997** | Mean ± SD | 12.1 ± 7.0 | 78.7 ± 27.7 |
| (n = 60) | (min–max) | (5.5–21.7) | (37.7–145.5) |
| **Annual**** | Mean ± SD | 11.0 ± 4.3 | 72.3 ± 27.1 |
| (n = 190) | (min–max) | (1.6–39.7) | (12.9–145.5) |

[a]1996 and 1997 > 1995 (ANOVA $p < 0.001$); 1995 > 1994 (ANOVA $p < 0.05$), [b]1996 and 1997 > 1995 (ANOVA $p < 0.001$ and $p < 0.01$)

*cula* sp. was measured to be 2.14 pg chl *a* $cell^{-1}$. Accordingly, the cell number increase of *Navicula* sp. during the incubation period was calculated from the mean of replicate measurements of chl *a* content $tube^{-1}$ (Fig. 6). The exponential growth of *Navicula* sp. lasted for 6 d before reaching steady state (Fig. 6). From these results, the growth rate factor of *Navicula* sp. was $\mu = 1.68\ d^{-1}$.

The photosynthetic rate was saturated at a light intensity of 165 µE $m^{-2}$ $s^{-1}$ at 21°C (Fig. 7a). At this light intensity, the $P_{max}$ (maximum photosynthetic capacity) was 7.9 µg C µg chl $a^{-1}$ $h^{-1}$. There was no photoinibition at higher light intensities of up to 400 µE $m^{-2}$ $s^{-1}$. The photosynthetic rate was expressed by the following equation from Jassby & Platt (1976):

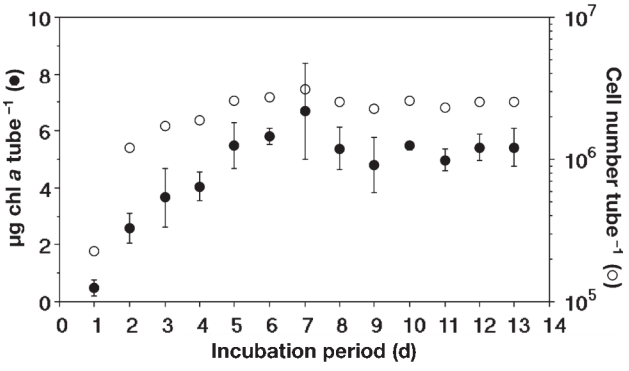


Fig. 6. Laboratory experiments on the growth rate by *Navicula* sp. Left scale: growth rate expressed as µg chl a $tube^{-1}$, SD of triplicate cultures of *Navicula* sp.; right scale: growth rate expressed as cell number $tube^{-1}$. This was calculated from the mean of triplicate measurements, where the chl *a* content of *Navicula* sp. is 2.14 pg chl *a* $cell^{-1}$

$$y = 7.85 - \tanh[(0.0785 - x)/7.85]$$

where $y$ is the photosynthetic rate and $x$ is the light intensity (Fig. 7a).

The relationship between temperature and photosynthetic rate was linear and positive within a field-relevant temperature range of 10 to 35°C at 55 µE $m^{-2}$ $s^{-1}$ (Fig. 7b).

### Elemental composition of *Navicula* sp.

The weight ratio between biophilic elements (C, N, P and Si) and chl *a* in *Navicula* sp. was: C/chl *a* = 33.7 ± 5.60 (n = 17), N/chl *a* = 5.58 ± 1.27 (n = 17), P/chl *a* = 1.15 ± 0.312 (n = 10), Si/chl *a* = 18.5 ± 1.31 (n = 4). Accordingly, the molar ratio was: C/N = 7.05 ± 0.81 and C:N:P:Si = 75.7:10.1:1:17.8.

## Patterns of microphytobenthos primary production

Our scaling up of production estimates to areal primary production of microphytobenthos was calculated

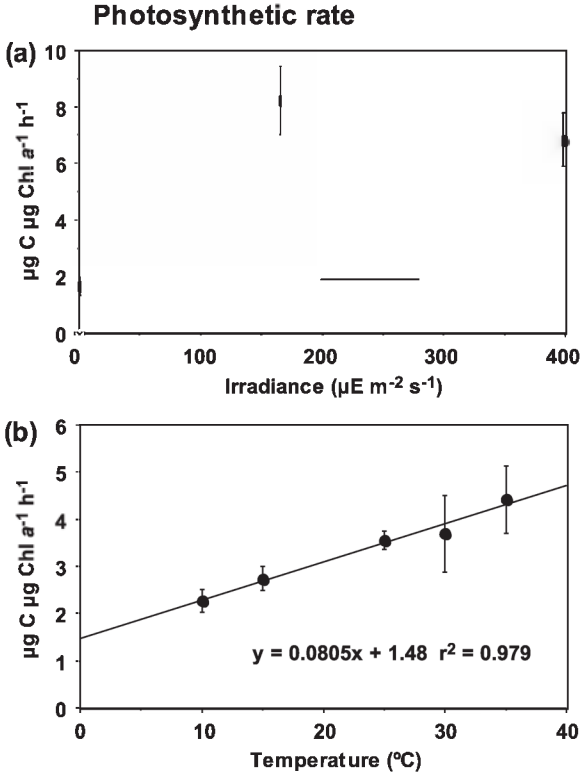


Fig. 7. Laboratory experiments on the photosynthetic rate of *Navicula* sp. (a) Photosynthetic rate irradiance-dependent at 21°C; indicated is the mean ± SD of 3 to 7 replicate measurements for each irradiance intensity (i.e. 0.7, 55, 110, 165, 225, 276 and 398 µE $m^{-2}$ $s^{-1}$). (b) Photosynthetic rate temperature-dependent at 55 µE $m^{-2}$ $s^{-1}$; indicated is the mean ± SD of 2 to 3 replicate measurements for each temperature level

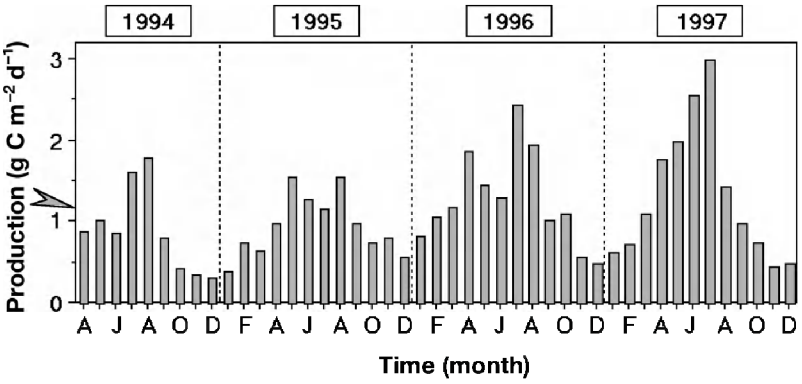


Fig. 8. Quantification of seasonal and interannual variability primary production (g C m$^{-2}$ d$^{-1}$) of microphytobenthos in the study area

and varyied up to an order of magnitude from 0.32 g C m$^{-2}$ d$^{-1}$ (December 1994) to 3.0 g C m$^{-2}$ d$^{-1}$ (July 1997), with an annual mean (1995 to 1997) of 1.2 g C m$^{-2}$ d$^{-1}$ (Fig. 8). Seasonal patterns and interannual variability were also observed. Uni-modal (summer) or bi-modal (spring and summer) peaks occurred in 1994 to 1997 and 1995 to 1996, respectively, with a marked decrease every year from late autumn to early winter, and a tendency to increasing annual estimates from 1995 to 1997. Regardless of the year, primary production was significantly higher both in spring and summer than in winter (ANOVA: $p < 0.001$) and fall (ANOVA: $p < 0.005$ and $< 0.001$, respectively) (Table 2).

## DISCUSSION

### Spatial and temporal distribution of microphytobenthos

Microphytobenthos biomass has been investigated in various intertidal sediments around the world at different latitudes (MacIntyre 1996 and references therein), spatial and temporal scales, and sediment types (Colijn & Dijkema 1981, De Jong & De Jonge 1995, Magni & Montani 1997). Although different methodological approaches may concur to limit comparisons among different studies, there is rather consistent literature on the quantification and variability of microphytobenthos biomass in these systems. The determination of chl *a* content is widely used to quantify the living fraction of microalgal assemblages in surface sediments. Chl *a* estimates of microphytobenthos biomass amounted to ca. 100 mg m$^{-2}$ (top 0.75 cm) in south New England (Marshall et al. 1971), 3.8 to 11.3 µg g$^{-1}$ (top 1 cm) in the Wadden Sea (Cadée & Hegeman 1974), 33 to 184 mg m$^{-2}$ (top 5 mm) in the Ems-Dollard (Colijn & De Jonge 1984), 50 to 330 mg m$^{-2}$ (top 1 cm) in Savin Hill Cove, Boston (Gould & Gallagher 1990), 72 to 102 mg m$^{-2}$ (top 5 mm) in the North Inlet of South Carolina (Pinckney & Zingmark 1993), 113 mg m$^{-2}$ (7.3 µg g$^{-1}$) (top 1 cm, De Jong & De Jonge 1995) or 5.9 mg m$^{-2}$ (sand) to 17.3 mg m$^{-2}$ (muddy) (top 1 mm, Barranguet et al. 1998) in the Westerschelde. In our study area, the annual mean of microphytobenthos biomass was 72.3 ± 27.1 mg chl *a* m$^{-2}$ or 11.0 ± 4.3 µg chl *a* g$^{-1}$ (Table 1). Whereas chl *a* estimates expressed either on a weight or an areal basis might be loosely correlated with each others if the sediment-specific weight varies considerably, this was not the case in our study area (see 'Results'), which was characterized by a relatively homogeneous sandy sediment. Sandy sediments tend to have a lower chl *a* content than muddy sediments (Colijn & Dijkema 1981, De Jong & De Jonge 1995, Magni & Montani 1997), comparison of our estimates with those from other sites indicate that microphytobenthic biomass at our site, expressed both as mg chl *a* m$^{-2}$ and µg chl *a* g$^{-1}$, rank in the mid-upper range of for intertidal sediments (see also Table 4 in Colijn & De Jonge 1984, and Table 3 in MacIntyre et al. 1996).

Although a lot of literature exists on the development of intertidal microphytobenthos at different sites, and on the multiplicity of abiotic and biotic controlling factors (but see also Pinckney & Zingmark 1991), little information is as yet available on the distribution of microphytobenthic assemblages within an individual system at various spatial scales (i.e. horizontal and vertical), in combination with an evaluation of seasonal

Table 2. Seasonal means (±SD) and ranges (minimum and maximum values) of microphytobenthos primary production (g C m$^{-2}$ d$^{-1}$). *1995 to 1997 period

| | | |
|---|---|---|
| **Winter** (Dec to Feb) | Mean ± SD | 0.63 ± 0.33 |
| (n = 10) | (min–max) | (0.32–1.07) |
| **Spring** (Mar to May) | Mean ± SD | 1.32 ± 0.44 |
| (n = 11) | (min–max) | (0.66–2.01) |
| **Summer** (Jun to Aug) | Mean ± SD | 1.75 ± 0.63 |
| (n = 12) | (min–max) | (0.88–3.00) |
| **Fall** (Sep to Nov) | Mean ± SD | 0.75 ± 0.25 |
| (n = 12) | (min–max) | (0.34–1.10) |
| **Annual*** | Mean ± SD | 1.19 ± 0.63 |
| (n = 36) | (min–max) | (0.40–3.00) |
| Spring > Winter | (ANOVA $p < 0.001$) | |
| Spring > Fall | (ANOVA $p < 0.005$) | |
| Summer > Winter | (ANOVA $p < 0.001$) | |
| Summer > Fall | (ANOVA $p < 0.001$) | |

and interannual patterns (Cadée & Hegeman 1974, Riaux-Gobin 1985, Peletier 1996). Our monitoring survey was carried out regularly on a monthly basis over an extended (interannual) period and at a controlled spring low tide of every month, in order to allow us to interpret the results in a coherent and consistent way. Both environmental variability and the spatio-temporal distribution of microphytobenthos in our study area could be thus evaluated from a seasonal and interannual standpoint and for that particular tidal state. Clearly, it was not our intention here to investigate the variability of these ecosystems inherent to shorter (i.e. hours, days) temporal scales (Stevenson 1983, Pinckney & Zingmark 1991, Barranguet et al. 1998, Kromkamp et al. 1998, Montani et al. 1998, Kingston 1999, Blanchard et al. 2001). This study indicated that there was a strong seasonal and interannual variability in the occurrence and development of microphytobenthic assemblages on the flat under investigation, displaying each year either uni- or bi-modal peaks. Late-winter (February) peaks occurred in 1995 and 1996, as was reported by several other authors (Colijn & Dijkema 1981, Pinckney & Zingmark 1993 and references therein) and also found in our previous study on the same flat (Magni & Montani 1997). In the Westerschelde, De Jong & De Jonge (1995) reported 1 large peak in early summer (May to June). In our study area, this period coincided with a seasonal reduction of irradiance (Fig. 2a) and a parallel decrease of microphytobenthic biomass (Fig. 5). Subsequently, summer peaks (i.e. July to August), noticeable in 1994 and highest in 1996 and 1997 (Fig. 5), also occurred as a second bloom, which was similar to that found by Montagna et al. (1983) at a higher latitude. In contrast, a summer peak was absent in 1995 and a sharp decrease of chl *a* content occurred in August 1997. In both cases, the marked reduction of microphytobenthic biomass coincided with periods of increased rainfall (Fig. 2b), suggesting that rainfall events effectively flushed away microphytobenthic assemblages, as has been documented elsewhere (Magni & Montani 1997).

## Laboratory experiments on microphytobenthos production

### Effect of light and temperature, and assimilation rates

Estimated photosynthesis-irradiance (*P-I*) curves for intertidal microphytobenthos have been shown to be variable in the literature, depending on species/assemblages composition. An early study by Colijn & Van Buurt (1975) indicated rates of photosynthesis similar to ours using cultures of *Amphiprora* cf. *paludosa* grown at 20°C and 74 or 129 µE $m^{-2}$ $s^{-1}$. Admiraal (1977) found that the minimum daily quantum irradiance for light-saturated growth of 4 intertidal microphytobenthic species was 85 to 170 µE $m^{-2}$ $s^{-1}$, and was not greatly influenced by the 2 experimental temperatures (i.e. 12 and 20°C). More recently, other authors reported light intensity saturation at ca. 1000 µE $m^{-2}$ $s^{-1}$. For instance in Pinckney & Zingmark (1991) and Blanchard et al. (1996), photosynthesis by microphytobenthos (intact cores and isolated motile diatoms, respectively) became light-saturated for scalar irradiance greater than 600 µE $m^{-2}$ $s^{-1}$, and was not photoinhibited up to 1800 and 1200 µE $m^{-2}$ $s^{-1}$, respectively. Conversely, lower $E_{max}$ (irradiance intensity at which $P_{max}$, maximal production rate, is reached) has been reported in the Wadden Sea. On the tidal flat of Keitum, Germany, photoinhibition occurred at irradiance higher than 861 µE $m^{-2}$ $s^{-1}$, with $E_{max}$ between 345 and 655 µE $m^{-2}$ $s^{-1}$ (at 18 ± 1°C) (Hartig et al. 1998). Even lower $E_{max}$ was found on a Danish flat in the Wadden Sea, where the photosynthetic rate was saturated at light intensity of 160 µE $m^{-2}$ $s^{-1}$ at 4°C, 175 µE $m^{-2}$ $s^{-1}$ at 18°C and 360 µE $m^{-2}$ $s^{-1}$ at 9°C (Rasmussen et al. 1983). In our laboratory experiments, *Navicula* sp. reached the $E_{max}$ at 165 µE $m^{-2}$ $s^{-1}$ at 21°C. Our values are therefore in close approximation with those reported by Rasmussen et al. (1983). These values are also rather similar to those for sublittoral microphytobenthic communities, measured as $^{14}C$ uptake and varying between 30 and 320 µE $m^{-2}$ $s^{-1}$ (Sundbäck & Jönsson 1988), and for planktonic diatoms reaching light saturation at irradiance greater than 220.6 µE $m^{-2}$ $s^{-1}$ (Ryther 1956).

As for temperature, Admiraal (1977) found that at 85 µE $m^{-2}$ $s^{-1}$ the highest growth rates of *Navicula arenaria* occurred at between 16 and 20°C, while *Amphiprora* cf. *paludosa*, *Nitzschia dissipata* and *Nitzschia sigma* had their optimum temperature at 25°C or higher. Blanchard et al. (1996) estimated that while $P_{max}$ was almost twice as high in September as in December, $T_{opt}$ did not change and was ca. 25°C. Our physiological measurements showing a linear increase of photosynthesis rate by *Navicula* sp. within a temperatures range of 10 to 35°C suggests that temperature might be less limiting than light.

Also assimilation rates are rather variable in the literature. Admiraal (1977) reported for *Nitzschia dissipata* and *Navicula arenaria* assimilation values of 2 to 6 µg $O_2$ µg chl $a^{-1}$ $h^{-1}$ (1.5 to 2.3 µg C µg chl $a^{-1}$ $h^{-1}$) at 20°C and 74 µE $m^{-2}$ $s^{-1}$. In a subsequent paper (Admiraal & Peletier 1980), the assimilation rates of 4 benthic diatom species (*Navicula salinarum*, *N. arenaria*, *Gyrosigma spencerii* and *Amphiprora* cf. *paludosa*) ranged between 7 and 13 µg C µg chl $a^{-1}$ $h^{-1}$. As described earlier, in our laboratory experiments, the assimilation number of *Navicula* sp. was 3.89 ± 0.45 µg C µg chl $a^{-1}$ $h^{-1}$ at 21°C and 55.2 µE $m^{-2}$ $s^{-1}$. We infer that discrep-

ancies between authors may be to some extent related to methodological differences and/or differences between species.

### Elemental ratios of *Navicula* sp.

The C/chl *a* ratio calculated in this study for *Navicula* sp. (33.7 ± 5.6, n = 17) is very similar to that earlier indicated for phytoplankton (Parsons & Strickland 1959) and more recently for Bacillariophyceae (Montagnes et al. 1994). Our results are also in good agreement with values previously reported for intertidal microphytobenthos. De Jonge (1980) reported a variability of the C/chl *a* ratio between 40.3 and 61.4, in Gould & Gallagher (1990) this ratio varied from 18.7 to 60.4. Accordingly, the N/chl *a* ratio (5.58 ± 1.27, n = 17) and the C/N ratio (7.05 ± 0.81) are also within the range reported in the literature (Perry 1976, Montagnes et al. 1994), whereas the Si/chl *a* and P/chl *a* ratios (18.5 ± 1.31 and 1.15 ± 0.31, respectively) indicate that *Navicula* sp. has a high P content relative to the Redfield et al. (1963) ratio.

### Areal primary production of microphytobenthos

Our extrapolation and scaling up of production estimates to a field-relevant situation principally was aimed at investigating the extent and magnitude of seasonal and interannual variability and/or patterns, if any. We concurrently acknowledge that our areal estimates may be referred as to 'potential' primary production of microphytobenthos for our study area, also considering that our derived rates are based on optimal conditions for photosynthesis of a predominant microphytobenthic species. However, we infer that the magnitude and scope of our approach, surpassing the short-term (hours, days) field variability and species-specific differences of physiological responses among microphytobenthic communities, allowed us to unravel long-term scales and patterns of intertidal microphytobenthos assemblages and development. Attempts to investigate the interannual distribution of both biomass and microphytobenthos primary production are very scarce indeed; this is also due to temporal restrictions and concrete difficulties in extrapolating physiological measurements to large-scale trends. In addition, although it is true that biomass measurements in the field were made during daytime at low tide when production rates of microphytobenthos have been shown to be maximal (Pinckney & Zingmark 1991), areal production estimates were obtained by applying the physiological rates of *Navicula* sp. to the minimal field-relevant fraction of microphytobenthos biomass in the photosynthetic active layer of sediments. This was calculated to be mainly restricted to the uppermost 0.2 mm of sediments which only represented 10% of total microphytobenthos biomass in surface sediments and where 77% of the total irradiance was available for primary production. Based on this theoretically derived approach, primary production of microphytobenthos indicated clear interannual patterns (Fig. 8), with significant differences between seasons (Table 2). Among the few examples of long-term variability, De Jong et al. (1994) reported for the Eastern Scheldt estuary that primary production of microphytobenthos increased from 1981 to 1990 from 150 to 242 g C $m^{-2}$ $yr^{-1}$, which accounted for 16 and 30% of the total primary production, respectively. In the Western Scheldt estuary, the contribution of the microphytobenthos to the total primary production was estimated to be at least 17% (De Jong & De Jonge 1995). Even stronger contribution to the autochthonous source of carbon from microbial production has been reported on an intertidal flat in SW England, where the annual primary production for the sediment and the water column was 143 and 81.7 g C $m^{-2}$ $yr^{-1}$, respectively. In our study, annual primary production of microphytobenthos (434 g C $m^{-2}$ $yr^{-1}$; Table 2) was higher than that reported on the phytoplankton in the eutrophic Seto Inland Sea (285 g C $m^{-2}$ $yr^{-1}$; Tada et al. 1998), suggesting a high productivity of this intertidal zone, in addition to the strong seasonality of both microphytobenthos biomass and primary production.

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