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Development of benthic microalgal assemblages on an intertidal flat in the Seto Inland Sea, Japan: effects of environmental variability

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Abstract : A bi-weekly survey lasting 13 months was carried out at low-tide on an intertidal flat in the Seto Inland Sea, Japan. We monitored the environmental conditions at a river and a low-tide shore-line stations and examined the factors affecting the development of benthic microalgal assemblages. At the two stations, the physico-chemical parameters of both emerged sediment (*i.e.* nutrient concentrations of the pore water) and the nearby low-tide water (*i. e.* salinity and dissolved oxygen concentration) showed strong but similar short- (days) and long-term (interannual) variability. However, the benthic microalgal standing stock was significantly higher at the river station (240.5±121.1mg m^{-2}, n=107) than at the low-tide shore line station (121.9±41.4 mg m^{-2}, n=108). Accordingly, estimated annual primary production was 634 g C $m^{-2}yr^{-1}$(1.74±0.65 g C $m^{-2}day^{-1}$) and 259 g C m^{-2} yr^{-1} (0.71±0.27g C m^{-2} day^{-1}), respectively. At the river station, more elevated than the low-tide shore-line station, the development of benthic microalgal assemblages was significantly limited by the washout caused by rainfall, but greatly enhanced during calm and fine weather. At the low-tide shore-line station, the reduced emersion of the surface sediment and the hydrodynamic energy (tidal currents, waves) were major factors responsible for keeping lower and less fluctuating microalgal biomass. Comparable primary production by intertidal microalgae (447gC m^{-2} yr^{-1}) and phytoplankton in the Seto Inland Sea (285gC m^{-2} yr^{-1}) is discussed as evidence of the high primary productivity of the intertidal zone, most likely underestimated in light of the grazing pressure by the macro-zoobenthos which is similarly abundant on this intertidal flat.

1. Introduction

The occurrence and the development of intertidal communities of the microphyto benthos, commonly represented by epipelic and epipsammic diatoms (COLIJN and DIJKEMA, 1981; COLIJN and DE JONGE, 1984; DE JONGE, 1985), are regulated numerous physico-chemical parameters. Such parameters include : temperature (COLIJN and BURT, 1975; ADMIRAAL, 1977a; ADMIRAAL and PELETIER, 1980; BLANCHARD *et al.*, 1996), salinity (ADMIRAAL, 1977b), gas diffusion and nutrient fluxes (ADMIRAAL *et al.*, 1982; WHILSHIRE, 1992), and organic enrichment (PELETIER, 1996).

Along a tidal estuary, noticeable differences in the benthic microalgal standing stock may also depend on the elevation of the station, which relates to the extent of the solar radiation available for the photosynthesis (ADMIRAAL and PELETIER, 1980; COLIJN and DE JONGE, 1984; DE JONG and DE JONGE, 1995) and the influence of the atmospheric condition on dessication and/or freezing of the emerged sediment (ADMIRAAL *et al.*, 1982) and flushing away of epipelic diatoms with the rainfall.

Further variability is related to the tidal currents and waves which influence the immigration rate (STEVENSON, 1983) and cause resu spension and transport of the microphytobenthos (MOSS and ROUND, 1967; CADÉE and HEGEMAN, 1974; DE JONGE and VAN DEN BERG,

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1987; GRANT *et al.*, 1988). Accordingly, physical sorting and grain-size composition of the sediment have been demonstrated important factors controlling the dynamics of the species composition of benthic diatoms (DE JONGE, 1985) and the microalgal biomass (COLIJN and DIJKEMA, 1981; DE JONG and DE JONGE, 1995), respectively.

All these physico-chemical and abiotic variables, along with the grazing pressure by the macro-zoobenthos (NICOTRI, 1977; LOPEZ and LEVINTON, 1987; BIANCHI and RICE, 1988; SMITH *et al.*, 1996), are likely to affect the benthic microalgal biomass independently from expected seasonal patterns which may justify an estimation of the annual microphytobenthic production on the basis of relatively few Chl *a* samples distributed over the year (CADÉE and HEGEMAN, 1977; COLIJN and DE JONGE, 1984). MACINTYRE and CULLEN (1996) recently suggested that the optimal use of resources to quantify the microalgal productivity would focus on between-day rather than within-day (PINCKNEY *et al.*, 1994) variability.

In light of such a variability, during the present study we made an intensive sampling effort (two times per week for 13 months) in order to assess the short-time (days) and seasonal environmental variability of an intertidal flat during low-tide in the Seto Inland Sea, and to examine the factors affecting the development of the microphytobenthos. A total of more than a hundred samples were collected at two stations, which allowed a more reliable estimation of the annual primary production. Values of annual primary production by the microphytobenthos reported from other intertidal flats and by the phytophankton in the Seto Inland Sea are compared and discussed within the frame of an integrated project which aims at quantifying the dynamics of biophilic elements (i.e. carbon, nitrogen and phosphorus) in this estuary and to assess the roles played by producers (microphyto benthos) and consumers (macrobenthos) on the processes.

2. Study area

The investigations were carried out at a river station (Stn.A) and a low-tide shore-line station (Stn. B4) of an intertidal flat in the Seto Inland Sea, south-western Japan (Fig. 1). The mean tidal range is *ca.* 2 m.

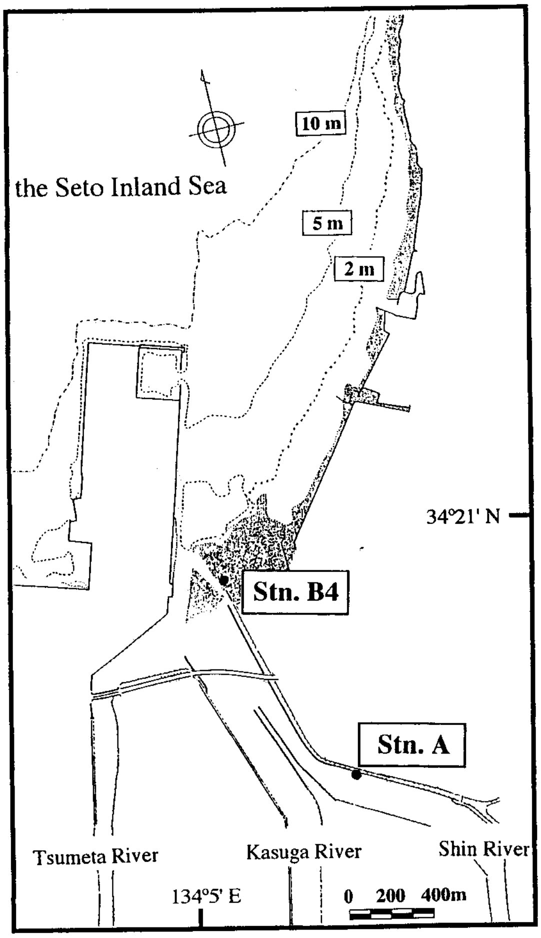


Fig. 1. Study area and location of the sampling stations.

The backwards Stn. A was more elevated and sheltered than Stn. B4, and was located about 1.1 km from the low-tide shore-line. The surface sediment emerged at a tidal level of about +140 cm. Stn. B4 was located near the low-tide shore-line, between the LWL (low water level) and the ELWL (extreme low water level), and therefore more exposed to the hydrodynamic energy of tidal currents and waves. The surface sediment emerged at a tidal level of about +70 cm.

Both stations were few ten meters away from the Shin River, which formed, during the low tide, a shallow pool (20 to 50 cm in depth) at

Stn. A and a reduced stream (20 to 50 cm in depth) at Stn. B4. The stream was flowing directly toward the low-tide shore-line and progressively mixing with intertidal and subtidal waters.

3. Materials and methods

3-1 Sampling procedure and meteorological parameters

Sampling activities always started from Stn. B 4. We monitored salinity (YSI portable salinometer), temperature and dissolved oxygen concentration (UK 2000 portable D.O. meter) of low-tide water close to the two stations. Emerged sediment samples were randomly taken at 7-8 spots of each station using acrylic core tubes (3 cm *i.d.*) gently pushed by hand into the sediment. Surface (0-0.5 cm) and sub-surface (0.5-2 cm) layers were carefully sliced off the sediment. Sediment samples from the same layer were pooled together and brought to the laboratory within 2 hours for chemical analysis. Sampling was carried out at low-tide twice a week from July 1993 to July 1994.

Data of daily rainfall and solar radiation were obtained from the Takamatsu Meteorological Agency Station, located near the intertidal flat under investigation.

3-2 Sediment treatment

In the laboratory, pigments were extracted from duplicate subsamples of wet sediment (*ca.* 1 g) using 90% acetone. After 24 hrs of darkness at 4℃, extracts were analyzed for Chl *a* and pheo-pigments by spectrophotometer, before and after acidification with 1N 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 of the sediment, expressed in grams. From the same pool of fresh sediment, acid-volatile sulfide (AVS) content was determined in duplicate subsamples (*ca.* 1g) with an AVS test column (Gastec, Model 201L and 201H). Values were expressed as $\mu g\ g^{-1}$ and $mg\ g^{-1}$, respectively, and corrected for porosity, as measured by the water content (obtained after drying sediment subsamples at 105℃ for 20 hours). The remaining sediment was then centrifuged at 3,000 rpm for 20 min. The extracted pore water was filtered on small filters (0.45 μm) fitted to a 10 ml syringe and the obtained dissolved phase was stored at −20℃ for later analysis of NH_4^+-N and $(NO_3^- + NO_2^-)$-N. Nutrients were determined with a Technicon autoanalyzer II, according to STRICKLAND and PARSONS (1972). Not all the samples collected for pigment analysis were analyzed for nutrients due to the insufficient amount of extracted pore water.

For the particle-size determination of the sediment, 0 to 10 cm in depth core samples were collected at each station. They were freeze-dried and sieved on 2 mm, 1 mm, 500 μm, 250 μm, 125 μm and 63 μm mesh-size sieves. Particle-size composition was expressed as a percentage of the dry weight of each fraction.

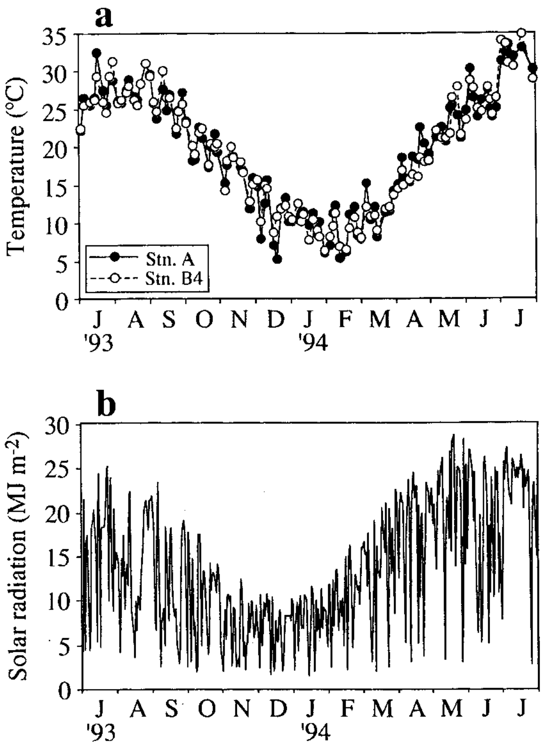
4. Results

4.1 Physico-chemical parameters

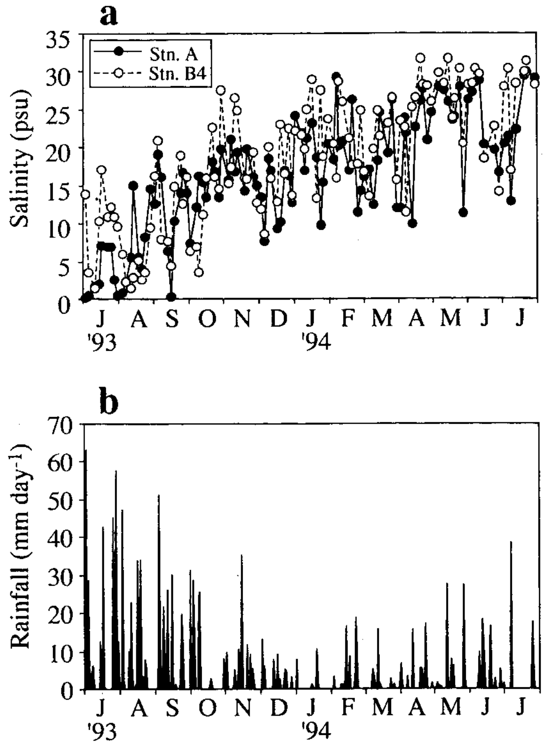
Low-tide water temperature (Fig. 2a) varied from 5.2℃ (December 18, 1993) to 34.8℃ (July 20, 1994) recorded at Stn. A and Stn. B4, respectively. It highly correlated with the temperature of emerged surface sediment ($r^2=0.973$ at Stn. A and $r^2=0.980$ at Stn. B4, data not shown; annual mean, ±S.D., in Table 1). Noticeable interannual differences were found, which corresponded to solar radiation higher in July 1994 than in July 1993 (Fig. 2b).

Low-tide water salinity (Fig. 3a) strongly fluctuated over both few days and the long period. The significant increase of salinity from the summer 1993 to the summer 1994 was caused by the progressive decrease of the rainfall, highest between July and early October 1993 (Fig. 3b). Values ranged from 0.1 psu (Stn. A, July 2, 1993) to 31.6 psu (Stn. B4, April 20 and May 14, 1994) and were slightly lower at Stn. A than at Stn. B4 (Fig. 3a, Table 1).

Low-tide water dissolved oxygen (D.O.) concentration (Fig. 4a) widely fluctuated. At Stn. A, it varied from 2.0 mg l^{-1} (22.0% saturation, December) to 16.7mg l^{-1} (232.5% saturation, May 18). At both stations, D.O. concentration showed a decreasing trend from mid-summer to autumn, but progressively increased from late-winter to early-spring, often resulting



Figs. 2a and b. Low-tide water temperature (a) recorded during each sampling occasion at Stns. A and B4 and daily solar radiation (b) obtained from the Takamatsu Meteorological Agency station where the intertidal flat is located.



Figs. 3a and b. Low-tide water salinity (a) recorded during each sampling occasion at Stns. A and B4 and daily rainfall (b) obtained from the Takamatsu Meteorological Agency Station where the intertidal flat is located.

oversaturated. D.O. concentration appeared to some extent influenced by the seasonal fluctuations of temperature (Fig. 2a) and solar radiation (Fig. 2b). A significant difference between Stns A and B4 was found in July 1994 (Fig. 4a), with a mean of 6.0±1.8 mg l^{-1} and 10.2±2.3 mg l^{-1}, respectively.

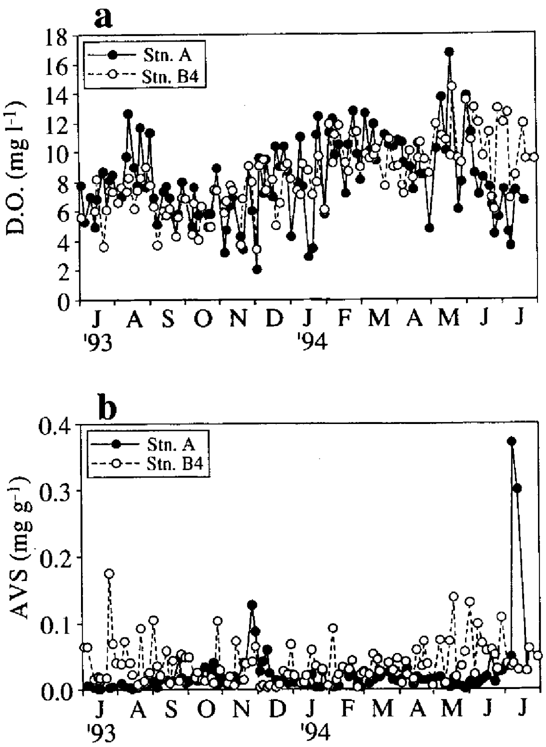
At the surface sediment (0–0.5 cm), acid volatile sulfide (AVS) level (Fig. 4b) was complexively lower at Stn. A (mean of 0.025± 0.047 mg g^{-1}) than at Stn. B4 (mean of 0.039± 0.031 mg g^{-1}). At Stn. A, it ranged from 0.001 mg g^{-1} (July 16, 1993) to 0.371 mg g^{-1} (July 8, 1994). At Stn. B4, it varied from 0.003 mg g^{-1} (February 23, 1994) to 0.176 mg g^{-1} (July 24, 1993). At both stations, no clear seasonal pattern was found. However, AVS level was less fluctuating at Stn. A, in spite of a sharp peak in July 1994 (Fig. 4b), while it varied the most at the less emerged Stn. B4 (Fig. 4b).

Fig. 5 shows the grain size composition of the sediment layer 0–10 cm at Stns A and B4. At both stations the sediment was sandy. However at Stn. A, the percentage of the fine (250–0.063 mm) and mud (<0.063 mm) fraction was significantly higher than that at Stn. B4 (37.4%–3.2% at Stn. A and 8.4%–1.5% at Stn. B4, respectively). The higher percentage of small-particle fraction at Stn. A went together with a higher water content (Table 1).

Table 1 reports the mean concentrations (± S.D.) of dissolved inorganic nitrogen (DIN) [NH_4^+-N and (NO_3^-+NO_2^-)-N] in the pore water of the surface layer (0–0.5 cm) of the sediment. (NO_3^-+NO_2^-)-N concentration was slightly higher at Stn. A, but overall, no significant difference in DIN concentration between the two stations was found.

Table 1. Average (±S.D.) of the physico-chemical parameters of low-tide water and the surface (0–0.5cm) sediment of Stns. A and B4.

	Water			Sediment			
	Temp. (℃)	Sal. (psu)	D.O. (mg/l)	Temp. (℃)	W.C. (%)	Amm. (μM)	Nitr. (μM)
Stn. A	19.6	16.4	8.1	19.2	33.4	116.6	8.6
S.D.	(±7.7)	(±7.8)	(±2.8)	(±8.2)	(±11.2)	(±65.8)	(±8.7)
n.	109	107	109	107	107	71	66
Stn. B4	19.5	18.4	8.3	19.1	25.5	99.1	5.3
S.D.	(±7.6)	(±8.3)	(±2.4)	(±7.8)	(±7.6)	(±50.3)	(±2.8)
n.	109	107	109	107	108	50	41



Figs. 4a and b. Low-tide water dissolved oxygen concentration (a) and emerged surface sediment-volatile sulfide level (b) recorded during each sampling occasion at Stns. A and B4.

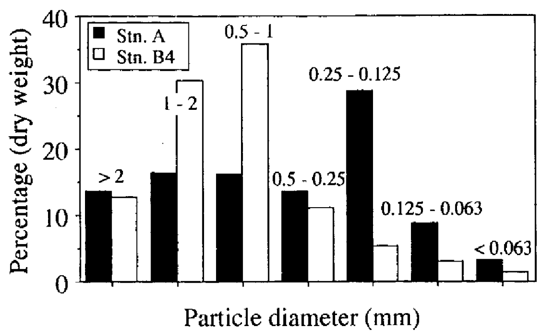


Fig. 5. Grain size composition of the 0–10 cm layer of the sediment at Stns. A and B4.

4-2 Photosynthetic pigment contents

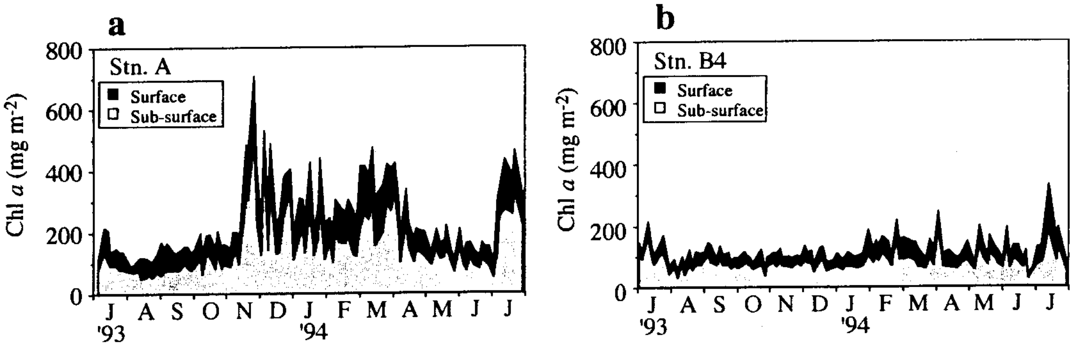
Chlorophyll *a* and pheo-pigment contents were obtained as a μg g^{-1} of dry sediment. They were then converted μg g^{-1} to mg m^{-2} by accounting the bulk-density of the sediment particle as 2.5 g cm^{-3} (MONTANI and OCHAICHI, 1984). A factor f was obtained from the ratio between the sediment particle bulk density and the total vulume, where the maximum value of 1 is for an hypothetical sediment with 0% of pore water. Thus, this ratio changed depending on the different water content of each sample, not corrected for salinity, and varied from 0.14 (water content 70.9%) to 0.69 (water content 15.3%).

The conversion equation was :

1. surface (0–0.5 cm) : mg m^{-2} Chl a = 5000cm^{3}m^{-2} $*$ 2.5 g cm^{-3} $*f*$ mg g^{-1} Chl a (or pheo-pigments)
2. sub-surface (0.5–2 cm) : mg m^{-2} Chl a = 15000 cm^3 m^{-2} $*$ 2.5 g cm^{-3} $*f*$ mg g^{-1} Chl a (or pheo-pigments)

Square regression lines of Chlorophyll a plots were :

for Stn. A : y(mg m^{-2}) = 2.5x(μg g^{-1}) + 45.4 (r^2 = 0.696, n = 107) at the surface and y = 12.4x + 35.6 (r^2 = 0.917, n = 105) at the sub-surface; for Stn. B4 : y = 5.4x + 8.1 (r^2 = 0.793, n = 108) at the surface and y = 16.8x + 16.1 (r^2 = 0.736, n = 108) at the sub-surface (plots not shown).



Figs. 6a and b. Chlorophyll *a* content (expressed as mg m^{-2} of dry sediment) of the surface (0 0.5 cm) and the sub-surface (0.5–2 cm) at Stns. A(a) and B4 (b).

Square regression lines of pheo-pigment plots were :

for Stn. A : y (mg m^{-2}) = 2.3x ($\mu g\ g^{-1}$) + 62.4 (r^2 = 0.842, n = 107) at the surface and y = 12.6x + 67.4 (r^2 = 0.868, n = 105) at the sub-surface; for Stn. B4 : y = 5.8x + 7.1 (r^2 = 0.762) at the surface and y = 15.8x + 48.5 (r^2 = 0.708, n = 108) at the sub-surface (plots not shown).

4-3 Chlorophyll a and pheo-pigment distributional patterns

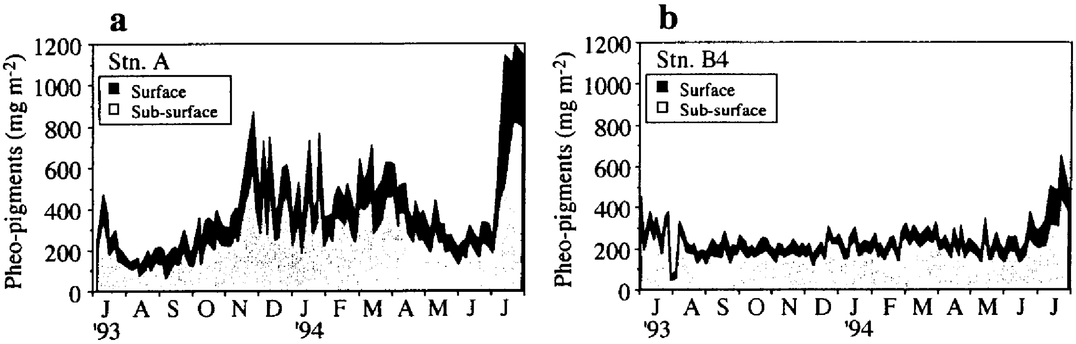
Chlorophyll *a* content of both surface (0–0.5 cm) and sub-surface (0.5–2 cm) sediment was complexively higher at Stn. A than at Stn. B4 (Figs. 6a and b). Mean Chl *a* content (as a sum of the two layers) was 241±121 mg m^{-2} and 122 ±41.4 mg m^{-2} at Stns A and B4, respectively.

At Stn. A, strong temporal (rather than seasonal) variability was found. Chl *a* content varied from 91.2 mg m^{-2} (August 3, 1993) to 708 mg m^{-2} (November 24, 1993) (Fig. 6a). From July to early October, the period of maximum precipitation rate (Fig. 3b), Chl *a* content was low and comparable to that of Stn. B4 (Fig. 6b). In mid-November, in spite of decreasing temperature (Fig. 2a) and solar radiation (Fig. 2b), but in coincidence with a reduced rainfall (Fig. 3b), a sharp peak occurred (Fig. 6a). From December to mid-February, Chl *a* content tended to decrease again, showed wide short-term (days) fluctuations, but remained constantly higher than that at Stn. B4 (Fig. 6a and b). From late February to March, at still low temperature (Fig. 2a) but with a progressive increase of solar radiation (Fig. 2b) and little rain (Fig. 3b), Chl *a* content significantly increased again. From April to the end of June, a new decrease occurred, which coincided with a more intensive rainfall (Fig. 2c) as also revealed by a rapid decrease of low-tide water salinity (Fig. 2d). During this period, as in summer 1993, Chl *a* content was similar to that found at Stn. B4 (Figs. 6a and b). In July 1994, atmospheric conditions were the most favourable for the development of the microphytobenthos. Indeed, during warm (Fig. 2a) and irradiated (Fig. 2a) days with no rain (Fig. 3b), a new sharp increase occurred (463 mg m^{-2} in July 22). However, the Chl *a* content at this time was not as high as that found in late-autumn and early spring (Fig. 6a).

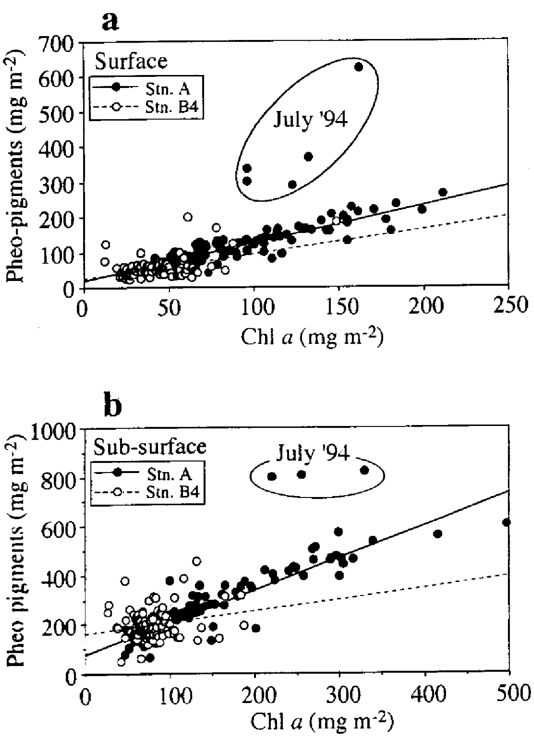
At Stn. B4, Chl *a* content ranged between 39.3 mg m^{-2} (June 24, 1994) and 333 mg m^{-2} (July 12, 1994) (Fig. 6b). The microalgal biomass remained constantly lower tha at Stn. A, and was rather uniform in spite of the strong changes of the environmental conditions which significantly affected the microalgal development at Stn. A. A slight increase occurred between February and March, at the surface sediment. In mid-July 1994, with the significant improvement of the meteorological conditions (Figs. 2b and 3b), a remarkable peak was found. However within few days, Chl *a* content was again as low as during the whole investigated period.

Pheo-pigment content (Figs. 7a and b) was generally higher than Chl *a* content. It averaged 414±218 mg m^{-2} and 251±84.1 mg m^{-2} (as a sum of the surface and sub-surface layers) at Stns A and B4, respectively.

Wide temporal fluctuations were found at



Figs. 7a and b. Pheo-pigment content (expressed as mg m^{-2} of dry sediment) of the surface (0–0.5 cm) and the sub-surface (0.5–2 cm) at Stns. A(a) and B4 (b).



Figs. 8a and b Correlation between Chlorophyll *a* and pheo-pigment content at the surface (0–0.5 cm) (a) and the sub-surface (0.5–2 cm) (b) sediment of Stns. A and B4. Square regression lines were significant at Stn. A (r^2 = 0.816 at the surface and r^2 = 0.843 at the sub surface), but not at Stn. B4 (r^2 = 0.210 at the surface and r^2 = 0.045 at the sub-surface). At Stn. A, some samples of July 1994 (delimited in the circle) were rather deviating from the linear correlation and excluded from the plots. See Discussion.

both stations. Pheo-pigment content varied from 143 mg m^{-2} (August 3, 1993) to 1192 mg m^{-2} (July 22, 1994) at Stn. A and from 84.8 mg m^{-2} (August 3, 1993) to 655 mg m^{-2} (July 22, 1994) at Stn. B4. Plots of Chl *a* against pheo-pigment at both the surface (Fig. 8a) and the sub-surface (Fig. 8b) indicated that the temporal variations of Chl *a* and pheo-pigment content (Figs. 6 and 7) were rather parallel at Stn. A (r^2 = 0.816 and r^2 = 0.843 at the surface and the sub-surface, respectively), but not at Stn. B4 (r^2 = 0.210 and r^2 = 0.045 at the surface and the sub-surface, respectively). At Stn. A, some samples collected in July 1994 were also deviating from a significant correlation (Figs. 8a and b) due to an exceptionally high content of pheo-pigments in respect to that of Chl *a* (Figs. 6a and 7a).

4-4 Primary production

Primary production by the microphytobenthos was estimated on the basis of the mean (±S.D.) monthly content of Chl *a*, assuming that the top 0–0.5 cm layer (surface sediment) was photo-syntetically competent. Monthly averaged light intensity incident on the sediment surface and photosynthetically available for the microphytobenthos was calculated as the sum of that fully available during the emersion period of the surface sediment and that partly available during the submerged period. For the submerged period we used an attenuation coeffcient of 0.6 Due to the differences in elevation between the two stations, the mean hours per day photosyntetically-available (including both periods of sediment emersion and submersion) varied from 6.9 to 9.1 hours and from 6.1 to 8.0 hours, at Stn. A and Stn. B4, respectively. On the basis of the production rate values reported by previous studies on microphyto-benthos (COLIJN and DE JONGE, 1984) and phy-toplankton (HARRISON and PLATT, 1980), factors of 2 (December to February), 2.5 (September to November and March to May) and 3 (June to August) were used as a minimum production rate per unit of Chl *a* (mg C mg Chl a^{-1} h^{-1}) in attempt not to overestimate of the annual primary production by the micro-phytobenthos of this intertidal flat.

At Stn. A, microalgal primary production varied from 1.21 ± 0.27 g C m^{-2} day^{-1} (May 1994) to 3.27 ± 1.49 g C m^{-2} day^{-1}(July 1994). Annual primary production was 634 g C m^{-2} yr^{-1}. At Stn. B4, on the other hand, it varied from 0.46 ± 0.14 g C m^{-2} day^{-1} (December 1993) to 1.71 ± 0.91 g C m^{-2} day^{-1} (July 1994). Annual primary production was 259 g C m^{-2} yr^{-1}.

5. Discussion

5-1 Environmental variability and development of benthic microalgal assemblages

The intensive and prolonged sampling effort enabled us to assess the short-time (days) and interannual variability of the physico-chemical parameters of low-tide water and emerged sediment and to examine possible differences between two stations as related to the development of the microphytobenthos. The constant low-tide status of samplings minimized possible misunderstanding of the results due to the high daily variability related to a complete tidal cycle (MONTANI *et al.*, 1998).

Plots of water and sediment temperature against Chl *a* (not shown) showed a broad distribution, with neither significative seasonal patterns nor differences between the stations (Table 1). On the other hand, temperature and solar radiation played an important role in determining a remarkable interannual variability (between July 1993 and July 1994, Figs. 2a and b) in the microphytoventhic development (Figs. 6a and b) at both stations. Temperature and solar radiation also affected the degradation rate of the primary products. It was particularly evident at Stn. A where the pheo pigment content, relative to that of Chl *a*, was higher during the warm and irradiated July 1994 than during the microphytobenthic blooms of autumn and spring (Figs. 6a and 7a). This fact resulted in values exceptionally deviating from the significant correlation found at this station between Chl *a* and pheo-pigments (Fig. 8a and b).

Salinity of low-tide water nearby the two stations (Fig. 3a) was rapidly influenced by the rainfall (Fig. 3b). Due to vicinity of the spots where sediment samples were collected, we infer that such variations were also representative of the temporal variations of the salinity of the pore water during low-tide, a factor possibly affecting the development and species composition of the microphytobenthos (ADMIRAAL, 1977b). Salinity was slightly but not significantly lower at Stn. A (Table 1), as it was more distant from the low-tide shore-line and relatively less affected by high-salinity water. Besides the strong short-term (days) fluctuations, a significant increasing trend of salinity from the summer 1993 to the summer 1994 was found, which indicated that interannual differences were more marked than seasonal ones, as strongly related to the rainfall (Figs. 3a and b).

Dissolved oxygen concentration (Fig. 4a) was enhanced by high solar radiation (Fig. 2b) and limited by the deterioration of atmospheric condition (*i.e.* rainfall, Fig. 3b) at both stations (average and S.D. in Table 1). A more distictive decline of D.O. concentration occurred at Stn. A in November 1993 and July 1994 (Fig. 4a) in coincidence with an increase of AVS (Fig. 4b) and pigment (Figs. 6a and 7a) content. We assume that the shallow low-tide water we monitored nearby the emerged sediment was rapidly influenced by the ***in situ*** benthic processes of oxygen production by the microphytobenthos and oxygen uptake due to the decay of plant material, respectively.

At the sediment level, $(NO_3^- + NO_2^-)$-N concentration was only slightly higher at the backward of Stn. A (Table 1), as relatively more affected by the river runoff which has been found a major source of nitrate+nitrite nitrogen to this tidal estuary (MONTANI ***et al.***, 1998; MAGNI and MONTANI, submitted to J. Oceanogr.). However, dissolved inorganic nitrogen concentration was com-plexively similar at the two stations (Table 1), indicating no nutrient limitation for the development of the microphytobenthos at Stn. B4.

Overall, the above mentioned physico-chemical parameters were not significantly different at the two stations as to explain the differences in development of the benthic microalgal assemblages.

Within the abiotic sphere, two factors resulted more distictive. Firstly, the different elevation between the two stations. Stns A and B4 emerged at a tidal level of *ca.*+140 and+70cm, respectively. This fact was also indicated by a generally lower AVS level at Stn. A than at Stn. B4 (Fig. 4b and Table 1), as an evidence of a longer exposure of the surface sediment to the atmosphere. Assuming that photo-inibition is almost absent in benthic microalgae (ADMIRAAL and PELETIER, 1980; PELETIER *et al.*, 1996), the higher elevation of Stn. A, and thus a better availability of solar radiation for the photosynthesis, favoured a higher microphytobenthic production. Our results support previous studies which showed higher standing stock of the microphytobenthos at higher elevation (ADMIRAAL and PELETIER, 1980; COLIJN and DE JONGE, 1984; DE JONG and DE JONGE, 1995). On the other hand, the higher elevation of Stn. A also resulted in a faster degradation of Chl *a* into pheo-pigments during warm and irradiated days, such as in July 1994 (Figs. 8a and b). Such a high pheo-pigment content coincided with a significant and unusual increase of AVS level as a result of the enhancement of anaerobic decomposition processes due to decaying microphytobenthos.

As related to the station elevation, the rainfall more strongly influenced the microphytobenthos standing stock at Stn. A. Rather than causing differences in salinity between the two stations, it appeared to be a major factor in limiting the blooming and/or precluding the existence of a stable microalgal vegetation (COLIJN and DE JONGE, 1984) during the summer 1993 and the late spring 1994 (Fig. 8a), as a possible result of a continuous washout (COLIJN and DIJKEMA, 1981). Indeed at Stn. A, the Chl *a* peaks of November, early spring and July 1994 occurred after minor rain and in coincidence or following days of fine weather. Neither dessication nor freezing of the sediment was observed during the course of this investigation.

A second abiotic factor significantly divergent between the two stations was the grain size composition of the sediment. As stated by DE JONG and DE JONGE (1995) the clay content is generally used to define the hydrodynamic energy (tidal currents and waves) of a location, a parameter difficult to measure. An area with low hydrodynamic energy will result in a smaller grain-size composition due to an easier settlement of the silt-clay particles. Accordingly, the sediment at Stn. A had a higher silt clay content than that Stn. B4 (Fig. 5), as it was more distant from the low-tide shore-line and less affected by the tidal currents and waves. A better water retention by smaller particles at Stn. A resulted indeed in a higher water content (Table 1). Differently, the bigger particle size of the sediment of Stn. B4 indicated an area with higher hydrodynamic energy. Thus, the processes of resuspension more strongly limited the primary production of the microphytobenthos at this station (DE JONGE, 1985; DE JONGE and VAN DEN BERGS, 1987). The different impact of the tidal currents on the physical sorting of the sediment (COLIJN and DIJKEMA, 1981; DE JONG, 1985) further contributed to determine the differences in Chl *a* content between the two stations, according to previous studies which found a good positive correlation between silt-clay percentage and pigment contents (COLIJN and DIJKEMA, 1981; DE JONG and DE JONGE, 1995).

5-2 Benthic microalgal standing stock, primary production and consumers

Both the surface (0-0.5 cm) and the sub-surface (0.5-2 cm) layers of the sediment were used to calculate the standing stock of the microphytobenthos, according to DE JONGE and COLIJN (1994) who showed that only 35% to 60% of the total biomass of the microphytobenthos is taken into account, if only the top 0.5 cm are used. In this study, Chl *a* content of the sub-surface (0.5-2 cm) sediment was reduced to 54.8±19.6% and to 67.4±21.1%, at Stn A and B4 respectively. We agree with DE JONGE and COLIJN (1994) that accounting the

0–2 cm layer, rather than the 0–0.5 cm layer, will result in a more accurate estimation of the microphytobenthic standing stock, a certain food resource for the macro-zoobenthos (NICOTRI, 1977; LOPEZ and LEVINTON, 1987; BIANCHI and RICE, 1988; SMITH *et al.*, 1996). At Stn. A, also pheo-pigment content was lower at the sub-surface (78.2±20.6%). Differently, at Stn. B4 it was higher (132.4±39.3%). This facts may be related to the hydrodynamic energy and the particle size of the sediment which allows a faster penetration of microalgal material into the sediment at Stn. B4.

Primary production by the microphytobenthos was estimated assuming the top 0–0.5 cm layer of the sediment as photo-synthetically competent (DE JONGE and COLIJN, 1994) in spite of a deeper presence of vital cells due to physical factors, migration (PINCKNEY *et al.*, 1994) and bioturbation (BRANCH and PRINGLE, 1987).

Both Chl *a* standing stock and annual primary production were generally higher than those reported for the Ems-Dollard estuary, Dutch Wadden Sea (CADÉE and HEGEMAN, 1977; ES VAN, 1982; COLIJN and DE JONGE, 1984), and the Western Scheldt estuary, SW Netherlands (DE JONG and DE JONGE, 1995). On the Western Scheldt estuary, the contribution of the microphytobenthos on the total primary production was estimated at least as 17% (DE JONGE and DE JONGE, 1995). In our study, annual primary production by the microphytobenthos (634 gC m^{-2} yr^{-1} and 259 gC m^{-2} yr^{-1}, at Stns. A and B4 respectively) was higher or similar to that reported by the phytoplankton in the Seto Inland Sea (285 gC m^{-2} yr^{-1}, mean euphotic depth 27.2 m) (TADA, 1997). The present study indicated the high productivity of the intertidal zone, most likely underestimated on the light of the grazing pressure by the macro-zoobenthos which is similarly abundant on the intertidal flat under invenstigation.

Besides the differentiated development of the benthic microalgal assemblages along the estuary, as related to abiotic factors (*i.e.* station elevation and sediment gran-size composition), further studies are in progresses to quantify the effects of the grazing pressure by the macro-zoobenthos on the microphytobenthic standing stock and primary production. Since April 1994, we extended the investigations on the seasonal changes of abundance and faunal composition of the benthic communities quantitatively. Stn. A is inhabited by brachyuran feeding on the surface sediment, while at Stn. B 4 the filter feeder bivalves *Ruditapes philippinarum* and *Musculista senhousia* are dominant (MAGNI *et al.*, in preparation). It is likely that an important food source for the filter feeders is represented not only by organic materials from the water column, but also by epipelic and epipsammic microphytobenthos resuspended with sediment particles (NUMAGUCHI, 1990). We suggest that not accounting the *potential* primary production of the microphytobenthos, as that masked by the grazing effect of the consumers, would lead to a significant underestimation of the autotrophic benthic processes of this intertidal flat.

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