



# Long-term mean annual microphytobenthos chlorophyll *a* variation correlates with air temperature

Victor N. de Jonge<sup>1,\*</sup>, Willem F. de Boer<sup>2,5</sup>, Dick J. de Jong<sup>3</sup>, Verena S. Brauer<sup>4</sup>

<sup>1</sup>Institute of Estuarine and Coastal Studies (IECS), University of Hull, HU6 7RX Hull, UK

<sup>2</sup>Koeman and Bijkerk, Postbus 14, 9750 AA, Haren, The Netherlands

<sup>3</sup>Rijkswaterstaat Directorate Zeeland, Poelendaesingel 18, 4335JA Middelburg, The Netherlands

<sup>4</sup>Laboratoire Ecologie des Systèmes Marins Côtiers ECOSYM, UMR 5119, CNRS, IRD, Ifremer, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier Cedex 05, France

<sup>5</sup>Present address: Resource Ecology Group, Wageningen University, Droevendaalsesteeg 3a, 6708 PB, Wageningen, The Netherlands

**ABSTRACT:** Long-term interannual variations in the mean microphytobenthos chlorophyll *a* (chl *a*) of the Ems estuary were investigated. Concentrations in the 1990s were on average 1.5 times higher than during the period from 1976 to 1978, but a trend in chl *a* over the entire period (1976 to 1999) was not found. In agreement with findings from the late 1970s, the mean chl *a* concentrations over the post 1990 period correlated significantly with the station elevation related exposure time. Over the entire 1976 to 1999 study period, the estuary mean annual chl *a* concentrations correlated strongly and significantly with the mean annual air temperature. Although the range of the variation in the mean annual air temperature of the studied years was only 7.5 to 10.2°C, it surprisingly corresponded with a ca. 2.5-fold variation in the mean annual microphytobenthos chl *a*. In addition to chl *a*, mean monthly C:chl *a* ratios from 1976 to 1977 also correlated positively with changes in temperature. Microphytobenthos carbon biomass [chl *a* × (C:chl *a*)] may, therefore, respond even stronger to air temperature than chl *a* does. The correlations are most likely a combination of direct temperature effects on microphytobenthos and stronger, probably complex, indirect temperature effects on the seasonal development and standing stock of young and adult grazers (bivalves) and their carnivores like the brown shrimp *Crangon crangon*.

**KEY WORDS:** Benthic diatoms · Long-term temperature effect · Carbon: chlorophyll *a* ratio

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## INTRODUCTION

Microphytobenthos contributes significantly to the total system primary production in tidal flat estuaries and other shallow coastal systems (Colijn & de Jonge 1984, de Jonge 1995a, MacIntyre et al. 1996, Cahoon 1999, 2006, Underwood & Kromkamp 1999, Baird et al. 2004). These microalgae, mainly pennate diatoms, live at or near the sediment surface, where they produce organic material predominantly during low tide when they are exposed. During high tide, a varying

part of the microphytobenthos can become resuspended in the water column by the scouring effect of wind-induced waves (de Jonge 1985, de Jonge & van Beusekom 1995, Koh et al. 2006). The importance of this phenomenon depends on the elevation of the intertidal flats in relation to the local tidal characteristics. Once resuspended, the tidal currents transport the diatoms between the submersed intertidal flats and the channels, during which they are mixed with other water masses and redistributed (de Jonge & van Beusekom 1995). When resuspended, these

\*Email: v.n.de.jonge@planet.nl

microalgae, interestingly, continue to contribute to the primary production in the water column (V. N. de Jonge and H. Peletier unpubl. experimental results).

The system-wide contribution of the microphytobenthos (benthic plus resuspended) to the total primary production is variable, but can, as established for the Ems estuary with ~50% intertidal flats, be as high as that of the 'real' phytoplankton (de Jonge 1995a). The estuary-wide microphytobenthos chlorophyll *a* (chl *a*) mass in the top 2 cm of the sediment can easily exceed the chl *a* mass in the water (de Jonge 1995a). There is clear evidence that microphytobenthos supports the benthic food web (Decho & Fleeger 1988, Hecky & Hesslein 1995, Herman et al. 2000, Middelburg et al. 2000, Moncreiff & Sullivan 2001) and that it is an important food source for certain benthic fauna species (e.g. Hummel 1985, Kamermans 1994) as well as pelagic copepods (de Jonge & van Beusekom 1992). This all indicates that these benthic microalgae play a pivotal role for the functioning of estuarine systems in general.

The primary factors that regulate microphytobenthos production and biomass are typically light and nutrients. Yet, during the investigation period, the nutrient concentrations in most of the water column and in the sediments of the Ems estuary were most likely not growth-limiting (de Jonge 1995b, 2000), a phenomenon that has been observed in many of the western European systems (de Jong et al. 1994, Brotas et al. 1995, Barranguet et al. 1998). In contrast, the underwater light conditions were and still are strongly growth-limiting for phytoplankton in most of the Ems estuary (Colijn 1982, 1983, de Jonge 1983, 2000, van Beusekom & de Jonge 1998), which increases the importance of microphytobenthos for the primary production of the entire ecosystem. For the intertidal flats, the light climate during submersion is determined by the ambient irradiance in combination with water turbidity, which is partly controlled by wind and wave action (de Jonge 1995a, de Jonge & van Beusekom 1995), and water depth. During low tide, the light climate of the intertidal flats is determined by weather and season in combination with the elevation of the intertidal flat and the tidal phase, which together determine the exposure time of the sediment surface (see also Colijn & de Jonge 1984). Apart from environmental factors, the microphytobenthos primary production is also determined by its packing density. Blanchard et al. (2001, 2002, 2006, Herlory et al. 2004) found that a net primary production only occurred when the chl *a* concentrations in the top 1 cm sediment layer were lower than ~160 to 170 mg m<sup>-2</sup>. Higher chl *a* densities mostly resulted in net algal res-

piration, which was ascribed to a limitation of inorganic carbon or nutrients (Admiraal et al. 1982, Cook et al. 2010) or severe light limitation. Also, biotic factors, like grazing, influence standing stock and primary production of the microphytobenthos. Since the interactions between all the above mentioned physico-chemical, physical and biological factors lead to complicated and interwoven 'bottom-up' and 'top-down' controls, it is not a straightforward process to determine the causes of measured variations in chl *a* in coastal systems (see de Jonge & Essink 1991).

Despite these problems and shortcomings, much is known and there is data available to qualitatively assess within year dynamics of microphytobenthos chl *a*. This is, however, not so for longer term (inter-annual) variations. Given the ongoing debate on the possible impact of climate change at all ecosystem levels, an interesting question is to what extent temperature may determine interannual chl *a* and related organic carbon biomass variations of the microphytobenthos and how this in turn affects the total food web.

The role of many factors involved in the seasonal regulation of the microphytobenthos growth and standing stock in the field has been clarified in some detail in the past, except for the effect of temperature. Therefore, in the present paper, we focus on possible temperature effects in relation to chl *a* mass variations, using data from the Ems estuary.

The role of nutrients (Admiraal 1977a, Colijn 1983, Helder 1983), light (Colijn & van Buurt 1975, Admiraal & Peletier 1980, Colijn 1982), system residence time (Helder & Ruardij 1982), interaction between tidal flats and water (de Jonge 1995a, de Jonge & van Beusekom 1995) and estuarine transport and exchange processes (Baretta & Ruardij 1988) have all been investigated and discussed at a system-wide scale for the Ems estuary. The overall conclusion was that light (and consequently elevation; see Colijn & de Jonge 1984, de Jonge & Colijn 1994) was the most important determinant for the available chl *a* and primary production of microphytobenthos and phytoplankton.

In short-term experimental investigations, Blanchard et al. (1997, 2001), Colijn & van Buurt (1975) and Admiraal (1977b) studied algal growth in relation to temperature. The latter 2 teams experimentally demonstrated a clear temperature effect on the growth of benthic diatoms, while Admiraal & Peletier (1980) found the same for developing natural benthic diatom films in microcosms under field conditions. Despite this research, up to now no clear effect of temperature on the chl *a* (or carbon biomass) of the

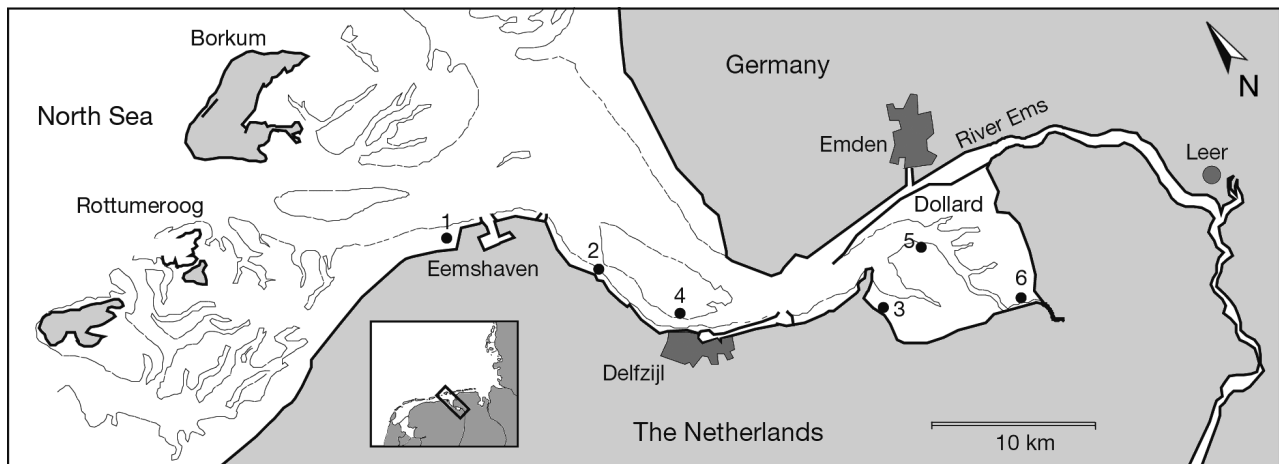


Fig. 1. The Ems Estuary with the 6 stations sampled over the periods 1976–1978 and 1992–1999. For more details see Google <http://earth.google.com>, Wadden Sea area in the northeastern part of the Netherlands

microphytobenthos was detected when considering field data.

The main focus of the present paper is to test whether there is a relationship between temperature and long-term variation in the mean microphytobenthos chl *a* content of the sediment in the Ems estuary and to discuss the possible implications of such a relationship. This is especially interesting as the temperatures in western Europe are rising faster than elsewhere on earth (van Oldenborgh et al. 2008).

## MATERIALS AND METHODS

### Study area

The Ems estuary (Fig. 1) is a coastal plain estuary, which crosses the Wadden Sea at the border between the Netherlands and Germany. Excluding the outer delta and including the freshwater tidal river, the surface area covers  $\sim 475 \text{ km}^2$ . The length of the estuary is  $\sim 70 \text{ km}$  and, including the freshwater tidal river, the total length is  $\sim 100 \text{ km}$ . Approximately 40% of the lower reaches (the area seaward of Stn 2 in Fig. 1) and as much as 80% of the Dollard (Fig. 1) comprise intertidal flats. The tidal prism of the estuary at the inlet is  $\sim 10^9 \text{ m}^3$  (de Jonge 1992a). The tidal range varies from  $\sim 2.3 \text{ m}$  in the tidal inlet to over  $3.0 \text{ m}$  near Delfzijl and Emden.

### Sampling stations

Microphytobenthos in the Ems estuary has been studied for many years with the aim of modelling the

ecosystem functioning dynamics (Baretta & Ruardij 1988). Within this context, a sampling scheme was developed based on the system's bathymetry and physical features. In 1992, Rijkswaterstaat started microphytobenthos monitoring at the 6 locations in the Ems estuary (Fig. 1) which had been previously sampled (de Jonge & Colijn 1994).

### Sampling

The corer based sampling procedure used from 1976 to 1978 has been described by de Jonge & Colijn (1994). Sampling was always done with a perspex core of 2.4 cm inner diameter. During 1992 and 1994 to 1999, the monthly samples were taken with a comparable corer. Three substations per sampling station (consisting of a  $500 \times 500 \text{ m}$  quadrat) were selected for sampling the elevation gradient. Based on the detailed study results from 1972 to 1986 (e.g. van Es 1982, Colijn 1983, de Jonge 1992b), 5 cores were taken at every substation in a circle to arrive at a representative pigment value per station. The cores were sliced and the upper 0.5 cm of the 5 cores pooled by substation for chl *a* analysis. Samples were stored at  $-30^\circ\text{C}$  and lyophilized before extraction in 90% acetone.

### Chl *a* analysis and conversions

From 1976 to 1978 (the 'pre-1990 period'), chl *a* was determined spectrophotometrically. Concentrations were expressed in weight per unit surface area ( $\text{mg m}^{-2}$ ) for the top 0.5 cm sediment layer. Pigment concentrations were measured using a modification

(addition of 60 µl of 10% HCl; Moed & Hallegraef 1978) of Lorenzen's method (1967) and an overnight extraction time at 4°C. During 1992 and 1994 to 1999 ('post-1990 period'), the sediment was sampled by comparable corers as above and again using an overnight period to extract the chl *a*, after which measurements were carried out by HPLC as described by Daemen (1986). The chl *a* values were expressed as a content on a weight per dry weight basis ( $\mu\text{g g}^{-1}$ ) for the top 0.5 cm sediment layer. The HPLC derived values are on average ~70% the values derived with the spectrophotometrical method of Lorenzen (Daemen 1986). To make the data of the entire 1976 to 1999 set mutually comparable and consistent with most of the literature, all data were converted to concentrations as measured by Lorenzen's method. Conversion was done by multiplying the HPLC values with a factor 1.43 (based on Daemen 1986). The conversion from weight per weight (of the post-1990 data) to weight per  $\text{m}^2$  was based on the specific density of the sediment at the 6 stations. These values were obtained during a full sedimentological analysis by D. J. de Jong (unpubl.) and carried out in 1992 and 1994. The effect of increasing mud content on the specific density of the sediment is illustrated in Fig. 2. The total range covers 0.63 to  $1.69 \text{ g cm}^{-3}$  and shows that the muddier the sediment is, the lower its specific density will be. The sediments with the lowest mud content (Stns 1 and 5) usually do not reach the mud content of the more muddy stations (Stns 4, 2 and 6). This means that, despite the variation in values, the gradient in Fig. 2 is a persistent one. There was unfortunately no data to illustrate any available seasonal effect.

### Environmental factors

Seasonal effects of environmental conditions and biotic interactions have been investigated before on a seasonal basis and were also incorporated in the ecosystem model of the Ems estuary (Baretta & Ruardij, 1988). This, however, does not hold for longer term interannual variations. To find an explanation for long temporal scale related variations in chl *a* concentrations among stations and years, the effect of station elevation, irradiance, turbidity (Suspended Matter concentration = SPM), wind speed and air temperature were investigated for the post-1990 period. The quality of the data on water temperature was too low to be used. This was due to the fact that water temperatures were only measured during water sampling. The elevations of the different sta-

tions and substations were derived from soundings carried out in the 1990s by Rijkswaterstaat. SPM data was obtained from the Rijkswaterstaat DONAR monitoring database. Data on air temperature, irradiance and wind speed were obtained from the KNMI (Royal Netherlands Meteorological Institute). Mean meteorological conditions were calculated based on 3 meteorological stations close to the coast in the study area (Lauwersoog, Eelde and Nieuw Beerta).

For the pre-1990 period environmental data were not available in the required detail for the above mentioned meteorological stations, so only mean annual air temperatures and mean annual chl *a* values were used. The post-1990 data on mean annual air temperature and mean annual chl *a* and the same data from the pre-1990 period (the years 1976 to 1978 from de Jonge & Colijn 1994) were combined to investigate the relationship between air temperature and chl *a*.

### C:chl *a* ratios

In addition to chl *a*, an existing data set on C:chl *a* ratios from 1976 to 1978 (de Jonge 1980) was re-analysed to investigate the possible effect of air temperature (Table 1). In de Jonge 1980, temperature was not considered as a possible relevant factor explaining the observed seasonal fluctuations in C:chl *a* ratios. De Jonge (1980) concluded that C:chl *a* varied seasonally per sampling period and that there were no spatial, i.e. no among-stations differences in C:chl *a*. Therefore, for the present paper, data from all 6 stations were pooled and the monthly mean C:chl *a* ratios calculated. The aim was to investigate whether the variation in the monthly mean C:chl *a* ratios correlated with air temperature. If so, then that result could be used to indicate to what extent any

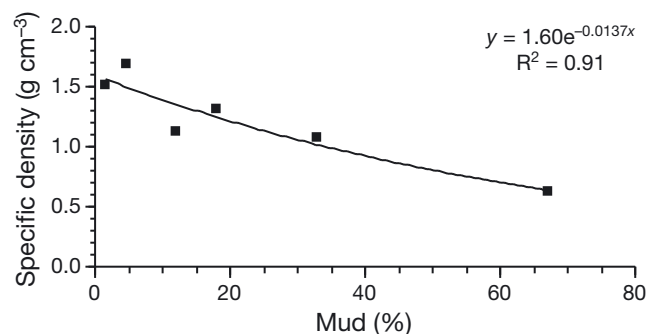


Fig. 2. Specific density of the sediment at the 6 stations against the mud content of the sediment, to illustrate the effect of the variations in mud content. See Fig 1 for station locations

Table 1. Description of the different data subsets used. Chl *a*: chlorophyll *a*

Set	Year	No.	Description	Source
1a	1992, 1994–1999	3493	Raw data	This study
1b		1135	Stn mean per sampling date	This study
1c		412	Stn mean per mo	This study
1d		41	Stn mean per yr	This study
2	1976–1978, 1992–1999	12	Estuary-wide mean per yr	de Jonge & Colijn (1994), this study
3	1976–1977	23	Estuary-wide mean C:chl <i>a</i> per mo	This study <sup>a</sup>

<sup>a</sup>C:chl *a* ratios from de Jonge (1980)

temperature related variation in C:chl *a* could contribute to a possible accumulated temperature effect via both chl *a* and C:chl *a* on the microphytobenthos carbon biomass [expressed as chl *a* × (C:chl *a*)].

#### Data sets

The data sets in this paper were derived from different sources, yielding different data sub-sets, except for mean annual chl *a* and mean annual air temperature. Table 1 provides an overview of the structure of the data sets used.

#### Statistical analyses

The chl *a* data for the top 0.5 cm sediment layer did not follow a normal distribution. To satisfy the requirements for the application of parametric tests (Zar 1984), the data were log-transformed. To test whether data from substations could be used as independent data in the analysis, the spatial autocorrelation was calculated, using Moran's *I* test (R Development Core Team 2007). A General Linear Model (GLM) was applied to test for differences between the means among Station (Stn), Year (Yr) and Month (Mo), followed by the Tukey multiple comparison test to determine the differences between the classes. The  $\eta^2$  statistic was used to describe the proportion of the total variation that is attributable to a specific predictor variable. The possible relationships between chl *a* concentrations and independent variables, such as air temperature, were tested using Spearman correlation tests and a multiple backward regression. The calculation of the independent contribution of each variable to the total explained variance of the regression model was carried out using hierarchical partitioning (Chevan & Sutherland 1991).

The chl *a* data series in the post-1990 period (1992, 1994 to 1999) were often incomplete. If this happened, the data were case wise deleted. All statistical analyses were carried out in SPSS 15.0, except for the calculations of the spatial autocorrelation and the hierarchical partitioning, which were carried out in R (R Development Core Team 2007).

## RESULTS

### Factors explaining spatial and temporal changes in chl *a*

#### Spatial autocorrelation of substations

As a first step, we determined whether the data collected at the 3 substations within each station were independent, by calculating the spatial autocorrelation. The chl *a* concentrations of the 3 substations were spatially autocorrelated in all cases (Moran's *I* = 0.088,  $p < 0.001$ ). The data from substations were, therefore, pooled, using the mean chl *a* concentrations per station for further analysis.

#### Trends

The mean monthly chl *a* concentrations for the 6 stations in 1992, and 1994 to 1999 (Fig. 3) show strong variation among Stns, mo and yr, without a visually clear seasonal pattern. A 3-way ANOVA (data set 1c in Table 1) with Stn, Yr and Mo as independent factors revealed significant effects of all 3 factors and their 2-way interaction terms, and explained 75 % of the observed variation (Table 2). The factor yr had a significant effect on the mean chl *a* concentration with the highest  $\eta^2$  of the 3 main factors (Table 2), with significantly higher values in 1992 and 1999 and lower values in 1996 (Tukey test,  $p < 0.05$ ). Stn and mo were also significant, with lower chl *a* concentrations measured at Stn 3 and 4, and between December and March, and higher values reported at Stn 6, and between May and October (see also Fig. 3). Interannual and seasonal effects were apparent with a high  $\eta^2$  (0.601) for the Yr × Mo interaction term (Table 2).

A distinct pattern, however, emerged when the mean, log-transformed annual chl *a* values per sta-

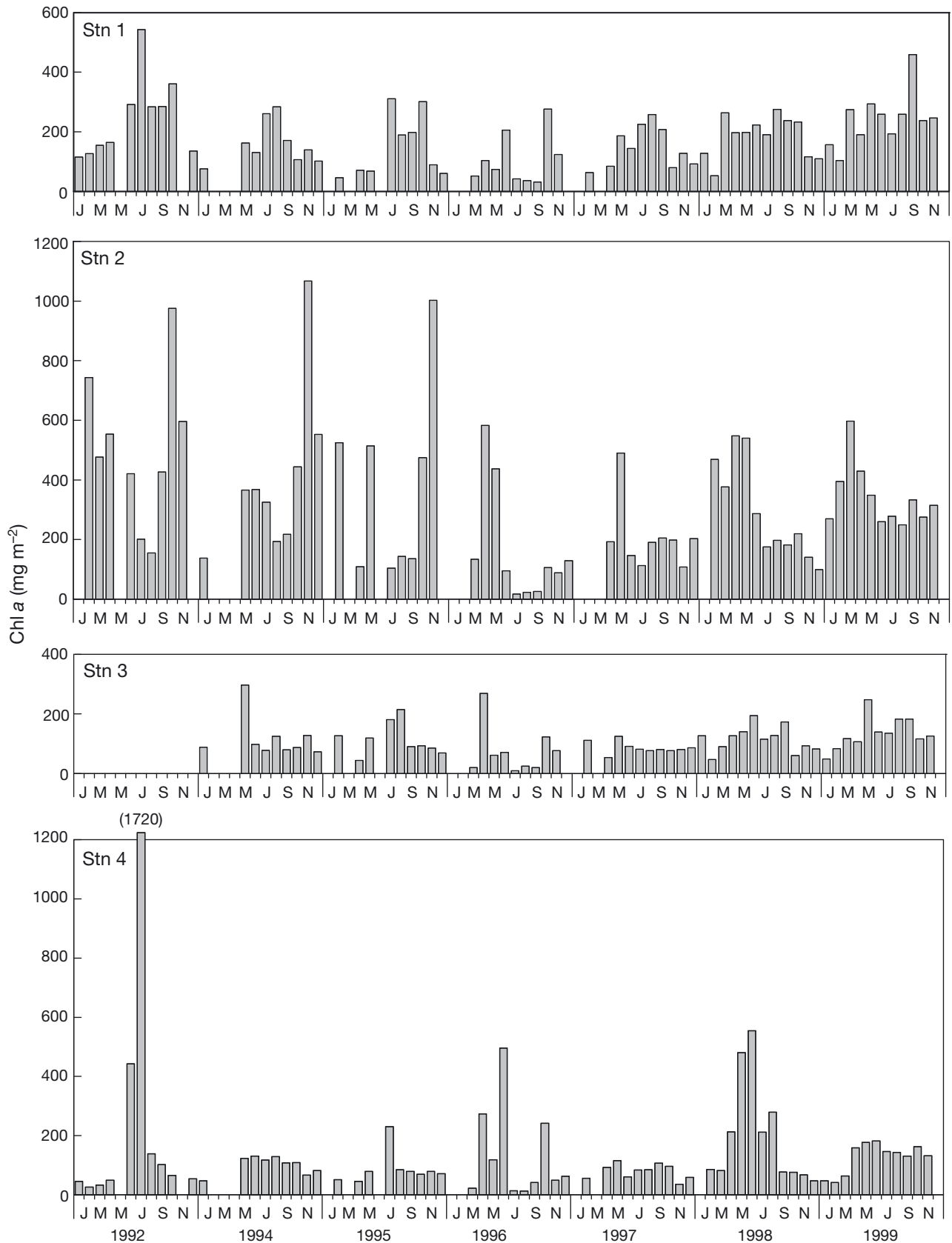


Fig. 3. Monthly fluctuations of chlorophyll a (chl a) concentrations in the top sediment layer (0.0–0.5 cm) for the 6 stations for 1992, and 1994–1999, n = 406. Data from 1993 is missing. Missing bars indicate missing values



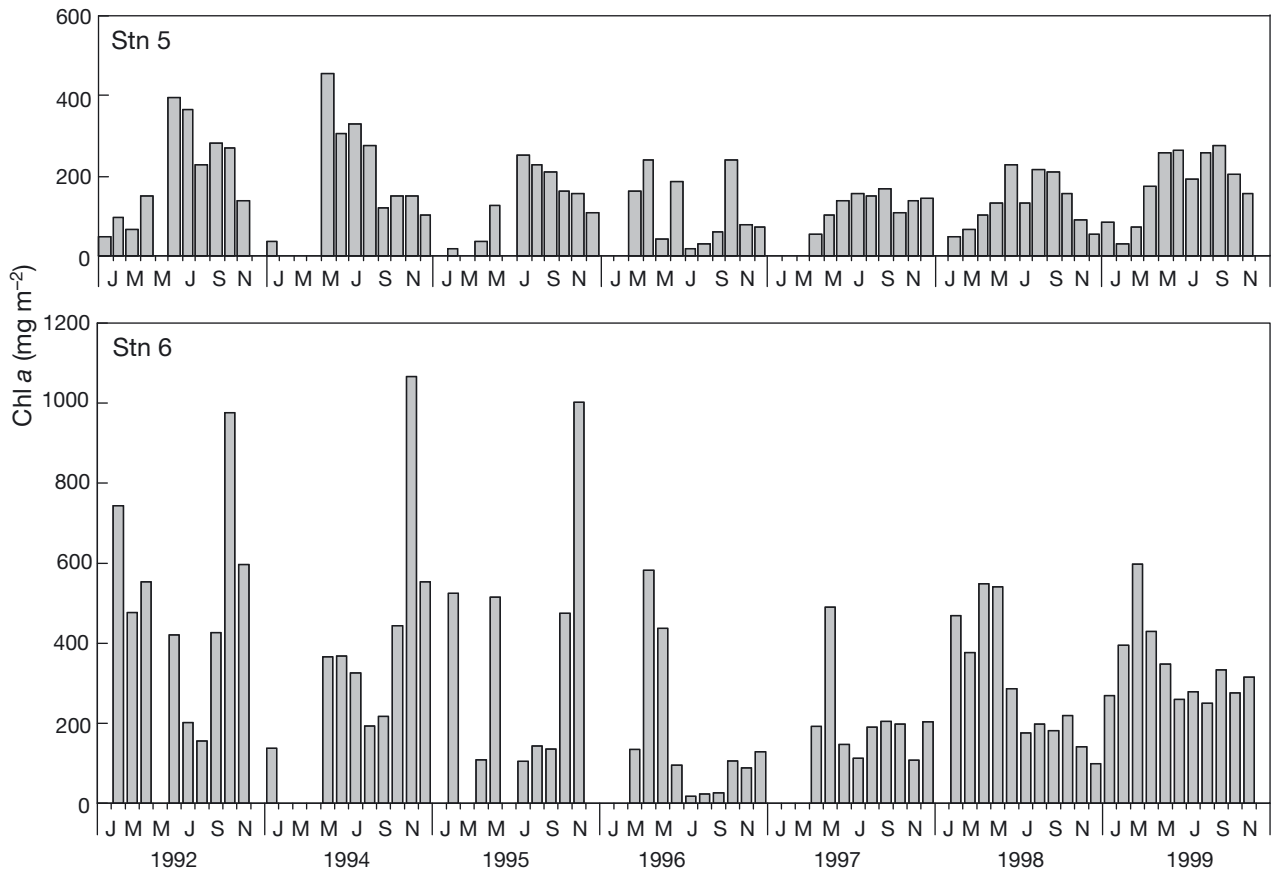


Fig. 3 (continued)

tion were plotted as a time series (Fig. 4). The values of all Stns decreased from 1992 to 1996, after which they increased again. Fig. 4 (see also the estuary-wide annual means in Fig. 6) illustrates that there was no overall increasing or decreasing temporal trend in chl *a* during 1992, and 1994 to 1999. Given the fact that the individual sampling stations are located in the ~50 km long main part of the estuary (Fig. 1) and are situated far from each other (max. distance ca. 40 km), it is clear that estuary-wide operating factors or processes must have been responsible for the observed interannual variations.

#### Station exposure time

The highest elevated station was Stn 6, and the lowest were Stns 2, 3 and 4. The maximal difference in elevation was 2.3 m which, compared to the local tidal range of ~3 m, results in a large difference in exposure times among stations. Within some stations (thus among substations), the difference in elevation was also relatively large, e.g. at Stn 2 the difference between highest and lowest

elevated substation was 1.0 m. When averaged over all years, the log-transformed chl *a* concentrations of all 18 substations were significantly and positively correlated with the average exposure time (Fig. 5; GLM:  $F_{1,17} = 33.774$ ,  $p < 0.001$ ), explaining 68% of the total variation. This suggests that exposure time related factors like irradiance, wind speed and air temperature might also be candidate factors, explaining a part of the differences in chl *a* concentrations among stations.

Table 2. Three-way ANOVA with factors 'Yr', 'Location', and 'Mo' on the mean monthly log-transformed chl *a* data per Stn (data set 1c in Table 1). The partial  $\eta^2$ -statistic is a relative index for the amount of variation that is attributable to a specific predictor variable

Factor	<i>F</i>	df	<i>p</i>	$\eta^2$
Yr	60.131	6,245	<0.001	0.596
Location	44.427	4,245	<0.001	0.476
Mo	12.133	11,245	<0.001	0.353
Yr × Location	1.602	29,245	<0.05	0.159
Mo × Location	3.344	55,245	<0.001	0.429
Yr × Mo	6.707	55,245	<0.001	0.601

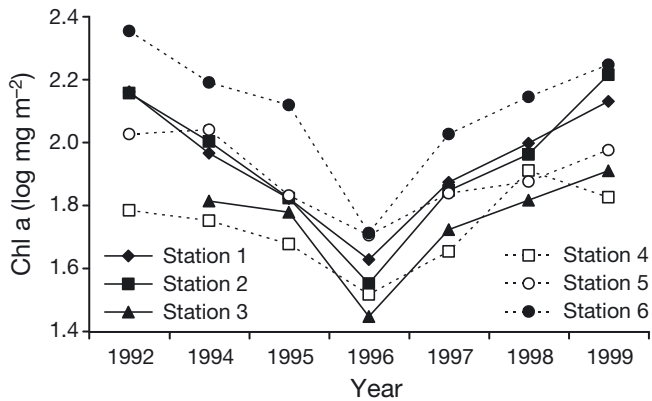


Fig. 4. Interannual variation of chl *a* concentration per  $\text{m}^2$  in the top layer of the sediment (0.0–0.5 cm) for the 6 stations sampled for 1992, and 1994–1999

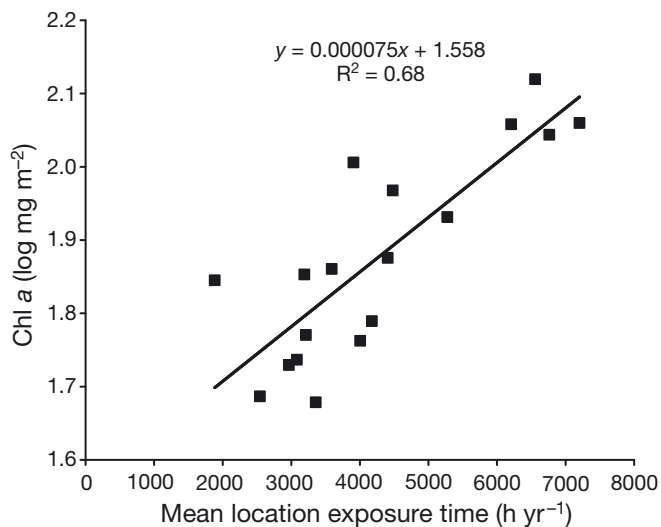


Fig. 5. Log-transformed total averaged chl *a* concentrations (per 0.5 cm sediment layer and  $\text{m}^2$ ) per sampled substation against the mean annual exposure time for 1994–1999 (3 substations per sampling station)

#### Effect of physical factors

Using the monthly mean chl *a* values, we tested for the influence of air temperature ( $T$ ), irradiance, suspended matter and wind speed, using the non-parametric Spearman rank correlation test (Table 3). All the presented correlations were highly significant. As expected, chl *a* values were positively related to  $T$  and irradiance and negatively to wind speed and suspended matter, with the highest rank correlation coefficient for  $T$  ( $r_s = 0.55$ ). These independent factors strongly co-vary, and a backward multiple regression only maintained  $T$  as the single significant factor in the model ( $t = 3.189$ ,  $n = 73$ ,  $p < 0.01$ ). A hierarchical partitioning confirmed this pattern, as the independent contribution of  $T$  to the total explained

Table 3. Spearman correlation results ( $r_s$ ) between the mean monthly estuary-wide chl *a* concentrations ( $\log \text{mg m}^{-2}$ ) and the abiotic variables. The meteorological data are obtained from the Royal Netherlands Meteorological Institute (number of mean monthly values,  $n = 73$ ), and the water quality data from Rijkswaterstaat (DONAR-database<sup>b</sup>) ( $n = 69$ ). The analyses are based on the data from 1992 and 1994–1999. Data are based on the estuary-wide mean chl *a* values in Table 1, data set 1c

Variable	$r_s$	$p$
Air temperature ( $^{\circ}\text{C}$ )	0.553	<0.001
Monthly % of days with mean daily air temperature $>0^{\circ}\text{C}$	0.498	<0.001
Irradiance ( $\text{J cm}^{-2}$ )	0.440	<0.001
Monthly % of days with maximum hourly mean wind speed $>10 \text{ m s}^{-1}$	-0.302	<0.001
Suspended matter ( $\text{mg l}^{-1}$ )	-0.317	<0.001

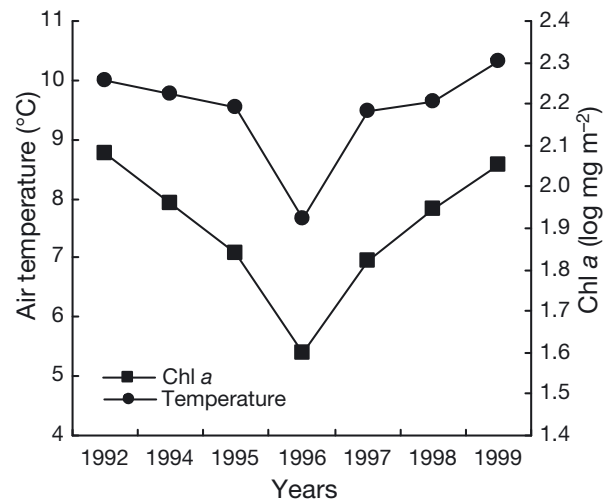


Fig. 6. Time series of the mean annual air temperatures ( $T$ ) ( $^{\circ}\text{C}$ ) and the estuary-wide averaged annual mean chl *a* concentrations ( $\log \text{mg m}^{-2}$ ) for 1992, and 1994–1999. Concentrations expressed per 0.5 cm sediment layer

variance was larger than that of the other 4 variables combined (respectively 0.074 and 0.064 %).

Supported by the above analyses, a plot representing the time series of the mean annual  $T$  and the mean log-transformed annual chl *a* values for 1992, and 1994 to 1999 was made (Fig. 6). The parallel trends in chl *a* and  $T$  of both curves are obvious, with a good correlation between chl *a* and  $T$ . This resulted in the decision to extend the 1992, 1994 to 1999 data sets with older data for a more thorough analysis of the correlation between mean  $T$  and estuary-wide mean annual chl *a* levels.

The mean annual chl *a* values for the period 1976 to 1999 showed a strong positive effect of the mean



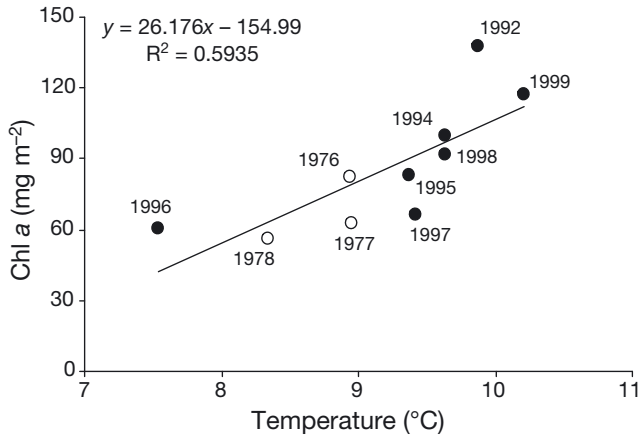


Fig. 7. Mean annual concentrations of estuary-wide averaged chl *a* against the mean annual air temperature (*T*). Concentrations expressed per 0.5 cm sediment layer. ●: 1992, 1994–1999 series, ○: 1976–1978 data taken from the literature (Table 4)

annual *T* (Fig. 7; GLM:  $F_{1,10} = 14.157$ ;  $p = 0.004$ ; based on data set 2 in Table 1, see also Table 4). Despite the relatively small range in the annual mean values (7.5 to 10.2°C), the explained variance is relatively high, amounting to nearly 60%. The lowest mean chl *a* value in the temperature range is 56.2 (1978) and the highest 137.8 (1992) mg chl *a* m<sup>-2</sup>, a difference of a factor of ~2.5. To demonstrate that this picture is not biased by extending the original 1992, 1994 to 1999 data set with the older data, a separate regression for 1992, 1994 to 1999 was carried out as well. This latter analysis resulted in a similar fit ( $y = 23.14x - 123.51$  and  $R^2 = 0.53$ ).

Important to note is that there was no consistent difference between the 2 chl *a* measurements methods (high performance liquid chromatography [HPLC] or spectrophotometric) in the *T* and chl *a* relationship (GLM,  $F_{1,9} = 0.009$ ,  $p = 0.925$ ).

Table 4. Mean annual microphytobenthos chl *a* concentration and mean annual air temperatures (*T*) from KNMI for the period 1976–1999. TE: total estuary

Year	Chlorophyll <i>a</i> (mg m <sup>-2</sup> )						TE	(°C)
	Stn 1	Stn 2	Stn 3	Stn 4	Stn 5	Stn 6		
1976 <sup>a</sup>	41.5	35.2	44.2	52.9	70.8	247.2	81.9	8.94
1977 <sup>a</sup>	60.2	33.2	50.2	33.2	73.6	124.0	62.3	8.95
1978 <sup>a</sup>	32.6	28.6	41.9	34.5	71.9	128.0	56.2	8.34
1992 <sup>b</sup>	128.3	175.7	59.6	124.7	103.4	235.2	137.9	9.88
1994 <sup>b</sup>	79.7	98.0	58.6	50.6	107.5	203.9	99.8	9.63
1995 <sup>b</sup>	74.2	80.1	57.1	43.8	68.2	173.2	82.7	9.38
1996 <sup>b</sup>	51.9	67.1	37.8	66.5	56.5	81.9	60.2	7.54
1997 <sup>b</sup>	73.5	74.2	43.3	39.5	64.4	102.5	66.2	9.42
1998 <sup>b</sup>	92.8	92.8	57.5	94.2	65.1	147.0	91.5	9.63
1999 <sup>b</sup>	121.6	190.9	67.6	62.9	89.2	170.6	117.1	10.21

Data from: <sup>a</sup>de Jonge & Colijn (1994), <sup>b</sup>this study

### C:chl *a* ratios and temperature

C:chl *a* ratios for 1976 and 1977 follow the temporal variation of the mean monthly *T* (Fig. 8a). Data from 1978 was not incorporated in this analysis because it was too fragmented to represent either a reliable temporal variation or an appropriate annual average value (see de Jonge 1980 for further details).

Fig. 8b shows a linear regression of the mean monthly C:chl *a* ratios to the mean monthly *T* over the same 2 yr ( $y = 1.28x - 29.94$ ; GLM,  $F_{1,21} = 12.568$ ;  $p = 0.0019$ ; 95% CI for the regression coefficient: 0.53–2.03; based on data set 3 in Table 1). Although the regression is significant, the relatively low explained variance of 37% indicates that factors other than temperature are also responsible for explaining the observed variation in C:chl *a* ratios.

## DISCUSSION

### Annual variations in benthic chl *a*

Causes for the large observed variation in the mean annual benthic chl *a* concentrations are typically grazing, resources and other environmental factors, as investigated here. The observed temperature related interannual variations are so strong (ca. 2.5-fold) that a combination of >1 temperature related effect is likely and, thus, should be considered.

It is not likely that nutrients played a major role, or that the water residence time in the system had changed. Thus, in case of the Ems estuary, other factors must be used to explain the major variations. These are temperature (Martens 2001, van Beusekom et al. 2009, present study), and light climate related factors caused by wind related turbidity changes (de Jonge 1995a), dredging activities (de Jonge 1983, 2000) and harbour spoil disposal (de Jonge & de Jong 2002).

Despite the fact that we have not been able to investigate the role of grazers due to lack of data, it is likely that grazers controlled the inter-annual chl *a* variations as well (e.g. Alpine & Cloern 1992, Hansen et al. 1993, Keller et al. 1999, Herman et al. 2000, Middelburg et al. 2000, Loebel & van Beusekom 2008). These factors will be discussed in some detail below in the section 'Temperature, macrozoobenthos and carnivores'.

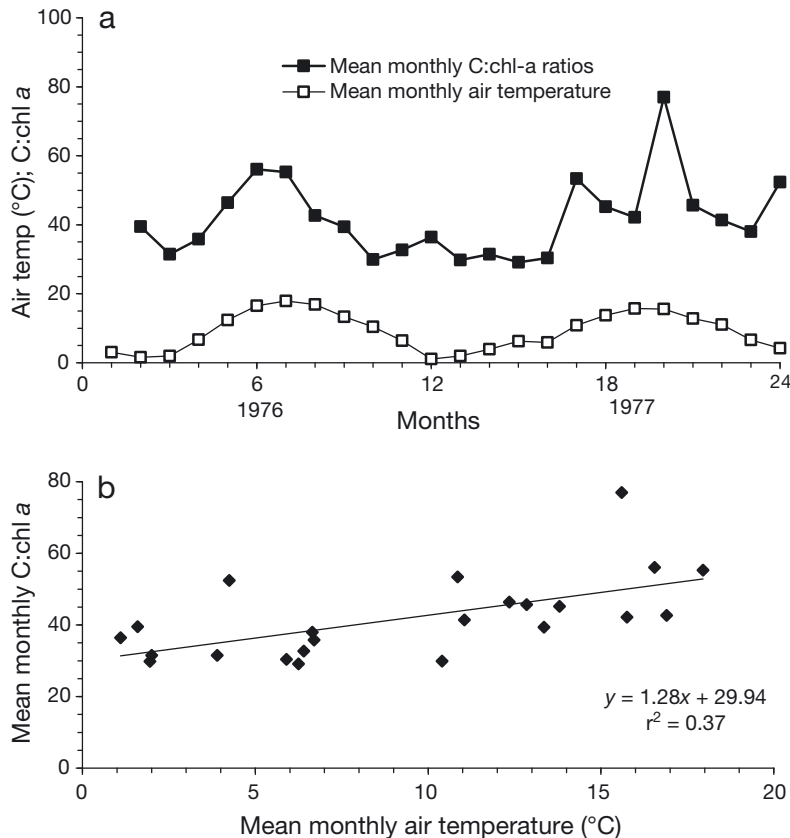


Fig. 8. (a) Time series of mean monthly C:chl a ratios (■) and mean monthly air temperature (T) (□) for 1976 and 1977. (b) Mean monthly C:chl a ratios against mean monthly T for 1976 and 1977

#### Temperature and microphytobenthos

In relatively turbid systems like the Ems Estuary, intertidal microphytobenthos is mainly photosynthetically active when low tides coincide with the daily photoperiod. This implies that the physiological functioning of these microalgae may indeed be more determined by the strongly fluctuating air temperatures and related water temperatures in the diatom films during exposure than the more stable water temperature, as measured in the main channels.

The periodic exposure which microphytobenthos experiences also implies that these microalgae need to be very well adapted to highly dynamic environments, where sometimes the sediment temperature in summer may increase by  $4^{\circ}\text{C h}^{-1}$  (Harrison 1985). Blanchard et al. (1997, 2006) performed field experiments on the photosynthetic capacity (P) of microphytobenthos during different seasons and different temperature increase rates (T) and observed invariable P – T curves over the year. The optimum temperature for photosynthesis was found at  $25^{\circ}\text{C}$ , while a

complete stop was measured at  $38^{\circ}\text{C}$ . These results may be used as an indication of the short-term chl a response of the microphytobenthos to temperature. Despite the very high local chl a values at times (Fig. 3), this may imply that there is the possibility for some further increase in chl a levels. However, loss terms such as respiration, grazing and resuspension (de Jonge 1995a, Guarini et al. 2006) and gain terms such as (strong local) sedimentation of wind-wave induced resuspended microphytobenthos (de Jonge 1995a) also determine if and when an increase in primary production is reflected in an increase in biomass. Thus, there is most likely a limit to the (future) development of the benthic chl a concentration which is caused by algal density related growth limitation (Blanchard et al. 2001, 2002, 2006, Herlory et al. 2004).

The above-mentioned maximum amount of chl a of  $\sim 160$  to  $170 \text{ mg m}^{-2}$  chl a (expressed by Blanchard and co-workers for their sampled 1 cm sediment layer, which corresponds to  $\sim 120$  to  $200 \text{ mg m}^{-2}$  chl a for the top 0.5 cm sediment layer as sampled by de Jonge & Colijn 1994) may be exceeded

considerably and reach values as high as  $500 \text{ mg m}^{-2}$  chl a (Colijn & de Jonge 1984, Cahoon 1999), while peak values can even reach  $1000 \text{ mg m}^{-2}$  (present study). These higher levels are not necessarily the result of higher production, but might be the result of the above mentioned accumulation due to sedimentation of microalgae since resuspended material is not deposited in a uniform fashion over the intertidal flats. Therefore, despite the linear regression presented in Fig. 7, we expect that the presented curve may bend to an upper saturation level, which may correspond with data as referred to above.

#### Temperature, macrozoobenthos and carnivores

Apart from influencing the microphytobenthos directly, the existing seasonal variations, interannual temperature variations and cold winters may also indirectly affect the chl a mass via influencing the behaviour, growth and reproduction of macrozoobenthos species like the cockle *Cerastoderma edule*,

the gaper clam *Mya arenaria*, the blue mussel *Mytilus edulis* and the Baltic tellin *Macoma balthica* (Beukema 1985, Beukema et al. 2001, Strasser et al. 2001, Beukema & Dekker 2005) and epifauna like the brown shrimp *Crangon crangon* (Beukema 1992, Jönsson et al. 1993, Strasser, 2002). Moreover, it is possible that what has been found here for the above species also holds for other benthic species like the mud shrimp *Corophium volutator*, the mud snail *Hydrobia ulvae* and the lug worm *Arenicola marina*. This point is very relevant as the above mentioned macrozoobenthos species are not evenly distributed over the Ems estuary. If the varying chl *a* masses of the microphytobenthos could be clearly related to variations in grazing pressure and related resuspension stimulating activity of epifauna (e.g. *Crangon crangon*, *Corophium volutator*) or macrozoobenthos, then we should have an important explanation for both the short-term and the long-term variations in the benthic and resuspended chl *a* mass.

#### Turbidity and light

Based on *in situ* measurements in the lower reaches of the Ems estuary, Colijn (1983) showed that during high tide the microphytobenthos production can benefit from clear water. He determined that this effect could increase the annual microphytobenthos production (and consequently the chl *a* concentrations) by ~35%, compared to a situation where the primary production only occurred during exposure of the same area. Despite this finding, the average water turbidity in our analysis was only a weak predictor for explaining the monthly variation in chl *a* concentrations. This is remarkable because apart from the reported wind driven natural variation in SPM concentrations (de Jonge 1995a, 2000, de Jonge & van Beusekom 1995), there is also a major (since 1970s) anthropogenic component in this estuary caused by channel maintenance dredging, the disposal of harbour sludge (de Jonge 1983) and major engineering works in the freshwater tidal river Ems itself since the mid 1980s with significant implications for the entire system (Schuttelaars et al. 2012). The result of the dredging activities was a ~2- to 3-fold SPM increase in the main channels of the main estuary during the early 1990s compared to 1954, and of which the effects are also measurable during high water above the submerged intertidal flats (de Jonge 1995a, de Jonge & van Beusekom 1995). If increased SPM affected microphytobenthos chl *a*, then one would have expected 2 independent

regression lines in Fig. 7, one for the 1970s to 1980s and one for the 1990s. This is clearly not the case and effects of increased SPM on microphytobenthos chl *a* concentrations, even in the lower reaches of the intertidal flats (Stn 1), can most likely be excluded.

#### Wind-induced resuspension

The resuspension of microphytobenthos into the water column is an important process determining the microphytobenthos chl *a* distribution between the intertidal flats and the water column (de Jonge & van Beusekom 1995, Koh et al. 2006). Due to windy, but not necessarily stormy, conditions a significant part of the microphytobenthos is resuspended into the water column where it continues to grow like phytoplankton (V. N. de Jonge & H. Peletier unpubl. experimental results). Under normal weather conditions, only ~25% of the total microphytobenthos chl *a* is present in the water column in this estuary (de Jonge 1995a). Under strong winds conditions (e.g. 12 m s<sup>-1</sup> wind speed), this value only doubles (de Jonge & van Beusekom 1995). Within the context of this paper, the effect of wind on resuspension is not strong enough to account for the 2.5-fold variation observed in the benthic chl *a*.

#### C:chl *a* ratios

In the literature, chl *a* is often considered a suitable proxy for algal biomass, but this assumption is not correct. In the late 1970s (de Jonge 1980), a significant (up to an order of magnitude) temporal variation in C:chl *a* ratios was measured. Consequently, algal biomass cannot simply be calculated from chl *a* values through linear transformation, nor can chl *a* values be directly converted into carbon biomass by using a constant (mean annual) C:chl *a* ratio. Thus, it is of importance to verify whether there is a possible amplification of the temperature effect on chl *a* by an additional effect via the C:chl *a* ratio. When using seasonal data, this is indeed the case, but the seasonal temperature effect on the C:chl *a* ratio is so modest (only 3% increase per 1°C air temperature; Fig. 8b) that the ultimate effect on the carbon biomass is considered to be marginal compared to the direct effect on chl *a*.

#### Future estuarine functioning

When the findings and suggestions by Martens (2001) and van Beusekom et al. (2009) for the List

tidal basin (German Wadden Sea) are combined with a part of our results for the Ems estuary, it may be that an overall increase in temperature may lead to a decrease in the spring bloom of the phytoplankton and to an increase in the benthic microphytobenthos biomass. Due to wind-induced resuspension of the microphytobenthos (de Jonge 1995a, de Jonge & van Beusekom 1995), an increase in the benthic biomass will consequently lead to an increase of microphytobenthos in the water column, therefore, the proportion between resuspended microphytobenthos chl *a* and that of phytoplankton will increase. This suggests that if the average temperatures or the water turbidity further increase, a gradual shift may occur in the relative importance of primary production from phytoplankton to microphytobenthos. However, based on the observations and calculations by Blanchard et al. (2001, 2002, 2006, Herlory et al. 2004), there is most likely a limit to this development by algal density related growth limitation.

The functioning of the phytoplankton is further dependent on water temperature and favourable turbidity and light conditions, while the microphytobenthos is more dependent on suitable growth conditions governed by favourable weather conditions. How this under the current human pressures (e.g. dredging and engineering works) and climate change related developments may work out in the future and also in systems with a lower turbidity than the Ems estuary is not clear and needs further research.

Additionally, the reproduction of autochthonous grazers and their carnivores, and also invaders like the rapidly developing bivalve *Crassostrea gigas* (Buschbaum et al. 2011), may be affected by changing temperatures. How this potential effect modifies the food web and, thus, the functioning of both the pelagic as well as the benthic system in the Wadden Sea needs further attention.

Despite the integrative modelling exercise carried out for this estuary in the past (Baretta & Ruardij 1988), it is still unclear how the above mentioned factors (temperature, light conditions, respiration, grazing and resuspension) interact on a more detailed level. Thus, there is an urgent need for an integrated approach to these issues in future studies.

In addition, we also need to give high priority to a clear coupling of strongly improved monitoring programs and further development of ecosystem models (de Jonge et al. 2006), because microphytobenthos is much more important for coastal areas than generally recognized.

## CONCLUSIONS

This long-term study produced two major conclusions: (1) The microphytobenthos in the Ems estuary shows a strong positive response to temperature, a ~2.5-fold variation in the mean annual chl *a* concentrations within a mean annual range in air temperature of 7.5 to 10.2°C and (2) the remarkably strong interannual response of microphytobenthos chl *a* to temperature seems only explainable from a combination of factors that, apart from direct 'bottom-up' temperature effects to the algae (e.g. C:chl *a* and unmeasured algal activity) also comprise temperature related variations in 'top-down' control via grazers and even their carnivores.

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