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Factors affecting microphytobenthic biomass, species composition and production in the Colne Estuary (UK)

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ABSTRACT: Biomass, species composition and primary production of the microphytobenthos (MPB) were investigated along environmental gradients in the Colne Estuary between August 1996 and March 1998. Mean monthly sediment chlorophyll *a* (chl *a*) concentrations varied between 6 and 241 mg m^{-2}. There was no seasonal pattern in chl *a* distribution. Low chl *a* concentrations were associated with the estuary mouth, which was characterised by exposed sandy sediments and low water column nutrient concentrations. Position on the shore affected biomass, with greater chl *a* concentrations on the high shore. Chl *a* concentrations followed the same pattern at both high and low shore positions. There was a significant relationship between concentrations of sediment colloidal carbohydrate and chl *a*. Epipelic diatoms dominated the MPB, with cluster analysis identifying 10 distinct species assemblages. *Navicula* spp. were an important component of all assemblages. Peaks in chl *a* biomass occurred when a single species made up >65% of the cell numbers. The distribution of the diatom assemblages was related to salinity, temperature and dissolved inorganic nitrogen (DIN) concentrations. Net production of oxygen occurred in sediments under illumination at all sites on almost all occasions. Oxygen uptake by sediment in the dark was positively correlated with temperature at the head of the estuary, but not at any other site. Mean annual chl *a* concentrations correlated positively with total annual dark oxygen uptake. Annual primary production was estimated by 4 models using chl *a* concentrations and a model based on gross O_2 fluxes across the sediment-water interface. Production was estimated to be in the range of 25 to 1199 g C m^{-2} yr^{-1}, depending on site and model. There was greater production on the high shore than on the low shore, due to higher chl *a* concentrations and longer emersion periods. Total production of MPB in the estuary was estimated to be between 117 and 852 tons C yr^{-1}. Assimilation by the MPB provided a temporary sink for DIN, equivalent to 12.8% of the annual 1996 load to the Colne Estuary. N assimilation rates by the MPB were in the same order of magnitude as denitrification, indicating that assimilation by the MPB is an important process affecting the flux of DIN through this mesotidal estuary.

KEY WORDS: Biomass · Production · Diatom · Chlorophyll *a* · Nitrogen · Estuarine ecology · *Navicula* · Carbohydrate · Exopolymeric substances · Oxygen flux

INTRODUCTION

Microphytobenthos (MPB) are a major component of intertidal sediment microbial communities in terms of biomass (Admiraal 1984, Wiltshire 1992, Underwood & Paterson 1993a,b, Underwood & Kromkamp 1999) and production (for review see Underwood & Kromkamp 1999). The MPB are a primary source of fixed carbon for food webs (Heip et al. 1995, Underwood & Kromkamp 1999) and provide a food source for animals such as deposit feeders (Admiraal 1984). Moreover, exchanges of energy and nutrients (e.g. carbon and nitrogen) occur between the benthic and pelagic components of the system (Middelboe et al. 1998).

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Annual MPB production rates may be high (Underwood & Kromkamp 1999); for example, annual production rates of 62 to 276 g C m^{-2} were estimated for the Ems-Dollard estuary in the Netherlands (Colijn & de Jonge 1984) and 47 to 178 g C m^{-2} for the Tagus estuary in Portugal (Brotas & Catarino 1995, Serôdio & Catarino 2000). Epipelic diatoms dominate the MPB assemblages found in cohesive sediments, and a proportion of the carbon assimilated by these organisms (up to 70%, Smith & Underwood 2000) may be used to produce carbohydrate-rich exopolymeric substances (EPS). This polymeric material is extruded from the diatom cell during locomotion and results in increased stability of the surrounding sediment (Paterson 1989, Underwood et al. 1995, Underwood & Smith 1998, Yallop et al. 2000).

Epipelic diatoms may grow rapidly, with daily doublings of standing stock *in situ* under favourable conditions (Underwood & Paterson 1993a). However, measurements of daily production may not correlate with biomass (Colijn & de Jonge 1984). Though epipelic diatom biomass often shows seasonal patterns of increase during summer months (Colijn & Dijkema 1981, Colijn & de Jonge 1984, Santos et al. 1997), peaks of biomass as chlorophyll *a* (chl *a*) can occur throughout the year, even in winter (Colijn & de Jonge 1984, Underwood & Paterson 1993b). Field observations and laboratory experiments have shown that the distributional patterns of some epipelic diatom taxa are related to salinity and nutrient gradients, particularly that of ammonium (Underwood et al. 1998, Sullivan 1999, Underwood & Provot 2000).

In this study, we set out to determine the seasonal patterns in MPB biomass and activity in the Colne Estuary, UK, and to test a number of hypotheses: (1) there is a seasonal cycle in MPB biomass, with the maximum occurring during spring or summer; (2) the relationship between colloidal carbohydrate and chl *a* concentrations at the sediment surface fits a previously published model (Underwood & Smith 1998); (3) there is a seasonal cycle in primary production, with maximum rates occurring during the spring and summer months; and (4) the species composition of MPB biofilms differs along the estuarine gradient over the annual cycle, and these differences are a result of preference for particular environmental conditions.

Nitrogen cycling processes have been extensively studied in the Colne Estuary (Ogilvie et al. 1997, Robinson et al. 1998, Dong et al. 2000), and a second aim of this study was to determine the importance of MPB production on the attenuation of the annual nitrogen loading to the estuary.

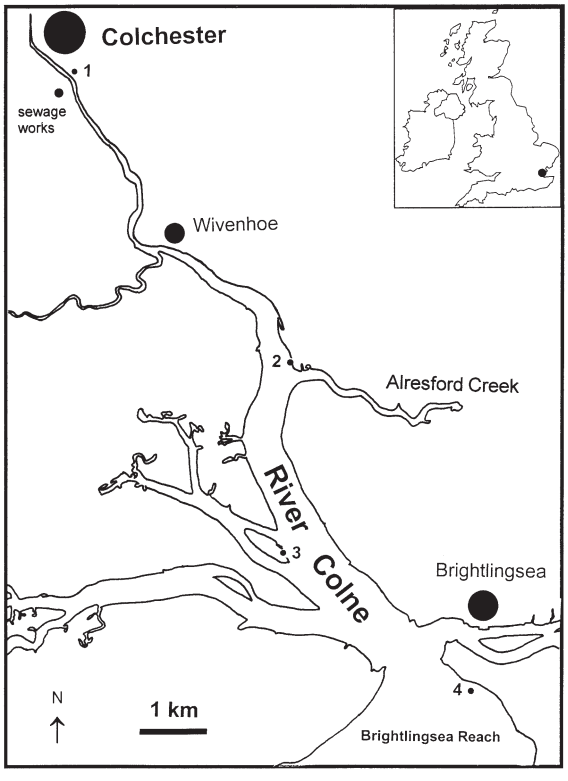

Fig. 1. Map of the Colne Estuary (Essex, UK) showing the position of 4 sampling sites, with 2 stations (high shore and low shore) at Sites 2 and 3

MATERIALS AND METHODS

Site. Monthly sampling was carried out between August 1996 and March 1998 in the Colne (51° 50′ N, 1° 0′ E), a mesotidal (tidal range 3.5 to 4.0 m) estuary that joins the North Sea on the east coast of England (Fig. 1). The Colne is relatively small (2335 ha area, Ogilvie et al. 1997), with a mean fluvial flow of 0.54 m^3 s^{-1} during 1996 (A. Sage pers. comm.). The estuary is muddy and highly turbid; Kocum et al. (2002) measured mean annual vertical light attenuation coefficients (K) of 3.2 m^{-1} at the head of the estuary and 1.4 m^{-1} at Brightlingsea (Fig. 1). The Colne is hypernutrified (concentrations of NO_3^- and NH_4^+ in the water column occasionally exceed 1 mM) and there are pronounced gradients in concentrations of nutrients (NO_3^-, NH_4^+, PO_4^{3-} and SiO_3^-) inversely related to the salinity gradient (King & Nedwell 1987, Ogilvie et al. 1997). A sewage treatment works serving the town of Colchester (Fig. 1) discharges into the estuary. During 1996 input of dissolved inorganic nitrogen (DIN) into the estuary from the sewage treatment works (24.62 Mmol N yr^{-1}) was more than twice that of the river (10 Mmol N yr^{-1}) (A. Sage pers. comm., calculated using UK Environment Agency data). Four sites were

sampled in the Colne, with both high and low shore stations at Sites 2 and 3 (Fig. 1).

***In situ* sampling.** An inflatable boat was used to access the mudflats at low tide. Cores were taken from the lower shore (mean low water neap) just above the low tide mark. At Sites 2 and 3 extensive mudflats were emersed at low tide. For better representation of environmental conditions at these sites, additional cores were taken from the upper shore at the level of mean high water neap tides. All cores were collected from emersed sediment.

Sediment cores were collected using 2 sizes of Perspex core tube. Four short cores (80 mm internal diameter, 20 cm length) were sealed at the bottom with the lids of petri dishes. Long core tubes (80 mm internal diameter; 65 cm length) were sealed with silicone rubber bungs after adjusting the headspace volume above the sediment core (30 to 40 cm long) to 500 ml by pushing the bung up the core tube. The long cores were used to measure rates of processes, with 3 replicate cores taken from each sampling station at each site. Therefore, 3 long cores were collected at Sites 1 and 4, and 6 cores (3 high shore and 3 low shore) were collected from Sites 2 and 3. High shore and low shore stations are denoted by H and L, respectively, in the text.

On return to the laboratory, sub-samples of the surface 0 to 5 mm depth were taken with minicore tubes (18 mm internal diameter) from the short cores (Smith & Underwood 1998). These 5 mm sections were placed in bijou bottles and frozen until analysis. The sediment from each site was characterised by measuring porosity (determined from the wet and dry weights of a known volume of sediment; Dalsgaard et al. 2000), chl *a* and carbohydrate fractions from 5 sub-samples. Chl *a* was extracted from lyophilised sediment with cold methanol over 24 h and measured spectrophotometrically, correcting for phaeopigments (Stal et al. 1984, Underwood & Paterson 1993a,b). Three carbohydrate fractions were measured: total, colloidal and extracellular polymeric substances (EPS). All fractions were extracted from lyophilised sediment and measured using the phenol-sulphuric assay (Dubois et al. 1956) with D-glucose as a standard. Results are expressed as glucose equivalents. Colloidal carbohydrate was extracted from the lyophilised sediment using 25‰ saline (Underwood et al. 1995). EPS was extracted from the colloidal carbohydrate extract by precipitation in cold 70% (v/v) ethanol (Underwood et al. 1995). Organic carbon content in the surface 5 mm of sediment was measured seasonally with a CHN analyser (model 2400, Perkin-Elmer) according to Nedwell & Trimmer (1996). Sediment density was measured seasonally (Dalsgaard et al. 2000), and grain size distribution was measured during February 1998 (Dalsgaard et al. 2000).

Site water (40 l) was collected from the shore during daylight high tide on the day before the cores were collected. It was not possible, particularly in winter, to collect and process sediment cores and site water during daylight within a single day. Water collected at high tide was representative of the water covering both the high and low shore in this well-mixed estuary (Ogilvie et al. 1997, Robinson et al. 1998). Site water was collected from Site 1, Alresford Creek (Site 2) and at Brightlingsea (which was representative, in terms of nutrient concentrations and salinity, of the water overlying both Sites 3 and 4). The site water was placed in aerated water barrels at *in situ* temperature and used in the oxygen exchange measurements (see below). Triplicate samples of site water (20 ml) were filtered through glass-fibre filters (GF/C, Whatman) and stored at –20°C for nutrient analysis. Nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, SiO_3^- and PO_4^{3-}; American Public Health Association 1995, Kirkwood 1996) were measured using a segmented flow autoanalyser (Skalar SAN^{plus}, Skalar Analytical). Site water temperature was measured using an alcohol thermometer and salinity with a hand-held refractometer.

Oxygen exchange. The long cores were stored (with caps removed) immersed in aerated site water at *in situ* temperature overnight in darkness, simulating nighttime immersion. Oxygen exchange measurements were carried out the next day (Fig. 2). As epipelic diatoms show rhythms of vertical migration within sediments (Pinckney et al. 1994, Serôdio et al. 1997, Smith & Underwood 1998) related to diel and tidal cycles, it was important to make the photosynthesis measurements during low tide emersion periods in the tidal cycle. Dark oxygen exchange was measured during the *in situ* immersion period. Illuminated oxygen exchange was measured under 500 W halogen lamps at a photosynthetically active radiation (PAR) incident on the sediment surface of 500 µmol m^{-2} s^{-1}, which approximates the mean daily PAR received by the Colne Estuary. Kocum et al. (2002) showed that mean daily PAR was 564 ± 264 µmol m^{-2} s^{-1} (mean ± SD) during the year September 1994 to September 1995, using light data collected by the University of Essex weather station (located within 1 km of the estuary). We did not scale PAR between months due to the warming effects of the lamps at high PAR.

Five water samples were taken with a glass syringe from the overlying aerated site water before the incubation. The water was placed in exetainers (Labco) and fixed for Winkler titration (Strickland & Parsons 1972). These samples represented both C_0 (initial oxygen concentration; µmol l^{-1}) and 100% oxygen saturation of the site water. All cores were capped, ensuring that no air bubbles were trapped in the 0.5 l headspace, and stirred with an induction motor driving

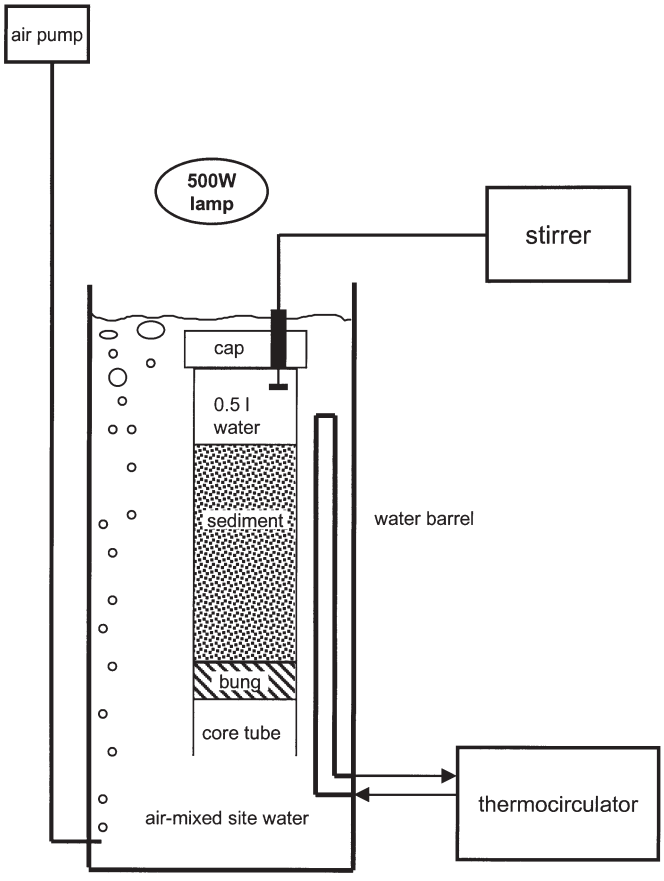

Fig. 2. Apparatus used to measure oxygen exchange across the sediment-water interface in intact sediment cores collected in long core tubes. Cores were immersed in air-mixed site water maintained at *in situ* temperature by a thermocirculator. The cooling system was closed; therefore, cooling water did not mix with site water in the barrel. Each barrel contained 3 replicate core tubes; only 1 (not to scale) is shown in the diagram

a magnetic follower (Rank Brothers) (Fig. 2). Water samples from above the sediment were taken with a glass syringe through a sampling port in the cap after 90 min. The C_{90} samples (oxygen concentration after 90 min) were immediately fixed for Winkler titration. Oxygen exchange across the sediment-water interface was calculated as follows:

$$\text{flux } (\mu\text{mol m}^{-2}\text{ h}^{-1}) = [198.9(C_{90} - C_0)h]/1.5 \quad (1)$$

where h is the volume (in litres) of water in the headspace above the core and 1.5 is incubation time in hours. The factor 198.9 scaled up the area of the core to a rate per m^2.

Production rates. Annual primary production rates (g C m^{-2} yr^{-1}) for the Colne were estimated from sediment chl *a* concentrations and oxygen production under illumination using 5 models, referred to as Maximum, Minimum, Regression, Photosynthesis and Gross O_2. The Maximum, Minimum and Regression models were from Santos et al. (1997). Santos et al. (1997) converted mean annual chl *a* (µg g^{-1}) to net production (g C m^{-2} yr^{-1}) from factors in the literature; a factor of 5 (Boucher 1977 cited by Santos et al. 1997) gave the minimum production and 25 (Schwinghamer et al. 1986 cited by Santos et al. 1997) the maximum. Finally, Santos et al. (1997) obtained the regression equation $\ln P = 0.419 + 0.974(\ln B)$, where P is net primary production (g C m^{-2} yr^{-1}) and B is chl *a* (mg m^{-2}), from literature measurements of production and chl *a* (Regression model).

Wolfstein & Hartig (1998) measured production rates of MPB extracted from lens tissue using ^{14}C and Clark-type oxygen electrodes, and expressed all production values as mg C (mg chl *a*)$^{-1}$ h^{-1}. Using their mean P_{max} value of 2.75 mg C (mg chl *a*)$^{-1}$ h^{-1} for measurements made with oxygen electrodes, we converted sediment chlorophyll into net production (Photosynthesis model), using the mean monthly chl *a* measurement for each site. Production per hour was scaled up to account for the number of hours in the month, assuming that high shore sites were emersed for 6 h d^{-1} in the light and low shore sites for 3 h d^{-1} (Dong et al. 2000). The monthly estimates were added together to produce an annual production rate. Gross production (Gross O_2 model) was calculated from oxygen exchange in the light and dark, and converted to carbon (mmol m^{-2} h^{-1}) assuming a photosynthetic quotient of 1.0 (Falkowski & Raven 1997). The hourly values were scaled up to annual rates as above. Gross rather than net primary production was calculated from the oxygen exchange data, as net oxygen exchange across the sediment-water interface in the light includes respiration of the total sediment community and chemical oxygen demand, and therefore would have severely underestimated net primary production.

Annual production in the Colne was estimated by dividing the estuary into 4 sectors (Ogilvie et al. 1997, Dong et al. 2000), and rates of production at the sites in each sector were used to estimate production for the whole sector. It was assumed that 72% of each sector was immersed throughout the tidal cycle (Ogilvie et al. 1997) and of the remaining, 18% was defined as high shore (emersed for 6 h) and 10% was defined as low shore (emersed for 3 h) (Dong et al. 2000).

Diatom identification. Two samples were taken from 2 different short cores using the lens tissue method (Eaton & Moss 1966) and fixed in 2% v/v glutaraldehyde solution. Diatoms collected by the lens tissue method were cleaned of organic matter using potassium permanganate solution and concentrated HCl. Cleaned valves were mounted in Naphrax (Northern Biological Supplies) to produce permanent slides. The

Table 1. Sediment characteristics (surface 0.5 cm) of sites in the Colne Estuary during summer (July 1997) and winter (February 1998). Data are for porosity (volume of water per volume of sediment), density (g cm^{-3}) and organic carbon content (percentage dry weight). Particle size is expressed as the percentage of particles (by weight) in the silt/clay fraction (<65 µm); the remainder of particles were sands. Data show the range of values (n = 5)

Site	Porosity		Density		Organic carbon		Particle size
	Summer	Winter	Summer	Winter	Summer	Winter	Winter
1	0.75–0.92	0.72–0.75	1.3–1.4	1.1–1.2	2.0–3.0	3.0–3.7	87–98
2	0.76–0.82	0.69–0.85	1.2–1.4	1.1–1.3	1.8–2.0	2.0–3.0	80–95
3	0.65–0.80	0.66–0.82	1.4–1.6	1.2–1.4	1.5–2.0	1.5–3.0	89–91
4	0.55–0.68	0.53–0.70	1.7–1.7	1.4–1.6	0.4–0.5	1.0–2.0	35–43

slides were examined by phase contrast light microscopy. Two hundred valves were identified and counted for each site on each sampling occasion between August 1996 and August 1997. These data gave the relative proportion of different taxa in the diatom component of the MPB. Microalgae from the 2 short cores were also sampled by taking duplicate 5 mm deep minicores, which were preserved in 2% v/v glutaraldehyde solution and stored in the cold (4°C) and dark. Preparation of the permanent mounts in Naphrax destroys organisms such as cyanobacteria and Euglenophyta. Whole sediment samples provided a source of reference material in which these organisms were preserved.

Statistical analysis. Canonical correspondence analysis (CCA) and cluster analysis were carried out using MSVP v3.1 (Kovach). The count data were log (n + 1) transformed before analysis. Correlation and ANOVA were carried out with SPSS 10.0.5 (SPSS).

RESULTS

Site characteristics

All sites were dominated by clays and silts, except the most exposed site towards the mouth of the estuary (Site 4), where fine sands dominated (Table 1). The proportion of organic carbon (percentage dry weight of sediment) was higher during the winter months (e.g. 1 to 2% in winter compared to 0.4 to 0.5% in summer 1997 at Site 4; Table 1). Organic carbon increased towards the head of the estuary, with 2 to 3% (summer) and 3.0 to 3.7% (winter) at Site 1 (Table 1). Water temperature showed summer maxima in July and August (22 to 23°C) and winter minima (4°C) in December. Water column salinity at high tide fluctuated throughout the year, although it was consistently lowest at Site 1 and highest at Sites 3 and 4 (Fig. 3). The largest range in salinity was observed at Site 1 (2 to 22‰) and the smallest range at Sites 3 and 4 (28 to 34‰). The water column showed both temporal and spatial patterns in inorganic nutrient concentrations (Fig. 4). There were decreases in all nutrient concentrations towards the estuary mouth (Sites 3 and 4). Ammonium concentrations peaked in spring and summer, exceeding 1.4 mM at Site 1 during April 1997, while nitrate + nitrite concentrations peaked in winter and exceeded 1 mM (Fig. 4).

MPB chl *a*, carbohydrates and production

There were no clear seasonal patterns in benthic chl *a* concentration at any site (Fig. 5). One-way ANOVA, treating all sampling locations equally and months as replicates, showed that there were significant ($F_{5,93}$ = 11.33, p < 0.0001) differences in chl *a* concentrations between locations. For example, there were generally higher concentrations at Site 1 than at Site 4. Post-hoc Tukey analysis showed that there were significant differences (p < 0.05) in chl *a* concentrations

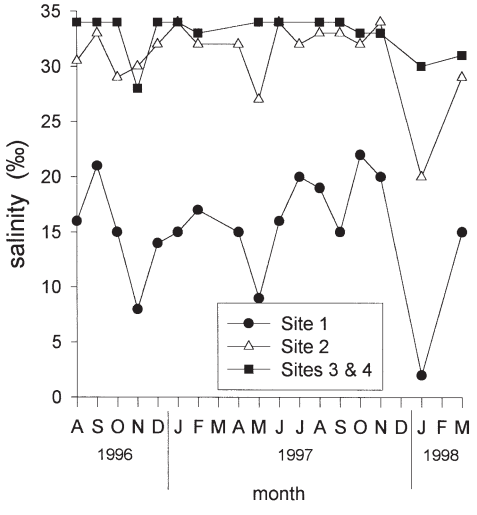

Fig. 3. High tide salinity (‰) at the surface of the water column in the Colne Estuary between August 1996 and March 1998

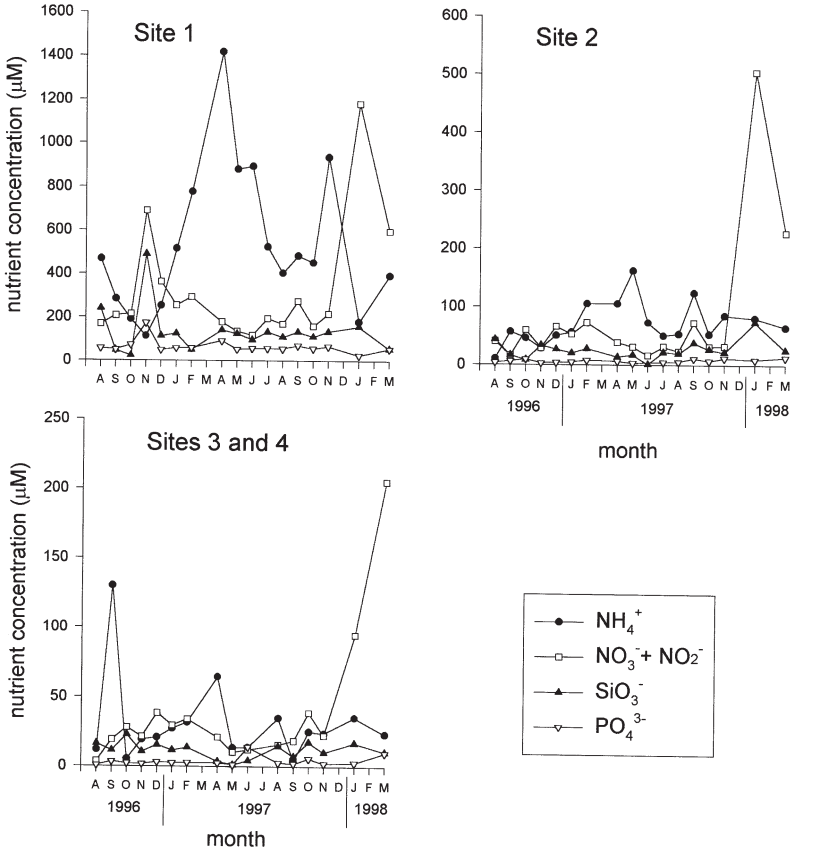

Fig. 4. High tide inorganic nutrient concentrations at the surface of the water column in the Colne Estuary between August 1996 and March 1998. Data points are means (n = 3); for clarity, error bars are not shown. Note the differences in values on the *y*-axis for the different sites

between Site 1 and all sites except Site 3H. Mean monthly chl *a* concentrations during the seasonal cycle were greater at high shore stations than at the low shore (at Sites 2 and 3) (Table 2) with significant differences in chl *a* concentrations between the high and low shore stations at Site 3 (Tukey, $p < 0.05$), but not at Site 2. Mean monthly chl *a* concentration on the high and low shore stations at both Sites 2 ($r = 0.53$, $n = 17$, $p = 0.028$) and 3 ($r = 0.58$, $n = 16$, $p = 0.018$) were significantly positively correlated. The highest monthly mean chl *a* concentration during an annual cycle (August 1996 to July 1997) was at Site 3H and the lowest at Site 4 (Table 2).

In the dark, all sediments showed net oxygen uptake throughout the year due to the respiration of organisms and chemical oxygen demand within the sediment (Fig. 6). Highest sediment oxygen consumption in the dark for a complete annual cycle (Table 2) occurred at Site 1 and the lowest at Site 4. There was a significant ($r = 0.93$, $n = 6$, $p < 0.01$) correlation between mean chl *a* concentration at the 6 sites and annual sediment oxygen uptake (data from Table 2). At Site 1 (Fig. 6), there was a significant ($r = -0.89$, $n = 17$, $p < 0.001$) linear relationship between oxygen uptake and temperature ($U = -0.40t + 0.34$, where U is oxygen uptake in µmol m^{-2} h^{-1} and t is temperature in °C). However, such a significant relationship was not observed at any other site. Under illuminated conditions (500 µmol m^{-2} s^{-1}) there was generally net oxygen production (Fig. 6). A problem in determining seasonal patterns in pooled data is that changes at one site, which may be significant, can be masked by a large data range across all sites. Therefore, to determine broad trends and relationships in gross primary production, data for each site were normalised by dividing by the average annual value for that particular site (set as 100%). There was a significant positive correlation between normalised gross primary production and temperature at Site 2H ($r = 0.64$, $n = 10$, $p = 0.045$; Fig 7a) and a negative correlation at Site 3H ($r = -0.75$, $n = 9$, $p = 0.019$; Fig 7b).

A proportion of carbon fixed by epipelic diatoms is extruded from the cell into the surrounding environment in the form of extracellular carbohydrate (see 'Introduction'). With the exception of Sites 2L ($r = 0.793$, $n = 13$, $p < 0.001$) and 2H ($r = 0.58$, $n = 13$, $p = 0.04$), there was no significant correlation between the log of sediment chl *a* and the log of colloidal carbohydrate concentrations in the Colne (Fig. 8). Colloidal carbohydrate concentrations were normalised to chl *a* and grouped

Table 2. Estimated annual oxygen uptake and mean sediment chlorophyll *a* (chl *a*) concentration in the Colne. Annual oxygen uptake for each site was calculated by integrating the mean monthly values for oxygen flux in the dark over 1 calendar year (August 1996 to July 1997). Missing values were accounted for by taking the mean of the oxygen uptake for the months before and following the missing value. H: high shore stations; L: low shore stations

Site	Oxygen uptake (mol m^{-2} yr^{-1})	Chl *a* (mean ± SE) (mg m^{-2})
1	−37.5	109.0 ± 18.6
2L	−20.5	37.8 ± 6.8
2H	−18.8	54.0 ± 8.9
3L	−22.1	54.9 ± 13.4
3H	−31.2	119.9 ± 21.3
4	−12.4	18.8 ± 4.6

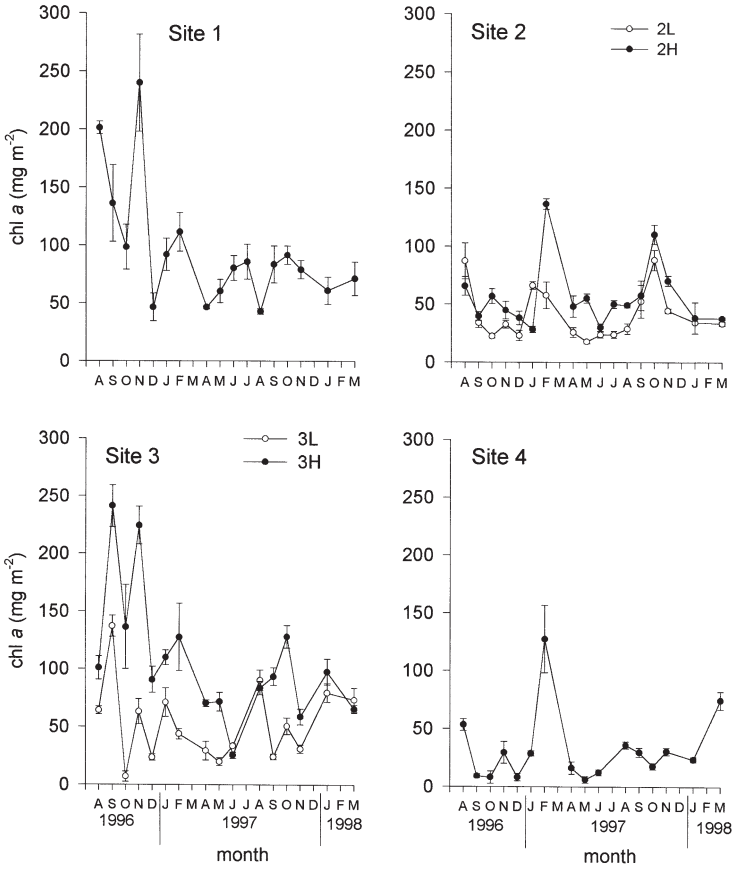

Fig. 5. Monthly sediment chlorophyll *a* (chl *a*; mg m^{-2}) concentrations in the Colne Estuary between August 1996 and March 1998. Data points are means ± standard error (SE; n = 3). H: high shore stations; L: low shore stations

according to the 10 diatom assemblage types identified in the Colne by cluster analysis (see the 'Characterisation of diatom assemblages' section below). There was no significant difference (1-way ANOVA) in the amount of colloidal carbohydrates produced per unit chl *a* by the different MPB assemblage types. Pooling the data from all sites showed a statistically significant linear relationship between sediment chl *a* and colloidal carbohydrate concentrations in the surface 0.5 cm of the sediment for the whole estuary (Fig. 9). This relationship was compared to the model derived from a number of estuaries (Underwood & Smith 1998):

$$\log[(\text{colloidal carbohydrate}) + 1] = \alpha + \beta \log[(\text{chl } a) + 1] \quad (2)$$

The values of coefficients for the Colne data were:

$$\log[(\text{colloidal carbohydrate}) + 1] = 1.820 + 0.312 \times \log[(\text{chl } a) + 1] \quad (3)$$

The regression was significant ($p < 0.001$); however, the coefficient of determination was relatively low ($r^2 = 0.27$, n = 418). Data from August 1996 were notably clustered as most samples contained relatively high concentrations of colloidal carbohydrate (Fig. 9).

Primary production in the Colne Estuary was estimated using the 5 models described in the methods. Estimates varied from 95 to 1199 g C m^{-2} yr^{-1} at Site 1 to 25 to 127 g C m^{-2} yr^{-1} at Site 4 (Table 3). These estimates were used to calculate annual production of MPB over the total area of the Colne Estuary. Annual production was calculated to be in the range of 1.17×10^8 to 8.52×10^8 g C yr^{-1} (Table 3). The mean estimate was equivalent to 352 tons of C fixed yr^{-1}. Using the Redfield C:N ratio of 6.625 (Redfield 1958) the production values in Table 4 were converted to nitrogen assimilation. Estimates of nitrogen assimilation by the MPB (Table 4) varied between 0.32 (Minimum model, Site 4) and 15.09 (Maximum model, Site 1) mol N m^{-2} yr^{-1}. Mean nitrogen assimilation was 3.26 mol N m^{-2} yr^{-1}. Using the same calculations as in Table 3, nitrogen assimilation was scaled up to calculate assimilation by the MPB for the total area of the estuary, producing a mean estimate of 4.43 Mmol N yr^{-1} (Table 4).

Table 3. Production (g C m^{-2} yr^{-1}) was estimated from 5 simple models (Minimum, Maximum, Regression, Photosynthesis and Gross O_2) using either chl *a* standing crop or sediment-water oxygen exchange (see 'Materials and methods') at 4 sites in the Colne Estuary between August 1996 and July 1997. Total production (g C yr^{-1}) was estimated accounting for the area of the estuary and tidal cycle (see 'Materials and methods')

Model	Site						Total estuary
	1	2L	2H	3L	3H	4	(g C yr^{-1})
Minimum	240	79	111	89	218	25	1.70×10^8
Maximum	1199	393	555	447	1091	127	8.52×10^8
Regression	141	53	78	73	151	27	1.17×10^8
Photosynthesis	631	115	345	161	678	115	4.84×10^8
Gross O_2	95	63	126	53	191	57	1.38×10^8
Mean							3.59×10^8

Table 4. Assimilation of dissolved inorganic nitrogen (DIN) (mol N m^{-2} yr^{-1}) by the microphytobenthos at 4 sites in the Colne Estuary. Estimates were made using 5 simple models of production and assuming a C:N ratio of 6.625. Total nitrogen assimilation (Mmol N yr^{-1}) was estimated accounting for the area of the estuary and tidal cycle (see 'Materials and methods')

Model	Site						Total estuary
	1	2L	2H	3L	3H	4	(Mmol N yr^{-1})
Minimum	3.02	0.99	1.40	1.12	2.74	0.32	2.14
Maximum	15.09	4.94	6.98	5.62	13.73	1.60	10.72
Regression	1.78	0.66	0.99	0.92	1.91	0.34	1.47
Photosynthesis	7.93	2.23	4.34	2.03	8.83	1.45	6.09
Gross O_2	1.19	0.33	1.58	0.67	2.40	0.72	1.73
Mean							4.43

Characterisation of diatom assemblages

At all sites diatoms dominated the MPB (>90% cell numbers), though other groups were present at lower densities, including euglenoids and cyanobacteria. Seventy-five taxa of benthic diatoms were identified in the samples counted. Of these, 23 taxa were present in sufficient abundance throughout the year (>0.5% of the total valves counted or, if less than this, at least 1% of the valves at a particular site) to be included in further analyses. Cluster analysis grouped the samples (at a similarity level of 50% or greater) into 10 different assemblage types, with the majority of species characterising the assemblages belonging to the genus *Navicula* (Table 5). Different diatom assemblages showed distinct patterns in their temporal distributions in the estuary (Table 6). Assemblage *A* occurred between June and Septem-

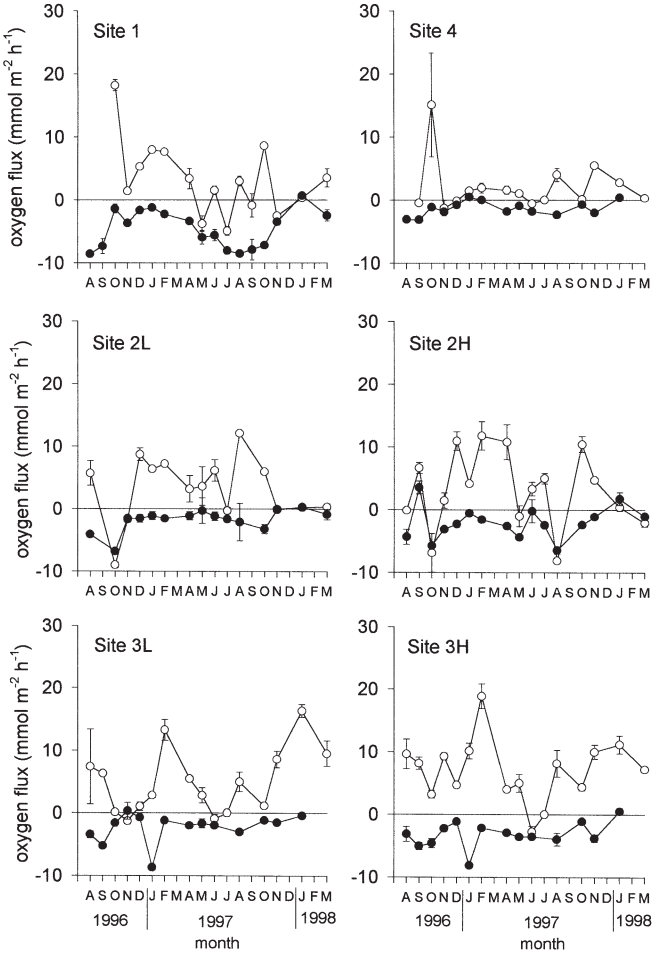

Fig. 6. Sediment-water oxygen exchange in the dark (●) and under illumination (○) in the Colne Estuary between August 1996 and March 1998. Data points are means ± SE (n = 3)

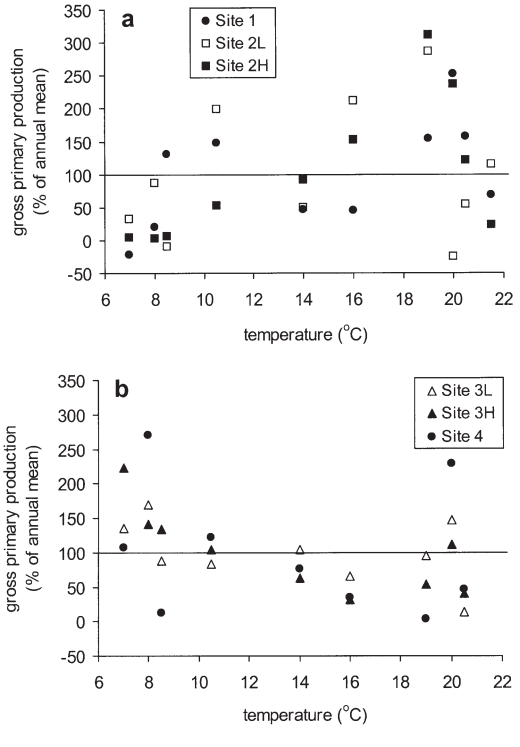

Fig. 7. Relationship between mean monthly primary production (gross oxygen exchange) normalised to the mean annual primary production and estuary water temperature. (a) Upper estuary (Sites 1 and 2); (b) lower estuary (Sites 3 and 4)

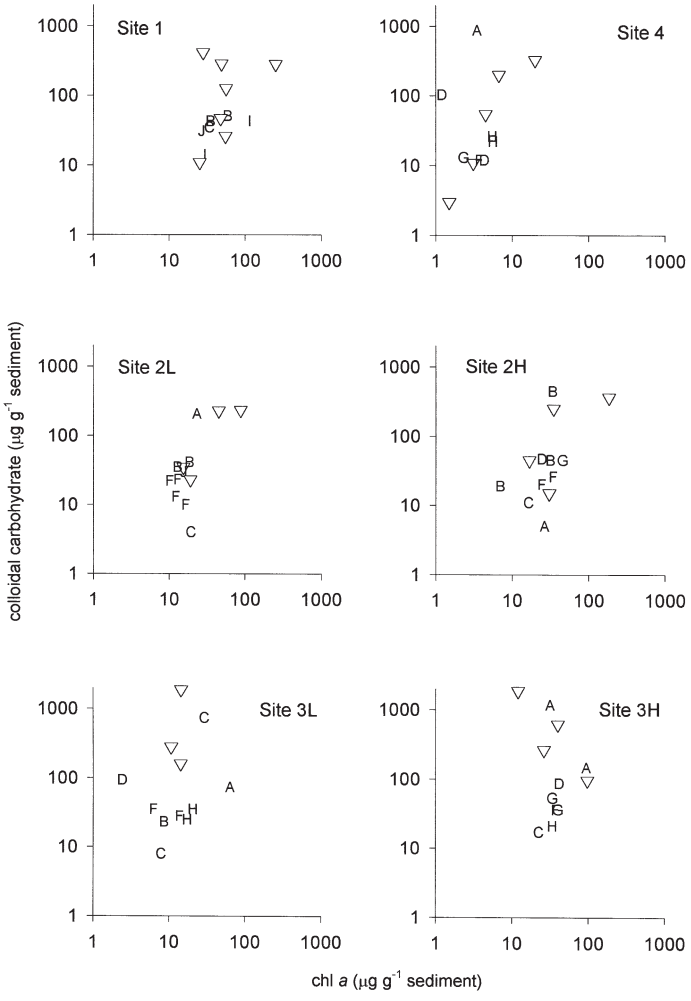

Fig. 8. Scatter plots of colloidal carbohydrate (µg g^{-1} sediment) against chl *a* (µg g^{-1} sediment) at each site in the Colne Estuary. Letters show the diatom assemblage type (Table 5) present on that sampling occasion. Data points marked with ▽ indicate that no assemblage data were available. Data points are means (n = 5)

ber, *F* and *G* occurred predominantly in the spring (February to May), and *I* and *J* occurred during the winter months (November to January). There was also evidence for spatial patterns: assemblage *H* was a lower estuary, winter grouping. There was a significant (r = 0.431, n = 53, p < 0.01) positive correlation between biomass and production (gross O_2 flux) for the 10 assemblage types (Fig 10). Generally, assemblage type was not related to patterns in biomass (chl *a*) and production (gross O_2 flux). However, with the exception of a single datum point, assemblage *B* was located near the origin of the graph, indicating low biomass and productivity. Gross oxygen-flux data were normalised to chl *a* and grouped according to assemblage type. Subsequent 1-way ANOVA showed no significant differences between the assemblages types.

There were significant differences (1-way ANOVA) between environmental variables with diatom assemblage type as the fixed factor (temperature, $F_{9,47}$ = 5.80, p < 0.0001; salinity, $F_{9,44}$ = 3.54, p < 0.002; NO_3^-, $F_{9,47}$ = 13.97, p < 0.0001; SiO_3^-, $F_{9,47}$ = 9.06, p < 0.0001; PO_4^{3-}, $F_{9,45}$ = 5.85, p < 0.0001; NH_4^+, $F_{9,47}$ = 0.737, not significant). Assemblages *H*, *I* and *J* were low temperature, while *A* and *E* were high temperature assemblages (Fig 11a), as expected from their seasonal occurrence described in Table 6. Salinity patterns were less evident, with most assemblages occurring at high tide salinities greater than 25‰. Assemblage *I* was present in low salinity periods, with *J* also associated with lower salinities. There was a pattern between assemblage occurrence and DIN concentrations. Assemblages *I* and *J* were high nutrient groups (Fig 11b), whereas assemblages *G*, *D* and *H* were associated with lower DIN concentrations. Assemblage *E* occurred at the lowest concentrations of both nitrate and ammonium.

Canonical correspondence axes 1 (CCA 1) and 2 (CCA 2) explained 47.6% of the species composition data set (Fig. 12). CCA 1 (32.7%) represented a gradient of decreasing temperature, salinity, nitrite concentrations and MPB biomass parameters (colloidal carbohydrate, EPS, chl *a*), with summer samples plotting out on the left-hand side of the plot. Taxa characteristic of these summer assemblages were *Navicula phyllepta*, *Nitzschia frustulum* and *Fallacia pygmaea*. Over

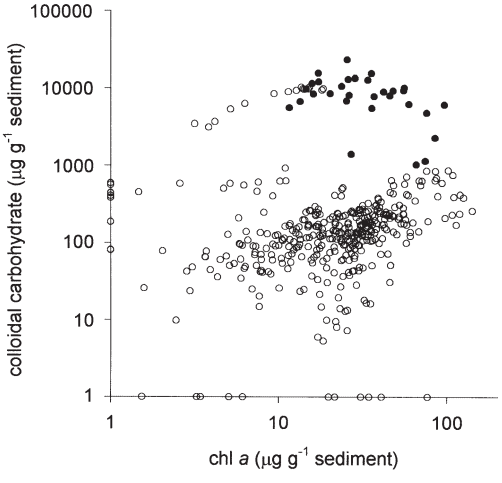

Fig. 9. Scatter plot of sediment colloidal carbohydrate (µg g^{-1}) against chl *a* (µg g^{-1}) for all sites in the Colne Estuary between August 1996 and August 1997. (●) Samples taken in August 1996

the whole year, there was no general clustering or separation of assemblages from Sites 2, 3 and 4 in the Colne based on species composition. However, differences in species composition between sites were found within individual sampling periods (see above). The November, December and January samples from Site 1 were separated by CCA 2 from the main cluster of points. CCA 2 (14.9%) was related to dissolved nutrient concentrations (NO_3^-, SiO_3^- and PO_4^{3-}), and the taxa associated with higher concentrations of these were *Navicula diserta*, *N. cryptotenella*, *N. salinarum* and *Gyrosigma littorale*.

Table 5. Epipelic diatom species assemblages (*A* to *J*) determined by cluster analysis in the Colne Estuary between August 1996 and August 1997. The major species characterising each assemblage and their abundances (mean percentage) are listed. Species abbreviations refer to those used in Fig. 12. Species listed in the bottom section of the table are plotted in Fig. 12; however, they were not diagnostic of the assemblages listed in the upper table

Assemblage	Species	Mean abundance (%)	Abbreviation
A	*Navicula phyllepta* Kützing	75	Nphy
B	*Navicula phyllepta* Kützing	35	Nphy
	Nitzschia parvula W. Smith non Lewis	5	Nzpa
C	*Navicula gregaria* Donkin	35	Ngr
	Navicula phyllepta Kützing	21	Nphy
	Navicula cincta (Ehrenberg) Kützing	3	Ncin
	Navicula peregrina (Ehrenberg) Kützing	3	Nper
D	*Rhaphoneis minutissima* Hustedt	25	Rhm
	Cymatosira belgica Grunow	20	Cbl
	Nitzschia parvula W. Smith non Lewis	5	Nzpa
	Rhaphoneis similis Hustedt	3	Rsim
	Amphora sp. 1	1	Amp
E	*Nitzschia frustulum* (Kützing) Grunow	48	Nzfr
	Navicula sp. 1	18	N-A
	Amphora sp. 1	2	Amp
F	*Plagiotropis vitrea* (W. Smith) Kuntze	57	Pvit
	Navicula rostellata Kützing	9	Nros
	Amphora sp. 1	1	Amp
G	*Navicula rostellata* Kützing	19	Nros
	Navicula cryptonella Lange-Bertalot	14	Ncc
	Navicula peregrina (Ehrenberg) Kützing	5	Nper
H	*Navicula flanatica* Grunow	44	Nfl
	Navicula cryptonella Lange-Bertalot	14	Ncry
I	*Gyrosigma limosum* Sterrenburg & Underwood	65	Glim
	Navicula cryptonella Lange-Bertalot	24	Ncry
J	*Nitzschia dubia* W. Smith	43	Ndb
	Navicula salinarum Grunow	25	Nsa
	Gyrosigma littorale (W. Smith) Cleve	20	Glit
–	*Navicula diserta* Hustedt		Ndi
–	*Navicula digitoradiata* (Gregory) A. Schmidt		Ndig
–	*Staurophora amphioxys* (Gregory) D.G. Mann		Sam
–	*Pleurosigma angulatum* (Quekett) W. Smith		Pan
–	*Gyrosigma fasciola* W. Smith		Gfa
–	*Fallacia pygmaea* (Kützing) Stickle & Mann		Fpg
–	*Navicula cryptocephala* Kützing		Ncc

DISCUSSION

Biomass

Chl *a* and carbohydrate were used as indicators of MPB biomass. All biomass values are estimates as no variable (e.g. photopigments, cell counts, protein, carbohydrate, etc.) unambiguously reflects algal biomass as organic carbon (de Jonge & Colijn 1994). The chl *a* concentrations were within the range of other investigations; de Jonge & Colijn (1994) also measured chl *a* to a depth of 0.5 cm and found annual mean chl *a* values in the range of 29 to 247 mg m^{-2} in the Ems estuary (the Netherlands). The vertical distribution of chl *a* and the depth to which it is sampled affects both biomass and productivity estimates. Sampling to a depth of 5 mm would have included chl *a* from photosynthetically active biomass (PAB) in the photic zone and photosynthetically inactive biomass (PIB) from below the photic zone (Kelly et al. 2001). Inclusion of PIB may mean that biomass-specific rates of primary production are inaccurate (Kelly et al. 2001), which could account for the relatively poor correlation between primary production and chl *a* (Fig. 10). PIB and PAB can be separated by sampling to a depth resolution of 0.2 mm using the Cryolander technique; however, it is time consuming and not suitable for processing a large number of samples (Kelly et al. 2001). In the Colne there were significant correlations between chl *a* on the high and low shores at both Sites 2 and 3 and significantly more chl *a* at the high shore station at Site 3 than at the low shore station. Greater biomass (chl *a*) on the high shore has been observed in the Gironde (Santos et al. 1997) and Severn estuaries (Underwood & Paterson 1993b). These data indicate that position on the shore affects MPB biomass (as chl *a*) and that where there is a significant difference, biomass is higher on the upper shore. Higher chl *a* at high shore positions may be related to the length of time available for photosynthesis. The Colne Estuary is highly turbid (Kocum et al. 2002); therefore, most photosynthesis would be restricted to daylight

Table 6. Spatial and seasonal occurrence of 10 epipelic diatom assemblages at 6 stations in the Colne Estuary (August 1996 to August 1997). Letters indicate the assemblage types defined in Table 5. –: no data were available for that month

Month	Site					
	1	2L	2H	3L	3H	4
1996						
August	–	*A*	*B*	*E*	*E*	*B*
September	*A*	*B*	*A*	*A*	*A*	*D*
October	*B*	*B*	*D*	*D*	*D*	*D*
November	*I*	*B*	*B*	*H*	–	*H*
December	*I*	*F*	*C*	*C*	*C*	–
1997						
January	*J*	*J*	*B*	*H*	*H*	*H*
February	*C*	*F*	*F*	*F*	*G*	*G*
March	–	–	–	–	–	–
April	–	*F*	*F*	*F*	*F*	*F*
May	*B*	*F*	*G*	*B*	*G*	*G*
June	–	*C*	*A*	–	–	*A*
July	–	–	–	–	–	–
August	–	*A*	*C*	*C*	*A*	–

emersion, which would be longer at high shore positions. The related factors of water movements, sediment resuspension and deposition, and grain size also affect the distribution of MPB across mudflats. MPB can sometimes be resuspended at current velocities as low as 10 cm s^{-1} (de Jonge & van den Bergs 1987) and may make a significant contribution to the phytoplankton (de Jonge & van Beusekom 1992). Sandy sites support less MPB biomass than sites dominated by fine cohesive sediments (de Jong & de Jonge 1995, Underwood & Smith 1998), and positive correlations between the proportion of clay particles and chl *a* have been

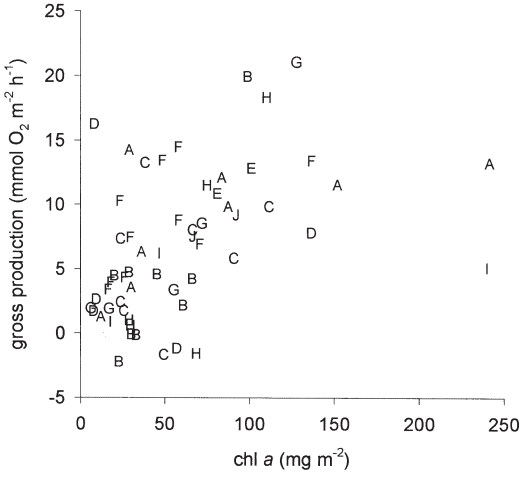

Fig. 10. Relationship between assemblage type (see Table 5), biomass (chl *a* in mg m^{-2}) and production (gross oxygen exchange in mmol O_2 m^{-2} h^{-1}) in the sediments of the Colne estuary. Data points are means (n = 3)

shown (Colijn & Dijkema 1981, de Jong & de Jonge 1995). The movement of sand grains reduces cell numbers of benthic diatoms in culture, probably due to collisions between the diatoms and the sand (Delgado et al. 1991). Sediment with a relatively low clay content indicates an area with high hydrodynamic energy as clay particles cannot settle out of the water column (de Jong & de Jonge 1995). In the Colne, MPB biomass (as chl *a*) was lowest throughout the whole sampling period at Site 4. This was the most exposed site at the estuary mouth and was associated with higher hydrodynamic energy and larger sediment particle size. Site 4 was also characterised by relatively low availability of organic carbon, and low rates of oxygen uptake and primary productivity. These factors indicate that rates of microbial processes and biomass were low at Site 4, correlating with the lower nutrient availability (e.g. organic carbon and DIN) and a greater potential for disturbance.

Although there was seasonality in factors such as temperature and day length, there were no seasonal patterns in chl *a* concentration. Some investigations have shown seasonal patterns in chl *a*, with increased biomass in the summer (Colijn & Dijkema 1981, Colijn & de Jonge 1984, Santos et al. 1997, Sundbäck et al. 2000). Other work has shown variation in chl *a* throughout the year (Colijn & de Jonge 1984, Underwood & Paterson 1993b). Over an annual cycle, chl *a* concentrations are a function of temperature, light and nutrient concentrations, which affect production and growth, and disturbance events such as grazing and resuspension. De Jong & de Jonge (1995) suggested that grazing by the amphipod *Corophium volutator* may cause the end of the MPB bloom in the Western Scheldt. In the Colne, although the mud snail *Hydrobia ulvae* and *C. volutator* were present, the polychaete *Nereis diversicolor* was the most abundant primary consumer (Cooper 1999). Although we did not measure the impact of grazers on the MPB in the Colne, Smith et al. (1996) estimated that *C. volutator* and *N. diversicolor* consumed 2150 to 3767 and 5476 to 12 184 diatoms d^{-1}, respectively, in the River Crouch, a similar estuary located in SE England. In the Colne, densities of up to 8000 *N. diversicolor* m^{-2} have been recorded (Cooper 1999). The feeding activity of benthic fauna in the Colne may cause patchiness and fluctuations in MPB biomass in both time and space.

There is a positive relationship between diatom biomass (as chl *a*) and colloidal carbohydrate in the surface of cohesive sediments (Underwood & Paterson 1993b, Underwood & Smith 1998). EPS tends to contribute 20 to 25% of colloidal carbohydrate (Underwood et al. 1995, Underwood & Smith 1998), and therefore it can be estimated from chl *a* concentrations. Underwood & Smith (1998) described the relationship

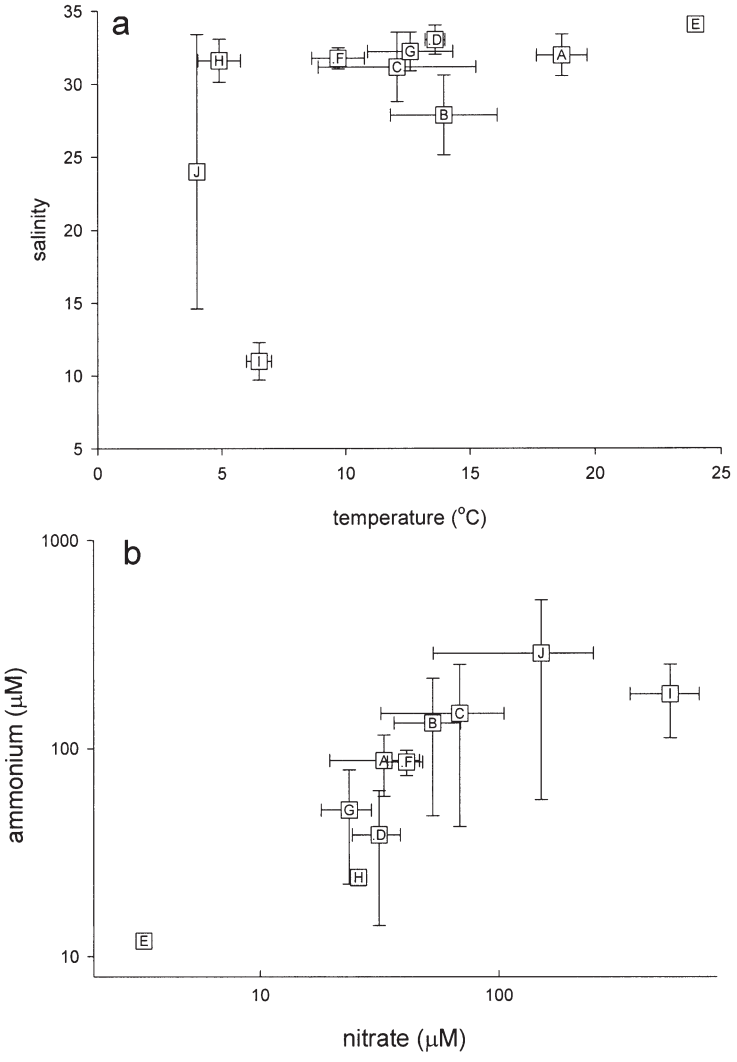

Fig. 11. Relationships between diatom assemblage types and environmental variables in the Colne Estuary. The letters in each data point indicate assemblage type, as defined by the species in Table 5. Data points show mean within the range of values (n = 2 to 10). (a) Temperature (°C) and salinity (‰); (b) Nitrate and ammonium concentrations (µM)

between colloidal carbohydrate and chl *a* (Eq. 2) and found values for α and β of 1.4 and 1.02, respectively. This model was found to be valid where epipelic diatoms constituted >50% of the MPB assemblage. In the Colne, a significant linear relationship between chl *a* and colloidal carbohydrate concentrations did not hold for individual sites, except at Site 2 for both low and high shore stations. However, pooling the data for all sites showed a significant relationship for the whole estuary (Eq. 3). Previous data from the Colne (January 1993) were not significantly different from the model of Underwood & Smith (1998); however, our data collected between August 1996 and August 1997 were different, with a relatively high value of α (1.820) and a low value of β (0.312). The intercept of the model (α) indicates a relatively high concentration of colloidal carbohydrate in the sediment, which was independent of diatom biomass. This may have been produced within the sediment by organisms such as invertebrates and bacteria, or be a result of allochthonous inputs. The clustering of data from August 1996 above the regression line (Fig. 9) was probably due to relatively high concentrations of colloidal carbohydrate from sources other than the MPB. The relatively low gradient (β) indicates that colloidal carbohydrate was being produced at a low rate or being removed from the sediment (e.g. by bacterial degradation) at a high rate relative to the biomass of MPB. This may have implications for the stability of Colne sediments, as a low value of β indicates low EPS concentrations per unit chl *a* and therefore relatively low biostabilisation per unit chl *a* (de Brouwer et al. 2000).

MPB species composition

While 75 species of benthic diatoms were recorded within the biofilms sampled, cluster analysis revealed that 10 different assemblages represented the biofilm composition in the Colne Estuary. Each of these assemblages was characterised by a few diatom taxa, especially when the biomass (chl *a*) was high. Dominance of a few species appears to be a feature of estuarine bio-films, where species diversity appears inversely proportional to MPB biomass (Colijn & Dijkema 1981, Underwood 1994). CCA (Fig. 12) showed that there was no distinct grouping of samples by field sites, although assemblage composition did show seasonal changes at each site throughout the year. Similar seasonal shifts in species abundance have been recorded on estuarine mudflats (Oppenheim 1991, Underwood 1994, Peletier 1996 and other references therein), but the environmental causes of such patterns are not well understood. Assemblage *J* contained *Navicula salinarum* and was associated with winter (temperature <5°C) and relatively high concentrations of nitrate and ammonium. *N. salinarum* appears to be a high nutrient species, as has been recorded in other field and culture studies (Peletier 1996, Sullivan 1999, Underwood & Provot 2000). Assemblage *I* was dominated by *Gyro-sigma limosum* and was associated with high nutrients, low salinity and low temperatures. As *G. limosum* was not associated with any of the other assemblages, these data indicate that this species favours those conditions. Similarly, *Nitzschia frustulum* was a high salinity, high nutrient species (Figs. 11 & 12).

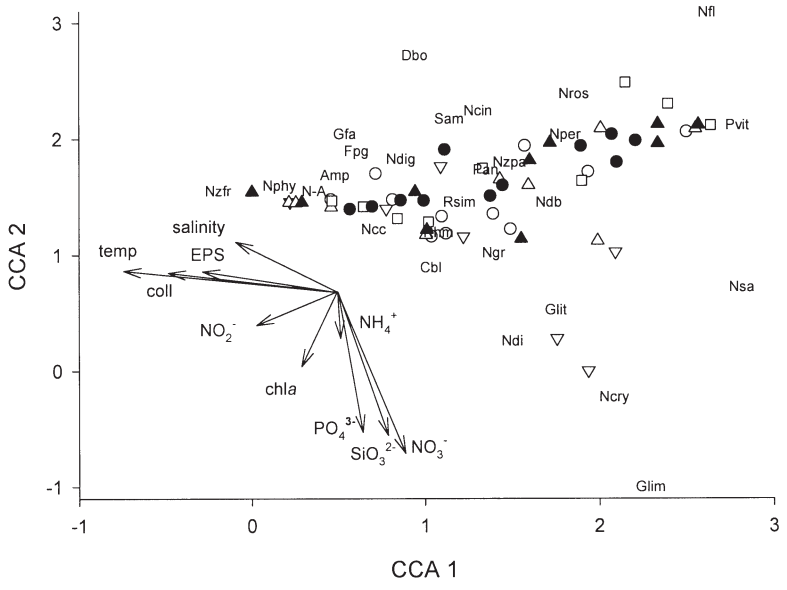

Fig. 12. Canonical correspondence analysis scatter plot of components 1 (CCA 1) and 2 (CCA 2) for microphytobenthos (MPB) samples from the Colne Estuary (∇: Site 1; ●,○: Sites 2H and 2L; ▲,△: Sites 3H and 3L; □: Site 4). Abbreviations indicate the centroids for diatom species listed in Table 5. Vectors show the direction of increasing values of particular environmental variables. Only environmental vectors that were significantly correlated ($p < 0.05$) with either CCA 1 or CCA 2 are shown

Particular species and assemblages did show preferences for high or low salinity conditions (Figs. 11a & 12), suggesting that salinity is a variable that affects biofilm species composition (Admiraal 1984, Underwood 1994, Sullivan 1999). However, one problem with estuarine salinity gradients is that they often covary with other variables (e.g. nutrient concentrations, Ogilvie et al. 1997), which also influence species composition (Underwood & Kromkamp 1999, Underwood & Provot 2000), so it is not always possible to decide what is the key factor. In an autecological study, Underwood & Provot (2000) showed that the growth rates of 4 species of benthic diatoms had different, though overlapping, salinity, ammonium and nitrate optima. *Navicula phyllepta*, for example, showed optimal growth rates at 10 to 20‰, and NH_4^+ and NO_3^- concentrations less than 120 µM. These conditions match the *in situ* distribution of *N. phyllepta* observed by Peletier (1996), Underwood et al. (1998) and Zong & Horton (1998). In contrast, our data (Figs. 11 & 12) indicate that *N. phyllepta* was found in assemblages associated with relatively high salinity and nutrient concentrations in the middle of the range observed in the Colne (Fig. 11). Cox (1995) found good growth of *N. phyllepta* clones from 7 to 35‰, with the maximum growth occurring at 28 to 35‰, which matches our assemblage data. Differences between studies may indicate that common species such as *N. phyllepta* contain populations or clones with different, though overlapping, distributions with re-spect to environmental factors. Alternatively, differences in *in situ* distribution of common species such as *N. phyllepta* be-tween locations or seasons with respect to factors such as salinity may be due to confounding factors or the interaction between factors. For example, in the Colne the distribution of *N. phyllepta* may be limited to higher salinities due to the toxic effects of high ammonium concentrations associated with the low salinities in the upper section of the estuary. Competition between species may also be important.

Respiration and primary production

Net oxygen uptake by the sediments in the dark was a result of respiration by organisms in the sediment and chemical oxygen demand; oxygen uptake was greatest at Site 1 and showed a linear relationship with temperature. Positive correlations between temperature and sediment oxygen uptake have been observed in macrotidal estuaries such as the Great Ouse (Trimmer et al. 1998), as well as microtidal sites in the NE Kattegat (Sundbäck et al. 2000). At other sites in the Colne there was no significant relationship between oxygen uptake and temperature, indicating that oxygen uptake may have been limited by nutrient or organic carbon supply. This conclusion is supported by relatively high overlying nutrient concentrations and sediment organic carbon at Site 1. There was a correlation between annual sediment oxygen uptake and mean MPB biomass (chl *a*) in the Colne. It is likely that this was an artefact of covariation rather than indicating that the MPB was the main consumer of oxygen. High MPB biomass was associated with relatively stable sediments, which have a high organic carbon content from a variety of sources (e.g. detritus, bacteria and fauna) and therefore a high potential for oxygen demand.

Illuminated sediments generally showed a net production of oxygen throughout the year, indicating that when light was available there was more carbon fixed through photosynthesis than lost from the sediment though aerobic respiration and chemical oxidation. Similar results have been observed at microtidal sites in the NE Kattegat (Sundbäck & Miles 2000, Sundbäck et al. 2000), where both sands and silty sediments were net autotrophic through most of the year. In contrast, in

the Tagus estuary (Portugal), oxygen uptake by the sediments was generally higher than production, particularly in the warmer summer months (Cabrita & Brotas 2000). There were significant relationships between temperature and monthly production rate normalised to the annual mean production. Temperature is a proxy for season; however, this relationship does not show a seasonal signal as temperature was both positively and negatively correlated with production, depending on the site. In the lower estuary (Site 3H) production decreased with higher temperatures. In the upper estuary there was a positive relationship between production and temperature at Site 2H. Positive correlations between temperature and oxygen production have been observed in both the Tagus (Cabrita & Brotas 2000) and the Kattegat (Sundbäck et al. 2000).

Annual production was estimated using simple relationships between chl *a* and production or more directly by converting gross oxygen flux across the sediment-water interface under illumination into production. The range of values at a single site was greatest at Site 1, varying from 95 (Gross O_2 method) to 1199 g C m^{-2} yr^{-1} (Maximum method). Production was always greater at the high shore stations at Sites 2 and 3, reflecting the higher chl *a* concentrations and longer emersion times on the upper shore. Previous investigations in a variety of estuaries have estimated primary production on intertidal sediments to lie within a range 29 to 314 g C m^{-2} yr^{-1} (Table 2 of Underwood & Kromkamp 1999). This broad comparison indicates that the estimates from the Colne were within the right range, with the exception of the Maximum model, which may significantly overestimate production. The models in Table 3 provide a mechanism by which benthic annual primary production can be estimated on the scale of a whole estuary or mudflat from chl *a* in the sediment surface. The simple models assume that both biomass and production rate per unit chl *a* do not change throughout the month. Recent research shows that fortnightly (spring neap tidal cycle) oscillations in primary production are comparable to those on a seasonal scale and that annual production is dominated by processes on hourly and fortnightly time scales (Serôdio & Catarino 2000). Despite the relative simplicity of the models used to calculate production, rates were the same order of magnitude as those calculated for the Tagus, a mesotidal estuary with semi-diurnal tides, as in the Colne. Mean annual production in the Tagus was 156 g C m^{-2} yr^{-1} (Serôdio & Catarino 2000), based on a model requiring the input of many parameters. Generally, estimates from the Gross O_2 model were lower than those from the biomass models. The Gross O_2 model was comparable to a model constructed by Pinckney & Zingmark (1993) for production in North Inlet estuary (South Carolina, USA), which also calculated gross production from measurements of oxygen exchange converted to carbon using a photosynthetic quotient of 1. However, Pinkney & Zingmark's model was more complex as it incorporated vertical migration and hourly measurements of irradiance. Estimates from the Gross O_2 model for the Colne were 53 to 191 g C m^{-2} yr^{-1}, similar to the values of 54 to 240 g C m^{-2} yr^{-1} for North Inlet (Pinckney & Zingmark 1993).

The MPB may make a significant contribution to the primary production of the water column through resuspension (De Jonge & van Beusekom 1992, 1995). Kocum et al. (2002) found that the phytoplankton in the upper Colne Estuary was dominated by flagellates throughout the year, although there was evidence of some resupension of epipelic diatoms such as *Navicula* spp. and *Nitzschia* spp. Water column primary production for the estuary was estimated to be 8.9 g C m^{-2} yr^{-1} (Kocum et al. 2002), which is at the lower end of values given in Underwood & Kromkamp (1999). Therefore, the MPB were the main source of primary production in the Colne Estuary.

Nitrogen assimilation

The simple models of production were used to estimate nitrogen assimilation by the MPB (Table 4). The importance of denitrification as a nitrogen sink in coastal marine and estuarine sediments is widely recognised (Seitzinger 1988, Nedwell et al. 1999, Herbert 1999); however, the role of the MPB has been overlooked until relatively recently. The assimilation of DIN by the MPB plays an important role in nitrogen cycling on an estuarine scale. Estimates for the amount of DIN assimilated by the MPB in the Colne varied between 1.47 and 10.7 Mmol N yr^{-1}, depending on the model used. The total inorganic nitrogen load to the Colne in 1996 was 34.62 Mmol (Dong et al. 2000); therefore, DIN assimilation accounted for between 4.2 and 30.9% of the DIN input into the Colne Estuary. The value of 30.9% was based on production calculated using the Maximum model, and should be regarded as the maximum possible. The mean estimate for DIN assimilation by the MPB was 12.8% of the 1996 DIN load, suggesting that DIN assimilation into MPB biomass is a significant process within the estuary. The pool of nitrogen locked in MPB biomass may be of the same order of magnitude as that lost to the atmosphere by denitrification. In the Colne, DIN loss to the atmosphere via denitrification has been estimated to be 18 to 27% of the DIN load (Ogilvie et al. 1997, Dong et al. 2000). Sundbäck & Miles (2000) estimated that the rate of nitrogen assimilation by the MPB was greater than denitrification by 1 to 2 orders of

magnitude and concluded that in northern microtidal sediments, MPB assimilation was more important than denitrification in determining DIN fluxes through the sediment. In the Tagus estuary (Portugal) assimilation by the MPB was of the same order of magnitude as denitrification (Cabrita & Brotas 2000). These data indicate that nitrogen assimilation by the MPB is at least as important as denitrification in affecting the flux of DIN through intertidal and nearshore sediments. However, denitrification is a mechanism by which nitrogen is removed from the system to the sink of the atmosphere, whereas the MPB assimilation results from the transformation of DIN to particulate organic matter (PON), which is a temporary sink. The subsequent fate of PON incorporated into the MPB is redistribution through resuspension and grazing (as discussed above), burial (Admiraal 1984) or remineralisation back to DIN, which may be denitrified.

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