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Algae and Associated Pigments of Intertidal Sediments, New Observations and Methods*

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With 18 Figures and 3 Tables

Key words: Cryolander, benthos, microphytobenthos, pigments, chlorophyll, microprofiles

Abstract

In intertidal sediments the presence of microphytobenthos in and on the surface affects the sediment biogeochemistry, can influence the flux of substances across the sediment interface and changes the physical surface structure and sediment stability. In this paper new methods of sampling and for examining the microphytobenthos assemblages of sediments have been implemented. This included the new Cryolander method for the fine scale (0.1 mm) sampling of intertidal sediments. Algal pigments in sediments were determined with a new HPLC method on a very fine-scale (µm) with depth. Such finely resolved pigment profiles are combined with electron micrographs of surface sediments to provide a new and radically altered understanding of the concentration of algal biomass in and on sediment surface layers. It is shown that benthic algal populations can be highly stratified in the top millimeters of sediments, with most of the algal biomass occurring in the top 200 µm (up to 700 µg chlorophyll *a* g^{-1}). Therefore, in order to determine vertical algal distribution accurately, in all investigations where the absolute pigment concentrations at the surface of sediments are important, sediments should be sampled and analysed using microtechniques. This can prevent underestimation of chlorophyll at the surface of sediments of a factor as high as 6, as sediment sampling techniques based on cores with a depth of mm/cm can give results representing a dilution of the actual surface algal populations and even water contents. If the extent of this dilution is unknown, this can cause problems in algal estimations in general. Specifically, this is of great relevance to ground truth data for remote sensing and to diffusive boundary layer and porosity assumptions in biogeochemical considerations.

Introduction

The biological and physical properties of the upper millimeters of surface sediments are closely inter-linked and are critical to the behaviour of the interface region. In intertidal sediments the presence of microphytobenthos in and on surface sediments affects the sediment biogeochemistry, can influence the flux of substances across the sediment interface and changes the physical surface structure and stability of sediments (SUNDBÄCK & GRANELI 1988; WILTSHIRE 1992a, 1992b; PATERSON 1995; WILTSHIRE et al. 1998). The fine-scale structure and properties of this layer have recently received increased attention in studies of sediment erosion (YALLOP et al. 1994; AMOS & MOSHER 1995; WILTSHIRE et al. 1998), biogeochemistry (REVSBECH 1993), mineralisation processes and as a tool for investigations in paleo-environments (PATERSON 1995). It has been shown that the study of sediment surfaces using low-temperature scanning electron microscopy (LTSEM) contributes to the understanding of the erosional process and the cohesive properties of sediments (YALLOP et al. 1994; PATERSON 1995; DEFARGE et al. 1996). In 1997, WILTSHIRE et al. published a new method for the fine scale (0.1mm) sampling of intertidal sediments. As a result of these sampling methods it is now possible to determine, for example, algal pigments in sediments on a very fine-scale (µm) with depth. In this paper such finely resolved pigment profiles are combined with electron micrographs of surface sediments to provide a new and radically altered understanding of the distribution of algae and chlorophyll concentrations in and on sediment surface layers.

Methods

Sampling

The sampling of intertidal sediments was carried out with the device known as the Cryolander, described in WILTSHIRE et al. (1997). The

* This paper is dedicated to Prof. Dr. HARTMUT KAUSCH on the occasion of his 60th birthday.

sediments were cryolanded *in situ*, at low tide. The sampling sites were in the Humber Estuary (LISP project site A, England, April 1995), the Eden Estuary (PRO-MAT project transect North Shore, Scotland, June 1995) and the Ems-Dollard Estuary (BOA project site Heringsplaat, The Netherlands, July 1996). The Cryolander was placed on the sediment surface and some liquid nitrogen dribbled on to the absorbent cotton in it. As the cotton was at ambient temperatures, the liquid nitrogen was instantly vaporised. This vapour freezes the immediate sediment surface without distortion even on a micrometer scale (see WILTSHIRE et al. 1997). After the surface had frozen, the liquid nitrogen was then poured onto it evenly through the Cryolander mesh. Once the fluid liquid nitrogen reached the sediment this froze quickly into an approximately 2 cm thick disc (area = 20.41 cm^2) of sediment which could be lifted and stored in liquid nitrogen until further use. These frozen samples were cut up in the laboratory into 1 cm^3 blocks using a diamond lapidary saw. Such blocks provided material for microslicing, subsequent pigment analyses and, unsliced, for LTSEM.

In order to obtain a comparative estimate of the pigments and dry weight in the surface sediments using a more usual method, a cylinder (diameter of 7.5 cm) was pressed into the sediment to a depth of 5 mm. The sediment within this cylinder was scraped into a preweighed vial and homogenised for two minutes with a spatula. Subsamples of this were taken for the pigment and water content analyses.

Microslicing and Pigment Analyses

The preparation of the sediments for pigment analyses is summarised in Table 1. The cryolanded sediment blocks were placed on the stage of a freezing microtome (LEITZ) and cut into slices at 200 µm intervals. This method was first implemented by TAYLOR & PATERSON (1998), but the alignment of the sediments under the blade and the removal of the sediments from the blade, were, however, modified by the author and CLAIRE HONEYWILL (St. Andrews, Scotland) to prevent material loss. The first modification involved aligning the top of sediment block in a transparent plastic cube placed under the blade on the microtome stage so that the sediment surface was absolutely horizontal. The base of the block was built up with water which froze on to the stage (–40 °C). The second modification included pre-weighing 4 mm^2 strips of glass fibre filter paper, which were then used to wipe the sample from the blade without sediment/water loss. The sediment and filter paper were weighed, frozen instantly in liquid nitrogen and freeze-dried allowing further measurements of parameters such as water contents and the pigment analyses.

Table 1. Cryolander sample preparation for subsequent pigment analyses.

1. Cut frozen Cryolander sample into 1 cm^3 blocks, with diamond lapidary blade.
2. Place frozen block onto a freezing (–40 °C) microtome stage, so that the sediment surface is absolutely horizontal. Use water at the base, to freeze block into position.
3. Wind down microtome blade until it rests on surface of sediment.
4. Cut a slice between 50 and 200 µm thick.
5. Wipe sediment off blade with pre-weighed 3 mm^2 piece of glass fibre filter paper.
6. Place sediment and paper in pre-weighed brown plastic vial.
7. Weigh (for water contents).
8. Freeze instantly in liquid nitrogen.
9. Place in freeze-drier and freeze-dry over night.
10. Remove from freeze-drier and determine dry weight.
11. Put 1 ml of acetone on sample, mix and extract for 72 hours in the dark and cold for subsequent pigment analyses.

HPLC methods

All the freeze-dried sediment samples were suspended in nanograde acetone (1 ml) and frozen at –70 °C for a minimum of 24 hours. A sequential extraction was carried out for isolated samples to calculate the residual amounts of pigments remaining in the sample. The extracts were filtered through 0.2 µm pore-size cellulose filters and then injected by an autosampler straight into an HPLC system consisting of a quaternary high pressure pump system (Perkin Elmer 410) and autosampler (Waters WISP 417), a diode array detector (Waters 910). Water was added to the sample to be injected in a ratio of water : sample 3:7. The flow rate used was 0.6 ml min^{-1}. Because of the difficulties of separating all the xanthophylls (polar oxy-substituted carotenoids), the chlorophyll pigments (including chlorophyll breakdown products) and the non-polar carotenes, present in sediments in one HPLC run, a new method was devised (VILLERIUS et al. 1996). The column used was a reversed phase non-endcapped 5C30, (YMC) column, 15 cm in length. This column was kept thermostated at 35 °C in a column oven. The gradient used was as in Table 2, whereby the solvent used as "A" consisted of a methanol : acetonitrile : water (45 : 20 : 35) solution and solvent "B" was a 30 : 50 : 20 methanol : acetonitrile : ethylacetate mix. All solvents were degassed nanograde HPLC solvents (FISONS/ MERCK). The pigments were identified and quantified using a diode-array detector, fraction collection techniques and normal scanning spectrophotometric methods. The relevant extinction coefficients were taken from JEFFREY et al. (1997). The standard error of the peak areas of the separate pigments was maximally 1% for five replicate measurements.

LTSEM methods

The LTSEM techniques were used to examine the sediment surfaces and sediment fracture faces according to the methods described by PATERSON (1995). The instrument used was a modified SEM (Joel 35FC SEM and Oxford instruments cryosystem). Basically the method involved fracturing the frozen sediment sample under liquid nitrogen into a small block (approx. 2 mm^3) and plac-

Table 2. HPLC Gradient. (Solvent mixture "A" consisted of an 45:20:35 methanol: acetonitrile: water solution and solvent "B" was a 30:50:20 methanol: acetonitrile: ethylacetate mix.)

Step	Time (min)	%A	%B
0	0	80	20
1	0.3	80	20
2	4	55	45
3	35	0	100
4	45	0	100
5	55	80	20

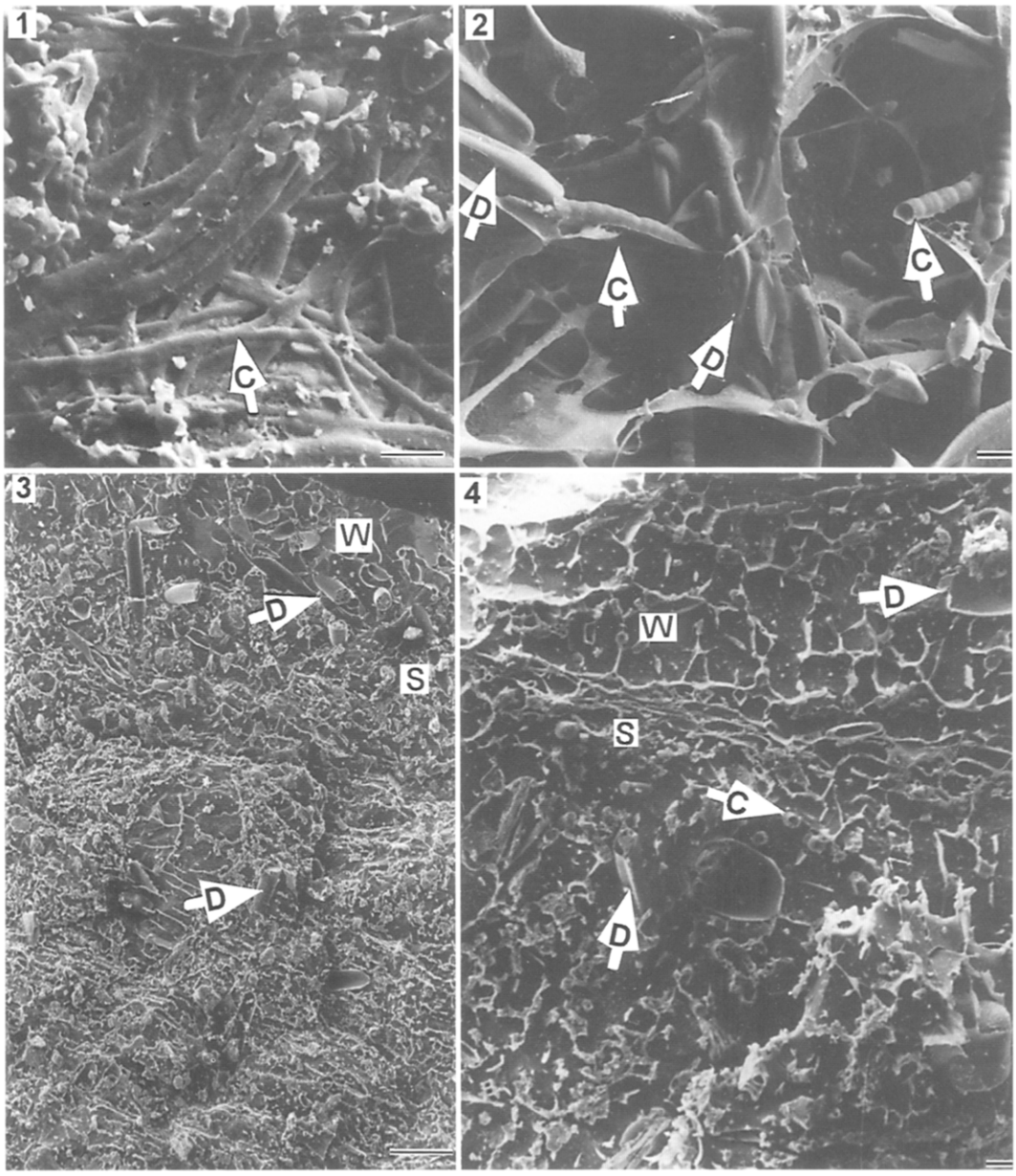
ing it in a sample holder. The sample was then introduced into the microscope and "heat" etched at –90 °C (see JEFFREE & READ 1991) in order to remove residual condensation water crystals from the sediment viewing surface. The sample was then moved into the sputter chamber (still at a low temperature) and coated with gold in an argon gas stream. After this treatment, the sample was reintroduced into the main chamber of the LTSEM, examined and photographed.

Results and Discussion

Three examples of different sediment algal assemblages are presented – Eden, Humber and Ems-Dollard estuarine sediments.

Eden Estuary sediment

The first example is of a sample taken from the Eden Estuary intertidal sediments (PRO-MAT EU project report 1997). These had a mean grain size of 110 µm (>30% < 63 µm) and consisted of mixed silt and sand. LTSEM of this sediment showed that it had a mixed population of diatoms and cyanobacteria at the surface (Figs. 1 and 2). From Figs. 3 and 4, which are images of a fracture made vertically through the surface sediments, it is evident that the algal layer was thin (10–100 µm) and patchy. From Fig. 4 it is clear that there were pockets of water on the surface; the broken ends of diatoms and cyanobacterial strands are evident just under the water patch. This algal layer was relatively dense by Eden



Figs. 1–4. LTSEM images of Eden surface sediments (D = diatoms; C = cyanobacteria; W = water; S = sediment). Scale bars: Fig. 1: 10 µm; Fig. 2: 10 µm; Fig. 3: 100 µm, Fig. 4: 10 µm. Figs. 3 and 4 are fracture faces.

standards – for most of the year these sediments are highly bioturbated by *Corophium volutator* and the algae mixed through the upper millimeter of sediment.

The sediment pigment profiles (μg g^{-1} dry weight) are shown in Figs. 5 and 6. The water contents of the sediment are given in Table 3 (mean values for four replicate Cryolander cores). In each figure the data for the surface sediments cored to a depth of 0.5 cm are also given at the top of the profile for comparative purposes. It is clear from Fig. 5 that the chlorophyll maximum was in the top 200 μm and that the values decreased by a factor of 5 in the material directly beneath this layer. There was a decreasing chlorophyll gradient down as far as 0.8–1.0 mm from the surface at which point the chlorophyll amounts remained the same until 1.8 mm. This means that algae were present at least down this depth (not shown in the electron micrograph). This was probably due to bioturbation and/or erosion and depositional sedimen-

Table 3. Mean water contents in Eden (n = 4), Humber (n = 6) and Ems (n = 4) surface sediments, g · g^{-1} , surface scrape and Cryolander sections with depth (mm). Standard error given in brackets.

Depth (mm)	Eden Sediment	Humber Sediment	Ems Sediment
Surface scrape	0.82 (0.02)	0.6 (0.002)	–
0–0.2	0.97 (0.009)	0.96 (0.01)	0.66 (0.017)
0.2–0.4	0.96 (0.009)	0.97 (0.006)	0.64 (0.017)
0.4–0.6	0.95 (0.003)	0.97 (0.007)	0.63 (0.015)
0.6–0.8	0.95 (0.006)	0.97 (0.01)	0.61 (0.021)
0.8–1.0	0.94 (0.007)	0.97 (0.007)	0.60 (0.027)
1.0–1.2	0.94 (0.004)	0.97 (0.005)	0.63 (0.027)
1.2–1.4	0.92 (0.02)	0.96 (0.008)	0.64 (0.032)
1.4–1.6	0.93 (0.02)	0.96 (0.009)	0.64 (0.029)
1.6–1.8	0.93 (0.007)	0.96 (0.008)	0.63 (0.028)
1.8–2.0	0.94 (0.003)	0.96 (0.006)	0.62 (0.02)

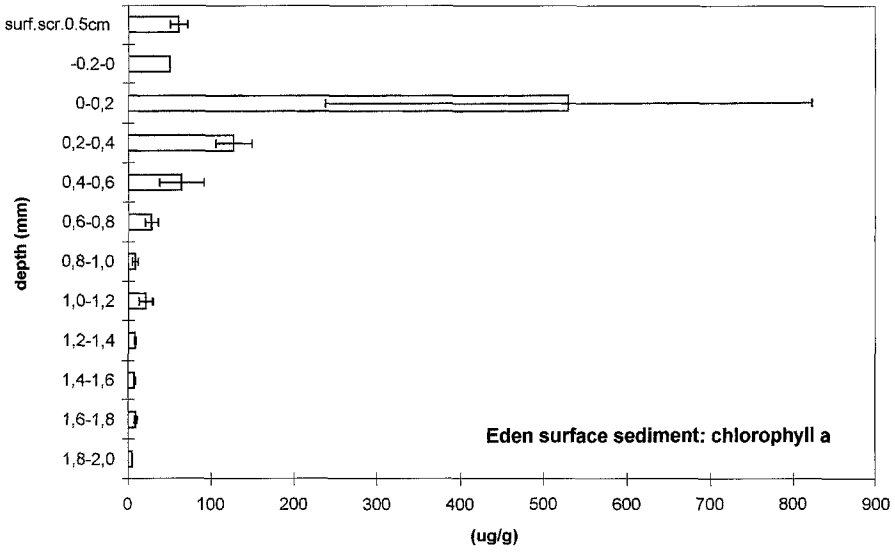


Fig. 5. Chlorophyll *a* concentrations in Eden surface sediments (μg g^{-1} dry weight), surface scrape and Cryolander sections with depth (mm).

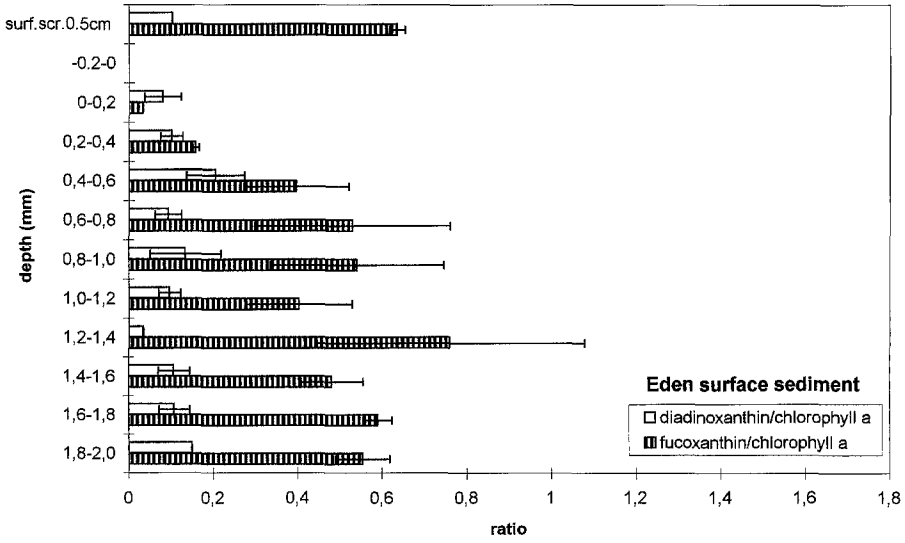
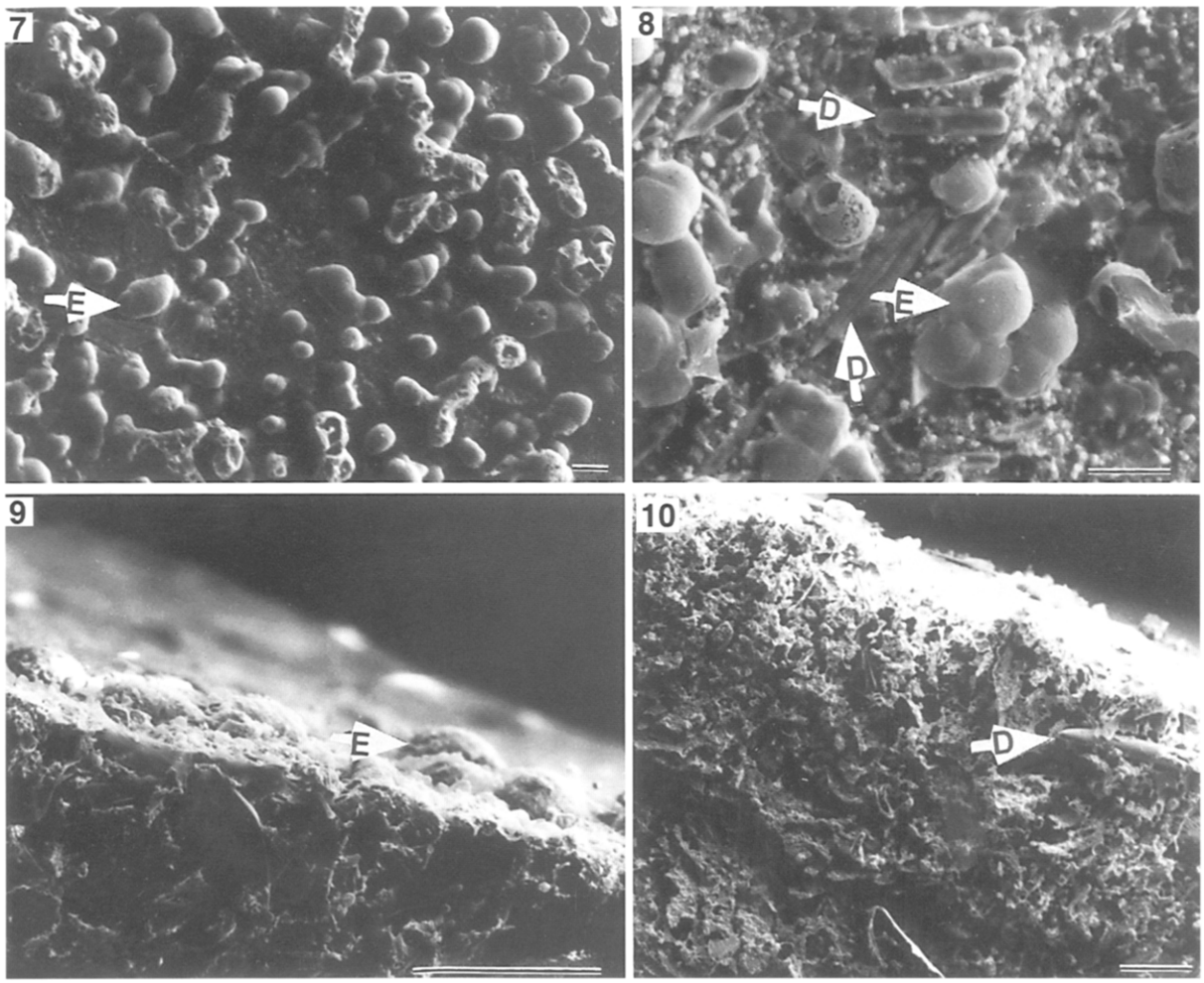


Fig. 6. Dominant pigment ratios to chlorophyll *a* in Eden surface sediments, surface scrape and Cryolander sections with depth (mm).

tary processes. As could be seen in Figs. 3 and 4, there was a layer of water and suspended matter on top of the sediment. This was also sliced off and analysed for pigments (slice –0.2–0 in the graphs). There was only a small amount of chlorophyll in this material. The other dominant pigments in the sediments were fucoxanthin and diadinoxanthin. These are marker pigments for diatoms. The only marker pigment which could have been detected for Cyanophyceae, zeaxanthin, was not found in detectable quantities, neither in the surface scrape nor in the Cryolander samples. It is of interest to note, however, that an increasing gradient of diatom pigment ratios (diadinoxanthin and fucoxanthin) was found in the first 0–0.6 mm of the sediment. The ratios were significantly higher with increased depth. Theoretically this could be due to the decrease in light levels and adaptive accessory pigment production. However, the diadinoxanthin is the 'low light' pigment in the xanthophyll cycle, and its high light counterpart to diatoxanthin was not detected in appreciable quantities at any depth. In addition, if the algae in deeper layers were specifically adapted to reduced light, an increase in chlorophyll *a* should have been detected. It is clear from the chlorophyll *a* profile in Fig. 5, that the just the opposite was the case. These lower chlorophyll concentrations with depth will have resulted in higher accessory pigment to chlorophyll ratios. Thus, the increase can be attributed to the fact that the chlorophyll at the surface originated from a mixture of algae (diatoms and Cyanophyceae), whereas the algae found in deeper layers were only diatoms and, consequently, the chlorophyll and accessory pigments had the same source (see LTSEM, Figs. 1 and 2). A comparison of the actual concentrations of chlorophyll *a* in the uppermost millimeter of sediment with the general surface scrape sample (first 0.5 cm) reveals that the surface scrape seriously under-represented the actual chlorophyll concentrations and, thus, possibly also algal biomass in the surface sediments. This will have been due to the simple dilution of the total chlorophyll amounts, which are in the uppermost µm, by the deeper sediments without chlorophyll in the deeper surface scrape sample. The relationships of the accessory pigments in the surface scrape also indicate that the sediment contained mainly

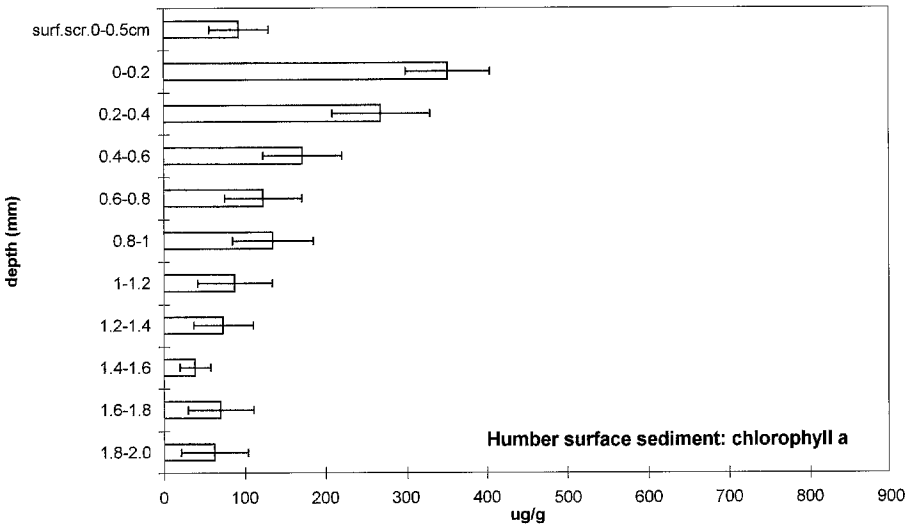


Figs. 7–10. LTSEM images of Humber surface sediments (D = diatoms; E = euglenids). Scale bars: Figs. 7 and 8: 10 µm; Figs. 9 and 10: 100 µm. Figs. 9 and 10 are fracture faces.

diatoms when such an integrated sample to depth of 0.5 cm was taken. Therefore, in this sample the thin mixed layer of cyanobacteria and diatoms at the surface was lost and the pigments did not reflect the actual algal population at the surface. The water contents of the surface layers were also found to be very much less for the surface scrape than for the cryolanded actual first millimeter.

Humber Estuary sediments

The second sediment assemblage example was from Humber Estuary intertidal sediments. The sediment was taken at the LISP sampling site A (LISP project report 1997). The sediment was a mixture of silt and fine sand. The mean grain size as measured with a laser particle size analyser was < 125 μm. At the time of sampling, the surface sediments had a mixed population of euglenids and diatoms (see PATERSON et al. 1998). This is shown in the LTSEM pictures in Figs. 7–10. The intact euglenids (which are relatively delicate) on the surface are evidence of the success of the cryolanding sampling method. These euglenids were highly migratory (see PATERSON et al. 1998) and during the day they formed a layer on top of the sediment surface. This can be seen in the surface images (Figs. 7 and 8) and in the high magnification, fracture face, image, Figs. 9 and 10. The chlorophyll *a* concentrations (Fig. 11) in the sediment were, as in the Eden sediments, highest at the surface (mean values for 12 Cryolander cores taken over the exposure period). The values were less than those found for the surface of the Eden sediments. However, for that the distribution was deeper and the gradient in the first 0.2 mm less pronounced. This probably was a result of the highly migratory behaviour of the euglenids over the exposure period. It was shown by PATERSON et al. (1998), that these algae moved up to and down from the sediment surface and in the first 1mm below the surface. In contrast, the algae in the Eden sediments did not visibly



Figs. 11. Chlorophyll *a* concentrations in Humber surface sediments (μg g^{-1} dry weight), surface scrape and Cryolander sections with depth (mm).

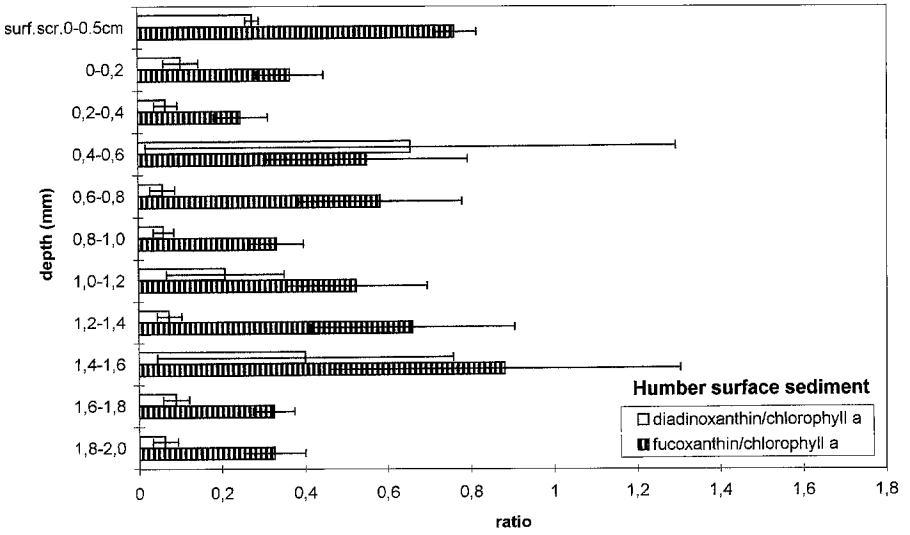


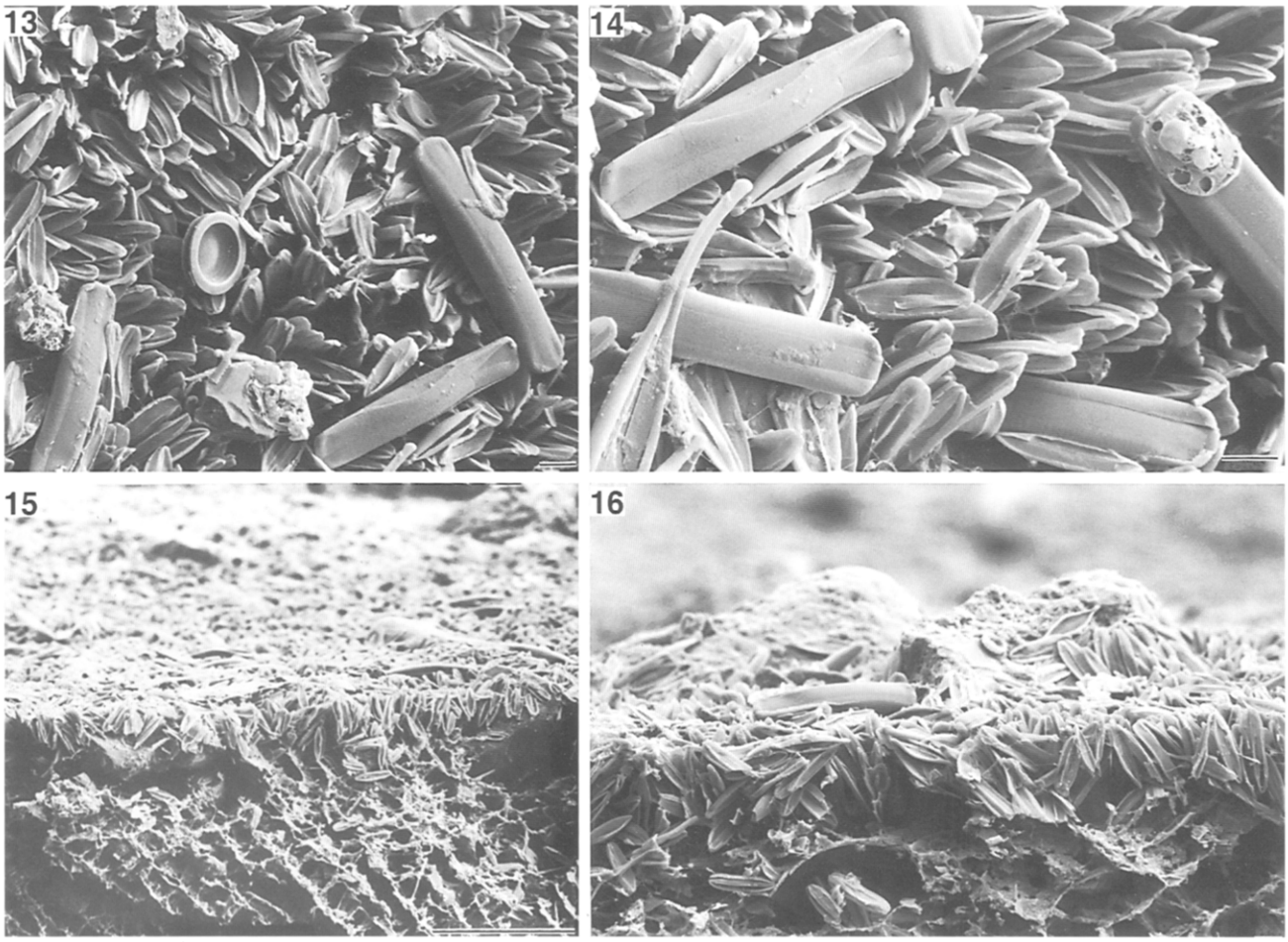
Fig. 12. Dominant pigment ratios to chlorophyll *a* in Humber surface sediments, surface scrape and Cryolander sections with depth (mm).

migrate. The absolute chlorophyll values found for the upper 0.2 mm layer of sediment in the Humber could be up to 1mg g^{-1} in isolated patches at site A (not shown here). Although a lot of euglenids were present on the surface of the sediments, diatoms were also present in large numbers (see PATERSON et al. 1998 and Figs. 8 and 10). These were found to a depth of 0.5 mm. They contained the pigment fucoxanthin. The pigment diadinoxanthin is found both in the diatoms and euglenids. The mean values of these pigments relative to chlorophyll *a* were insignificantly different with depth (Fig. 12). This indicates that overall the relationship of euglenids to diatoms was the same with depth in the cores. This is backed up by the chlorophyll *b* to chlorophyll *a* ratios (not shown here) which also were not significantly different for a comparison of the slices in the first 2 mm. Chlorophyll *b* is found in euglenids and green algae.

As was found for the chlorophyll *a* quantities in the uppermost millimeter of Eden sediment, the general surface scrape sample (first 0.5 cm) significantly under-represented the actual chlorophyll concentrations which were found at the surface and, consequently, algal biomass at the surface of the sediments. The sum of the chlorophyll concentrations (unit volume) in each slice is very much more than that found for the scrape. The chlorophyll concentrations were 2–3 times higher in the uppermost 0.4 mm. Unlike the Eden sediments, the relationships of the accessory pigments in the surface scrape are similar to those in the upper 2 mm suggesting that the algal composition was the same with depth in the cores and over a mixed depth sample of 0.5 cm. As was found in the Eden, the water content of the surface layers was significantly less (20%) for the surface scrape than for the actual first millimeter. No difference was found for the water contents with depth over the first 2 mm in the Cryolander slices (Table 3).

Ems-Dollard Estuary sediments

The third microphytobenthos and sediment assemblage example was from the Heringsplaat, an intertidal mudflat in the Ems-Dollard Estuary. The sediment was mixed sand with 20% of the particles under 63 µm in size. This mudflat was rather interesting as it was covered by an extremely dense



Figs. 13–16. LTSEM images of Ems-Dollard surface sediments; showing only diatoms. Scale bars: Figs. 13, 14 and 16: 10 µm; Fig. 15: 100 µm. Figs. 15 and 16 are fracture faces.

microalgal mat. The LTSEM photos of the sediment surface show that this algal mat consisted of a mixed population of only diatoms (Figs. 13 and 14) which were densely packed, often in a vertical manner. From Figs. 15 and 16, the electron micrograph images of a fracture made vertically through the surface sediments, it is evident that the layer was concentrated at the surface and that it was 100–150 µm thick. The mat was continuous over the sediment surface except where it had been eroded, i.e., where it had been peeled off by tidal movement/wave action, exposing the bare sediment underneath (see WILTSHIRE et al. 1998).

The sediment pigment profiles and the water contents for the Ems-Dollard surface sediment are given in Figs. 17, 18 and Table 3 (mean values for two Cryolander cores). In each figure the data found for a sample of the surface sediments taken with the small core to a depth of 0.5 cm are also given at the top of the profile for the pigment data. It is clear from Fig. 17 that the chlorophyll maximum was, as for the Eden and Humber sediments, found to be in the top 200 µm and that the values decreased by a factor of 6 in the layer directly beneath this. The LTSEM photos (Figs. 13-16) corroborate this. The algae were highly concentrated at the surface. Unlike the Humber sediments no gradient worth speaking of was found beneath this layer. Significant numbers of algae were found at greater depths though too: at 2 mm there were still 50 µg g^{-1} of chlorophyll *a* present in the sediments. The other dominant pigments presented here are, again, the diatom pigments fucoxanthin and diadinoxanthin, whereby fucoxanthin was the more important. No difference was found with depth in either pigment's relationship to chlorophyll *a* (see Fig. 17). This could indicate that the diatoms were from the same population as at the surface. The comparison of the actual concentrations of chlorophyll *a* in the uppermost millimeter of sediment and the general surface scrape sample (first 0.5 cm)

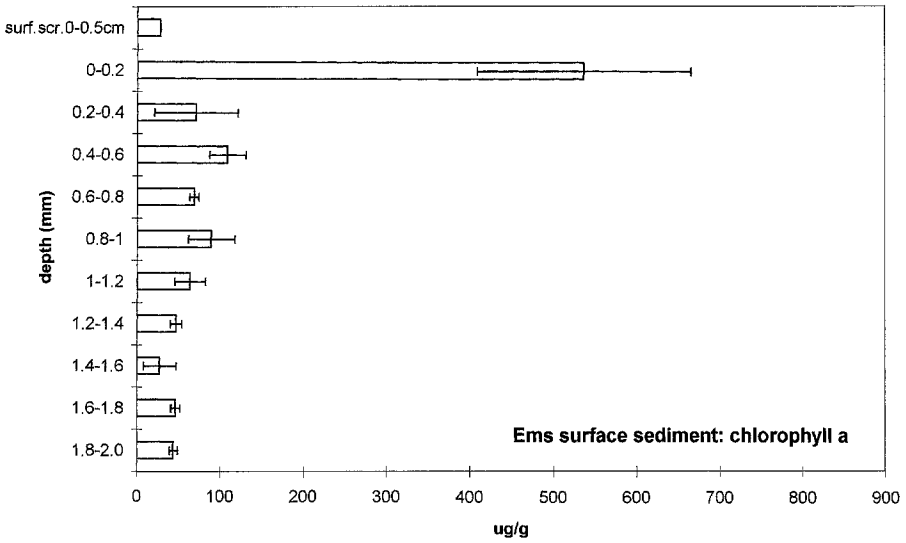


Fig. 17. Chlorophyll *a* concentrations in Ems-Dollard surface sediments (µg g^{-1} dry weight), surface scrape and Cryolander sections with depth (mm).

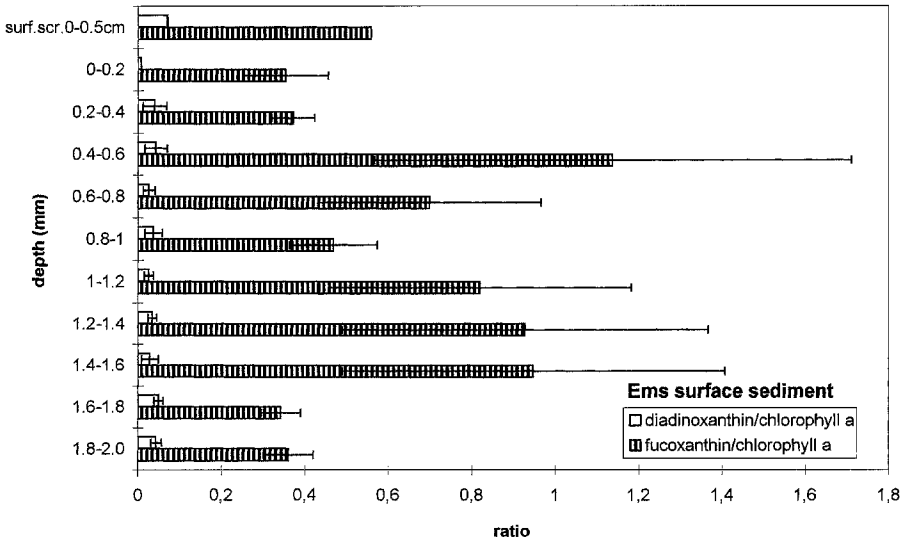


Fig. 18. Dominant pigment ratios to chlorophyll *a* in Ems-Dollard surface sediments, surface scrape and Cryolander sections with depth (mm).

reveals, once again, that the general surface sample significantly under-represented the actual chlorophyll amounts in the sediments at the absolute surface. The relationships of the accessory pigments in the surface scrape did not differ from the ratios found with the Cryolander samples.

General discussion

The combination of LTSEM and thin slice pigment analyses for the three different types of sediment algal assemblages revealed, conclusively, that in order to estimate algal biomass in terms of chlorophyll *a* at the sediment surface, this must be carried out in as thin a slice of sediment as possible. If this is not done in such a manner, the estimations for a surface sediment could result in underestimation due to the "dilution" of the surface sediment with deeper non-pigmented sediments. Of course, it is theoretically possible to express the pigment concentrations in terms of sediment surface area, where the algae in a particular volume of sediment beneath the surface are used for algal biomass estimations. However, this method is subject to substantial errors resulting from surface sediment topography, setting the depth of sampling and differences in sediment porosity/density. Indeed, it has been shown recently by KELLY et al. (unpubl. results), that except at extreme differences in algal biomass levels, coarse coring (5 mm depth) does not detect statistically significant differences in chlorophyll *a* between obviously diverse sampling sites, whereas Cryolander samples do. In addition, where pigment concentrations at the surface of sediments are of great importance to particular questions such as ground truth data for remote sensing (PATERSON et al. 1998; DOERFFER & MURPHY 1986), it is of little use to take values from a sample not representing the surface. It has been shown that whereas normal thick surface samples (0.5 cm) of sediment can correlate very poorly with optical signals of hand held spectrometers employed to measure sediment surface colour, pigment values from cryolanded slices correlate well (see PATERSON et al. 1998). It is not only surface differentiation which is often of importance, but the assessment of the light climate in surface sediments would also benefit from such a fine resolution of algal and pigment assemblages (see JØRGENSEN & DES MARAIS 1988; LASSEN et al. 1992).

The accurate differentiation of sediment algal populations and sediment water contents is of importance to all work on the estimation of algal production using microelectrodes and sediment oxygen and nutrient measurement/ modelling (JØRGENSEN et al. 1979; REVSBECH & JØRGENSEN 1983; REVSBECH 1993; WILTSHIRE et al. 1996). In particular, the LTSEM photos of Ems-Dollard sediment algal assemblages show that these can have a distinct structure at the surface. This is likely to effect the diffusion properties and porosity of sediments. This will affect the fluxes of substances across sediment-water interfaces and thus also diagenetic processes (JØRGENSEN et al. 1979; GLUD et al. 1992; JENSEN et al. 1994). It is known from the work of YALLOP et al. (1994), PATERSON (1995) and WILTSHIRE et al. (1998) that the cohesive properties and stability of intertidal sediments are often determined by the microphytobenthos. The erosion of sediments in aquatic systems can be followed using the pigment markers from the thin upper sediment layer (WILTSHIRE et al. 1998). Thus, it is clear that there is a multitude of topics involving sediment interfaces, particularly in intertidal sediments which would benefit from the techniques and observations presented above.

Conclusions

In this work new methods of sampling and examining the microphytobenthos assemblages of sediments have been implemented. It has been shown that benthic algal populations can be highly stratified in the top millimeters of sediments, with most of the algal biomass occurring in the top 200 µm. Therefore, in order to determine algal sediment biomass accurately and to prevent underestimation of chlorophyll in surface sediments, these must be sampled and analysed using microtechniques. Sediment sampling techniques based on cores with a depth of mm/cm can give results representing a dilution of the actual surface algal populations. This can cause problems in algal biomass estimations in general and specifically in ground truth data of remote sensing and diffusive boundary layer and porosity assumptions in biogeochemical considerations.

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