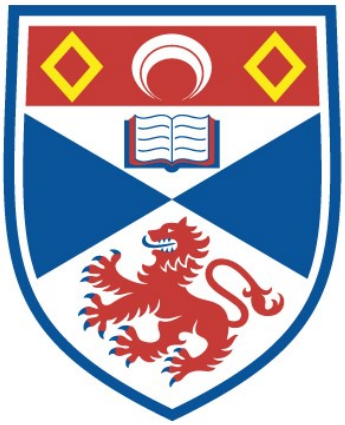
FINE-SCALE DISTRIBUTION OF CARBOHYDRATES ON INTERTIDAL SEDIMENTS IN RELATION TO DIATOM BIOMASS AND SEDIMENT PROPERTIES

Irene Sarah Taylor

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1998

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Irene Sarah Taylor

A thesis submitted in partial fulfilments of the requirements for the degree of Doctor of Philosophy, at the University of St Andrews.

School of Environmental and Evolutionary Biology,
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ST ANDREWS UNIVERSITY

Declarations

I, Irene Sarah Taylor, hereby certify that this thesis which is approximately 45,000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

10/8/98

I was admitted as a research student in September 1994 and as a candidate for the degree of Doctor of Philosophy in October 1995; the higher study for which this is a record was carried out in the University of St Andrews between 1994 and 1997.

10/8/98

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

10/8/98

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ABSTRACT

Extracellular polymeric substances (EPS) are produced by epipelic diatoms as a function of their locomotive mechanism and also protect cells from desiccation and heavy metal toxicity. EPS are carbohydrate-rich and form an important carbon source for heterotrophic microorganisms. In addition, the polymeric structure of EPS results in sediment inter-grain binding, thereby increasing the resistance of sediments to erosion forces. Despite the importance of generic carbohydrates (measured as an index of EPS), there is little information on their spatial distribution or factors influencing their abundance. In this study, a fine-scale sectioning technique was developed and provided the first depth profiles of sediment carbohydrates at a scale relevant to microphytobenthos. The operational separation of sediment carbohydrates into bulk and colloidal fractions was examined and fractions were shown to vary in spatial and temporal characteristics. Colloidal carbohydrates were concentrated in the surface 200 μm layer of intertidal mud flats and therefore influenced sediment interface processes such as diffusion and sediment erosion. Colloidal carbohydrates were positively correlated to sediment chlorophyll *a* concentrations and also varied with tidal height and with sediment topography. During the emersion period, colloidal carbohydrate concentrations increase significantly, however, bulk carbohydrate concentrations remain unchanged. Biochemical analysis of the carbohydrate fractions showed marked, but not statistically proven, differences in the proportion of monosaccharides present and suggested these operational fractions may arise from different sources and are subject to varying turnover rates. Bulk sediment carbohydrates increase in concentration with sediment depth in the upper millimetres of intertidal sediments and were positively correlated to increases in sediment density. The increase in density with depth reflects post-depositional compaction and has important implications for models which predict sediment erosion based on sediment density. These findings are discussed in relation to contemporary thinking on sediment processes.

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ABBREVIATIONS AND SYMBOLS

C	dry mass concentration (kg m^{-3}, also known as dry density, ρ_d)
CSM	cohesive strength meter
$D_{sediment}$	apparent sediment diffusion coefficient (cm^{-2} s^{-1})
D_{water}	water diffusion coefficient (cm^{-2} s^{-1})
EPS	extracellular polymeric substances
GC-MS	gas chromatography-mass spectrometry
HPLC	high-performance liquid chromatography
J	flux (according to Fick's 1st Law of Diffusion, nmol O_2 cm^{-2} s^{-1})
*J/J**	test statistic for Jonckerre's test of ordered alternatives
LISP	Littoral Investigation of Sediment Properties
LOIS	Land Ocean Inter-action Study
LN_2	liquid nitrogen
LTSEM	low-temperature scanning electron microscopy
M_s	mass of solids (kg)
NAPR	net average production rate (nmol cm^{-3} s^{-1})
NMR	nuclear magnetic resonance
PAR	photosynthetically active radiation (400-700 nm)
rpm	revolutions per minute
SE	standard error (also known as standard error of the mean, SEM)
SD	standard deviation
SPM	suspended particulate matter (g l^{-1})
TIC	total ion chromatograms
TFA	tri-fluoracetic acid
UDOM	ultrafiltered dissolved organic matter
U_{*crit}	critical shear velocity (m s^{-1})
V	volume (m^{-3})
X_{ph}	depth of photosynthetic zone
Φ	porosity
τ_{cr}	critical erosion stress (N m^{-2})
$\delta C/\delta x$	oxygen concentration at depth x (μM)

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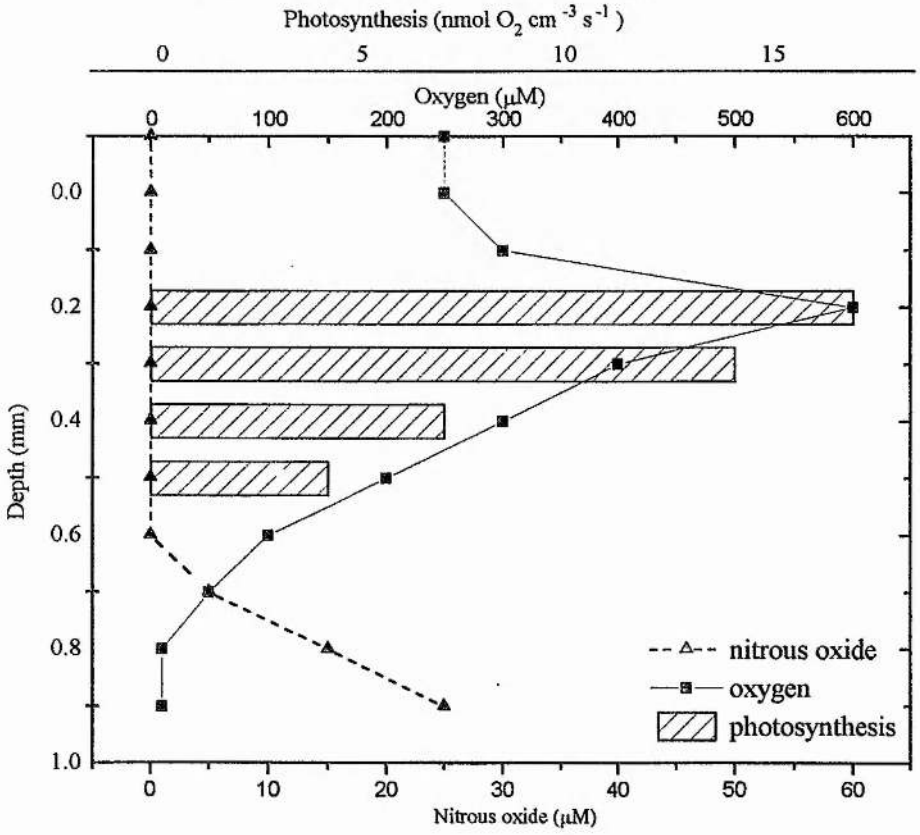
Chapter 1

1. General Introduction

1.1 Intertidal depositional environments

Intertidal mud flats are complex, highly productive environments which serve as important feeding grounds for migrating birds and juvenile fish. In addition, they are highly dynamic systems which may undergo erosion or deposition with each tidal cycle. Regular exposure to the air results in rapid temperature changes and desiccation of intertidal regions. The tidal variation in exposure leads to large scale gradients or zonation across the surface of intertidal flats. Microsensors studies have also shown significant microscale variation in light and sediment oxygen, sulphide and nitrous oxide concentrations in the upper millimetres of intertidal and subtidal sediments (Fig. 1.1) (Jørgensen *et al* 1983, Kühl and Jørgensen 1994). These microscale gradients may vary in response to local conditions and biological activity. Therefore, surface sediments contain extreme and rapidly changing microclimates and organisms growing there have adopted numerous strategies to cope with these stresses.

Figure 1.1 Schematic diagram of microspatial variation in the upper 1 mm of sediment (based on Revsbech 1989).



Diatoms (*Bacillariophyceae*) are well suited to growth in dynamic conditions and are often the main primary producers in depositional environments. In addition, diatoms colonise saltmarsh sediments, plants and any submerged surfaces. Diatoms contribute to 20-25 % of global primary production (Hoek *et al.* 1995). The annual production of fixed carbon by diatoms in temperate oceans is 200-400 g C m^{-2} and the amount of carbon fixed by a maize crop is 1000-2500 g C m^{-2} y^{-1} (Hoek *et al.* 1995). An understanding of the dynamics and ecology of estuarine mud flat systems is a fundamental requirement to predict the consequences of human activities and the influence of global environmental change in coastal waters.

Diatoms growing on sands have been termed "epipsammic" and those which thrive in mud have been termed "epipelic" diatoms (Round 1971). Most benthic diatoms (both epipelic and epipsammic) range in size from 10 μm to 300 μm and are commonly pennate (symmetrically lanceolate) (Admiraal 1984), whereas, planktonic diatoms are dominated by centric forms (radially symmetrical, Admiraal 1984).

Diatoms have adapted to withstand a wide range of temperatures and light intensities. For example, laboratory studies have shown *Navicula salinarum* and *N. pygmaea* to divide equally rapidly when grown at 15, 100 or 700 μE m^{-2} s^{-1} (Admiraal *et al.* 1984). However, on intertidal sediments, winter and summer species can often be identified (Colijn and Dijkema 1981) suggesting ecological factors other than light may influence natural communities. Estuarine diatoms can grow at salinities between 4 and 60 ‰ (Admiraal 1984). This tolerance is especially important on exposed mud flats where rainfall can cause a sudden decrease in salinity and evaporation can increase salinity to > 40 within 1 hour of exposure (Rasmussen *et al.* 1983). In addition, hydrological features of estuaries such as tidal scour, turbidity and sediment particle size can affect the growth of diatoms (Round 1979a). However, diatoms exhibit great versatility and can grow on a wide variety of sediments (sands, silts and muds). Sands are highly mobile and can lose moisture rapidly and epipsammic diatoms have adapted to this instability by adhering firmly to sand grains. The fine sediment particles which form muds are held closely together by small surface charges (Van der Waals

forces) resulting in a more stable environment for benthic diatoms. However, epipelic diatoms must cope with the lower porosities of muds and the consequent limitation of oxygen and light, which may penetrate only the upper 0.4 - 0.8 mm (Jørgensen *et al.* 1983, Kühl and Jørgensen 1994).

During photosynthesis, conditions of high pH, low CO_2 availability and saturated oxygen concentrations commonly occur on intertidal diatom biofilms (Jørgensen *et al.* 1983, Admiraal 1984). Diatoms cope with these stresses by exhibiting some characteristics of C_4 or CAM metabolism (i.e. β carboxylase activity and low $\delta^{13}C$ values) which may contribute to their success on intertidal flats (Morris 1980, Schwinghammer *et al.* 1983, Admiraal 1984). Another defence against changing environmental conditions and grazing is the ability to exist as resting cells. These resting stages are more suited to long periods of low-temperatures and short photoperiods than parental vegetative cells (McQuoid and Hobson 1995). However, the production of resting cells is almost entirely limited to centric diatoms and the lack of resting cells among pennate diatoms cannot be attributed to more favourable conditions but rather the possession of alternative protection against environmental changes, such as migration (see below).

Intertidal sediments hold plentiful nutrient supplies for autotrophic growth and diatoms, in turn, support a wide range of meiofauna and macrofauna including the amphipod *Corophium volutator*, the polychaete worm *Nereis diversicolor*, nematodes, copepods, harpacticoids and ostracoids (Montagna *et al.* 1995, Smith *et al.* 1996). One strategy to cope with this grazing pressure and other environmental stresses is the development of migration patterns. The diatom migratory rhythm is influenced by both lunar and solar cycles and diatoms surface shortly after the sediments are exposed and return (1-3 mm) down into the sediment before tidal inundation (Perkins 1960, Hay *et al.* 1993, Paterson *et al. in review*). This allows diatom cells to receive sufficient light for photosynthesis without the risk of being resuspended by the incoming tide (Palmer and Round 1967). The locomotive force for motility in diatoms involves the secretion of polymeric fibrils (extracellular polymeric substances, EPS), across the plasmalemma into the raphe fissure (Edgar and Pickett-Heaps 1984). The fibrils become hydrated and expand to make contact with external objects, whilst

maintaining a distal connection to the plasmalemma. Actin filaments in the cytoplasm are thought to act on the fibril attachment site to move the fibrils along the raphe fissure. Since the fibrils are also attached to external objects, the cell is displaced (Drum and Hopkins 1966, Edgar and Pickett-Heaps 1984). The migration pattern and speed of motility has been found to vary among species (Round 1979b, Hay *et al.* 1993). For example, *Gyrosigma limosum* (formally *G. spencerii*) can move across sediments at a speed of 17 mm h^{-1} and was notably faster than *Nitzschia* or *Navicula* sp. (Hay *et al.* 1993). Furthermore, Happey-Wood and Jones (1988) found greater average speeds in *Pleurosigma angulatum* during the time corresponding to low tide (32.4 mm h^{-1}) and slower speeds during tidal immersion(9 mm h^{-1}), although this has not been verified in subsequent studies.

1.2 Extracellular polymeric substances (EPS) and colloidal carbohydrates

The copious production of EPS by motile diatoms makes them the most likely source of colloidal carbohydrates in the intertidal zone. Hoagland *et al.* (1993) defines EPS as any macromolecule secreted external to the plasmalemma. In addition, non-motile diatoms commonly produce EPS in the form of stalks, pads and cell coatings (Hoagland *et al.* 1993).

EPS has been examined using many analytical techniques and histochemical stains have shown EPS from 30 taxa to consist of carbohydrates which are carboxylated or sulphated to varying degrees (Decho 1990, Hoagland *et al.* 1993 and references therein). These carbohydrates may be structurally altered with NaCl (Allan *et al.* 1972) resulting in structural variation within an estuary. The monosaccharide composition of the carbohydrates have been identified using separation techniques including paper, liquid, gas-liquid and gas chromatography - mass spectrometry (GC-MS) (Handa and Mizuno 1973, Cowie and Hedges 1984, Sigleo 1996, McCarthy *et al.* 1996). In addition, nuclear magnetic resonance (NMR) has provided information on colloidal carbohydrates isolated from many different water bodies and has shown the molecules to have very similar linkage and branching patterns (Mcknight *et al.* 1997). The structures of colloidal carbohydrates from water bodies were closely related to the exudates of *Thalassiosira weissflogii* grown in culture (Aluwihare *et al.* 1997).

Spectrophotometric assays have been employed for the rapid quantification of carbohydrates. The phenol-sulphuric acid assay (Dubois *et al.* 1956) has advantages over other rapid techniques (Table 1.1) since pentoses are detected as well as hexoses. Furthermore, the reaction agents are not affected by other organic materials or inorganic ions present in the marine environment (Handa 1966). Therefore, the phenol-sulphuric acid assay remains the most commonly used assay (Underwood and Smith 1998b, Sutherland 1996).

Diatom carbohydrates have been artificially separated into fractions depending on their solubility (Allan *et al.* 1972, Haug and Myklestad 1976, Underwood *et al.* 1995). Firstly, those carbohydrates which are soluble in water and an insoluble fraction. Colloidal carbohydrates are those extracted in aqueous solution and remaining in solution following centrifugation and they are measured as an index of EPS. Approximately 20-25 % of colloidal carbohydrates are polymeric and the other carbohydrates are monomers or short chains. The majority of biochemical studies on algal polysaccharides have involved carbohydrate extractions from planktonic mono-cultures (Hoagland *et al.* 1993, Allan *et al.* 1972).

Table 1.1 *Comparison of spectrophotometric carbohydrate assays.*

Assay	Author/s	Comments
Phenol-sulphuric	Dubois *et al.* 1956	simple, stable and sensitive (Handa 1966)
Anthrone-sulphuric	Spiro 1966	anthrone unstable in sulphuric acid, expensive
N-ethylcarbazole	Lewis & Rakestraw 1955	affected by non-carbohydrate materials (McLauglin *et al.* 1960)
L-tryptophan	Grasshoff 1978	reaction not chemically understood
MBTH *	Johnson & Sieburth 1977	many addition steps highly toxic

*3 methyl-2-benzothiazolinone hydrazone

Of the few studies that have isolated extracellular material from the culture medium, most have found the carbohydrates to be composed of fucose, rhamnose

and galactose with glucose, mannose, xylose and arabinose present to a lesser extent (Hoagland *et al.* 1993 and references therein, Allan *et al.* 1972). In this study the composition of *in situ* sediment carbohydrates associated with benthic diatoms were investigated for the first time. EPS represents 11-39 % of cellular carbon during stationary phase and 2-8 % during exponential growth (Claus 1988). Laboratory findings suggest that diatom cells produce more EPS in the stationary growth phase than during exponential growth (Myklestad and Haug 1972). In addition, EPS is often produced in greater concentrations in conditions of high nitrate to phosphate ratios, where nutrients are available but perhaps not in the ratios necessary for growth (Myklestad and Haug 1972).

1.3 EPS synthesis

The internal mechanism of synthesis and release begins with cytoplasmic vesicles adjacent to the Golgi apparatus (Edgar 1980). Variation in vesicle morphology suggests progressive stages in synthesis and preparation for secretion. In the case of fibril synthesis, vesicles containing fibrilar material migrate to the plasmalemma where fusion occurs and the vesicle contents are released into the raphe fissure (Herth 1979, Sicko-Goad *et al.* 1989). Radiolabelled ^{14}C uptake studies suggest that the percentage of photoassimilated carbon used to produce EPS is low (0.18-0.48 %, Underwood and Smith 1998).

Since high concentrations of EPS are produced by motile cells, carbohydrate concentrations are likely to be related to epipelic diatom biomass. Indeed, colloidal carbohydrates, measured as an index of EPS, have been positively correlated to chlorophyll *a*, (a photosynthetic pigment which is commonly used as a measure of biomass) (Sutherland 1996, Underwood and Smith 1998b). A model describing the relationship predicts the concentration of colloidal carbohydrates from chlorophyll *a* concentrations in several intertidal mud flats where diatoms constitute > 50 % of the microphytobenthic population (Underwood and Smith 1998b). Although these two variables are clearly related, the concentration of EPS is not strictly dependant on photosynthetic activity. Smith and Underwood (1998b) detected elevated levels of EPS during periods of darkness, which corresponded with a decrease in the diatom storage product, glucan.

1.4 The Functions of EPS

As well as motility, EPS allows habitat stabilisation, sessile adhesion, colony formation and protection against desiccation and toxic substances.

1.4.1 Habitat stabilisation

Diatoms have long been associated with a reduction in the resuspension of sediments (Delgado *et al.* 1991). In early work, the stabilisation effect was only found to be significant for diatoms which secreted mucilage (Holland *et al.* 1974) and in a more recent study, a positive correlation was found between colloidal carbohydrates (an index of EPS) and the critical shear velocity (U_* crit) (Grant and Gust 1987, Sutherland 1996). These authors speculate on the possible use of this relationship as a means to predict erosion thresholds. Furthermore, in some cases, biological activity can have a greater effect on sediment stability than physical parameters such as bulk density, which are typically used to predict stability (Sutherland 1996).

The stabilisation effect of EPS occurs as diatoms migrate through the sediment causing EPS to accumulate, forming a matrix in the upper millimetres which increases inter-grain binding. There is no information on the fate of EPS or colloidal carbohydrates when sediments are under erosion stress, whether they uncouple from the sediment fabric and enter the water column before sediment erosion or remain associated with sediment particles and settle out after erosion. The drying of sediments over the emersion period increases the stability of surface sediments. In addition, drying may stimulate the production of more EPS (Peterson 1987) which would further stabilise the sediment, a positive feedback mechanism. Furthermore, EPS smoothes the surface sediment, reducing shear stress and turbulence, thus contributing to stability (Delgado *et al.* 1991). EPS release creates a more stable environment for the assemblages, preventing sediment erosion and thereby reducing turbidity and light attenuation in overlying waters. By consolidating the sediment, diatoms may encourage the colonisation of other algae and plants. Such a succession of organisms would further stabilise the sediment and is important in the formation of salt marshes (Coles 1979).

1.4.2 Sessile adhesion

Diatoms also use EPS to adhere to submerged surfaces where they can maintain a stable position and where nutrient levels may be greater than in the water column (Brock and Madigan 1991). Diatoms are the most common biofouling microalgae found on submerged surfaces (Characklis and Cooksey 1983). The attachment strength and concentration of EPS was found to vary with the surface tension of the submerged material (Becker 1996). In addition, EPS concentration is reduced by cathodically polarising the surface and this procedure is used to protect marine structures from biofouling and corrosion (Bhosle *et al.* 1994). EPS secretion in the form of apical pads allows colony formation in many planktonic forms e.g. *Asterionella formosa*. The formation of a colony structure may reduce the sinking rate and affect the longevity of a diatom bloom.

1.4.3 EPS as a carbon source and binding agent

EPS and colloidal carbohydrates are an important carbon source for the microbial community (Freeman and Lock 1995) and they may encourage the establishment of complex bacterial communities (Stehr *et al.* 1995). Heterotrophic bacteria degrade EPS, colloidal carbohydrates and other organic matter using extracellular enzymes, notably α amylase, α and β-glucosidase (Decho 1994). The production of these enzymes varies diurnally and seasonally, mirroring changes in diatom biomass and ambient temperatures (Meyer-Reil 1983, Karner and Rassoulzedegan 1995, Ruddy *et al.* 1996). Furthermore, ion exchange across the EPS matrix results in the incorporation of inorganic nutrients (Freeman and Lock 1995). The anionic nature of EPS and colloidal carbohydrates makes them highly adsorptive to cationic metals such as Zn, Co, Cu, Fe, Al and Ni (Dugan 1987, Decho 1994, Stehr *et al.* 1995). These metals are toxic to cells. However, the high metal binding affinity of EPS and colloidal carbohydrates prevents metal uptake into the cell. Similarly, chlorinated hydrocarbon pesticides and the herbicide, Diclofop (Wolfaardt *et al.* 1995), are adsorbed into the EPS matrix. This protection allows micro-organisms to grow in toxic and polluted environments such as mine drainage channels. The growth habits associated with EPS production (stalks and rosettes) may confer an

advantage to these cells in biofilms where competition for light and nutrients occur (Hudon and Bourget 1981).

1.5 Conclusion

It is apparent that EPS and colloidal carbohydrates are important in many biogeological and physical processes. Yet, very little is known of the spatial distribution of EPS or carbohydrate fractions within surface sediments. This may be partly due to the difficulties associated with non-destructive analysis and sampling of soft sediments. While the processes of primary production, respiration and denitrification can be measured at a microscale of 100 μm (Revsbech 1989a), there is no device available to quantify similar microscale variations in EPS production and the distribution of carbohydrates. Carbohydrates may influence many of the biogeological processes which vary significantly over short distances, therefore, a knowledge of the microspatial distribution of carbohydrates would improve our general understanding of sediment ecology. In addition, there is no information regarding microscale properties of the physical environment in which epipelic diatoms occur, such as sediment density or water content. This study aims to address these shortfalls by firstly, characterising the sediment environment at the scale relevant to the microorganisms using low-temperature SEM and high-resolution sectioning techniques and secondly, by conducting parallel measurements of carbohydrate concentrations. This study seeks to provide biochemical information on *in situ* carbohydrate fractions commonly measured and to assess this information in the context of heterotrophic degradation processes involving extracellular enzymes. Such information is necessary for determining the lability of EPS and carbohydrates in sediments and the likelihood of long-term effects. In addition, the possible influence of some environmental conditions on the distribution of carbohydrates on intertidal mud flats will be addressed through the use of laboratory experiments and the applications of non-destructive sampling methods.

Chapter 2

2. Materials and Methods

2.1 Site selection

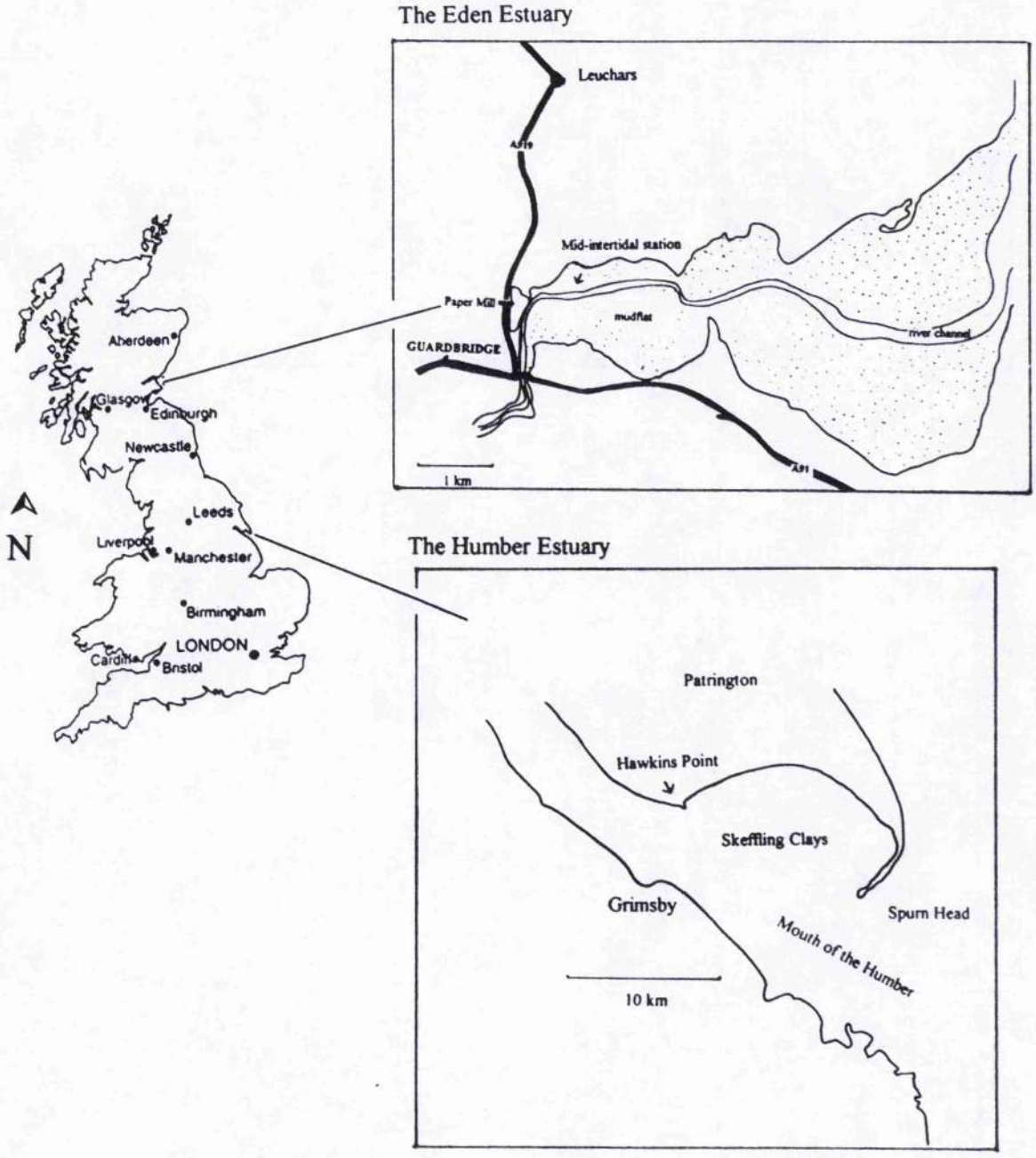
The three main field sites were selected, two on the Humber Estuary and one site on the Eden Estuary (Figure 2.1). Background data including sediment origin, nutrient concentrations, diatom migration behaviour were available for the Eden Estuary (Perkins 1960, Ahmad 1990, Mathieson and Aktins 1995). In addition, data on sediment transport, bathymetry, nutrient loading and infaunal species composition were readily available for the Humber Estuary.

2.1.1 The Eden Estuary

The Eden Estuary is the main research site used by the Sediment Ecology Research Group (SERG) of St Andrews University since it is a local estuary with reasonable vehicular access. At the time of this research, the Eden Estuary was designated as a primary investigation site for an EU environment programme, PROMAT, allowing multi-disciplinary studies to be carried out. The Eden Estuary, (56° 22' N 02° 51' E) is a small estuary situated between St Andrews and the Firth of Tay. The total area of the estuary is 8 km^2 and the majority of this area is exposed at low water with the exception of the narrow channel of the River Eden. The maximum height of water above the central channel does not exceed 5 m and the hydrodynamics are dominated by tidal currents rather than wave action. This is due to the presence of a sand bar across the mouth of the estuary and acts to reduce the force of storm waves (Wilson 1910). Fine sediments are brought into the estuary with the flood tide and are deposited on the tidal flats during the still period of high water.

The human population affecting the estuary is 11,000 and the estuary is important for local paper manufacturing, fishing and wading birds and waterfowl. The numbers of migrating birds exceeds 16000 and includes a significant percentage of the UK Shelduck, Redshank and Oyster Catcher populations. There are two sites of special scientific interest (SSSI) and an important dune system nature reserve adjacent to the Estuary. Part of the River Eden has been identified as a nitrate vulnerable zone under the EC directive 91/676/EEC (Scottish Office 1994) and the estuary is likely to be affected by eutrophication (Mathieson and Aktins 1995). A review of Scottish Estuaries showed the Eden Estuary to have the highest concentrations of total oxidised nitrogen between

Figure 2.1 The location of the Eden and Humber Estuaries in the UK.



1990-1994 (8 mg TON l^{-1}, Mathieson and Aktins 1995). 75 % of the estuary catchment land is agricultural and the high nutrient concentrations may be due to the increasing use of inorganic fertilisers in cereal crop farming.

2.1.1.1 Sediment biological communities

Sediment fauna in the Eden Estuary includes marine polychaete worms (*Nereis diversicolor* and *Arenicola marina*), the amphipod *Corophium volutator* and the gastropod *Hydrobia ulvae*. In addition, there are mussel beds on areas of fine sediments. Mussels are known to produce mucopolysaccharides (MPS) to facilitate the processing of suspended particles (Beninger and St Jean 1997). The MPS associated with mussels and other invertebrates may contribute to carbohydrate concentrations on intertidal flats however, this has not been investigated. The primary producers present in the Estuary include the seagrass *(Zostera* spp.*)*, macroalgae *(Fucus* sp., *Enteromorpha* spp. and *Ulva* spp.*)* and microalgae. *Enteromorpha* forms extensive blooms in the spring which remain until late summer on most intertidal flats. The degradation of *Enteromorpha* blooms may be a seasonal source of carbohydrates on intertidal flats. The fact the algal growth continues over the summer months suggests that the *Enteromorpha* growth may be due to high nutrient concentrations in the estuary (Mathieson and Aktins 1995, European Communities 1991). *Zostera* can be seen to fringe saltmarshes along the margins of north and south shores and *Fucus spiralis* exists only on boulders and extends up to Guardbridge paper mill. The dominant microphytobenthos are diatoms *(Bacillariophyceae)* which often form biofilms in the surface layers although cyanobacteria (mainly *Oscillatoria* spp.) are often present.

2.1.1.2 Sediment characteristics

The mud flats extend 100 - 300 metres to the Eden River channel and are bordered at the high shore with a sea-wall of boulders (Figure 2.2). The sediments of the estuary are heterogeneous and grain size increases towards the estuary mouth where 90 % of the sediments are quartz material. The upper estuary contains muds and silty sands (Figure 2.3). The organic content of the Eden sediments are within the range of other estuaries (Table 2.1) and increase with decreasing grain size (Ahmad 1990). The organic content of sediments at Baie de Marennes-Oléron was more than double the sediments of the Eden Estuary and this may be because the average particle size at Baie de Marennes-

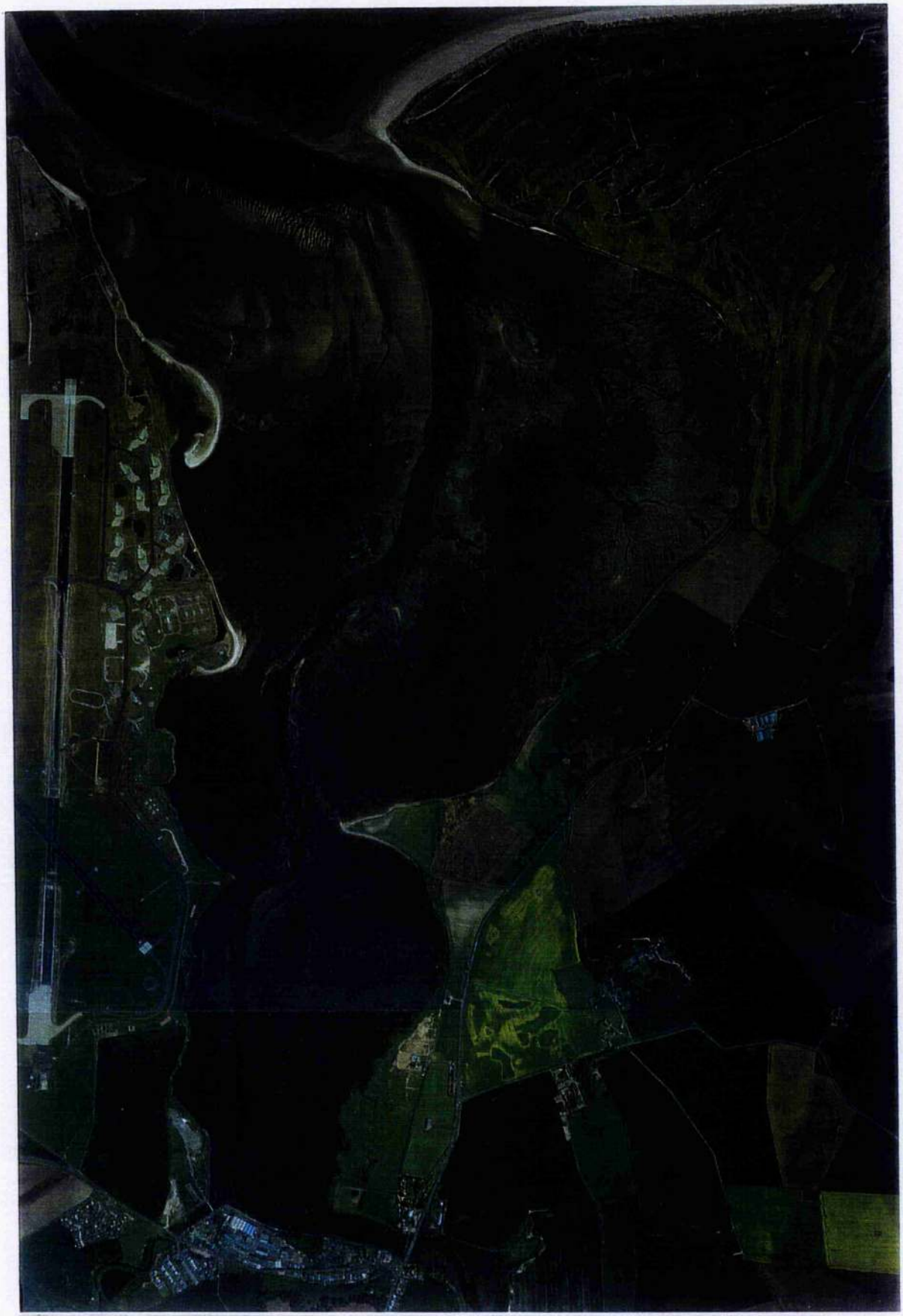


Figure 2.2 An aerial photograph of the Eden Estuary at low tide (ITE 1994).

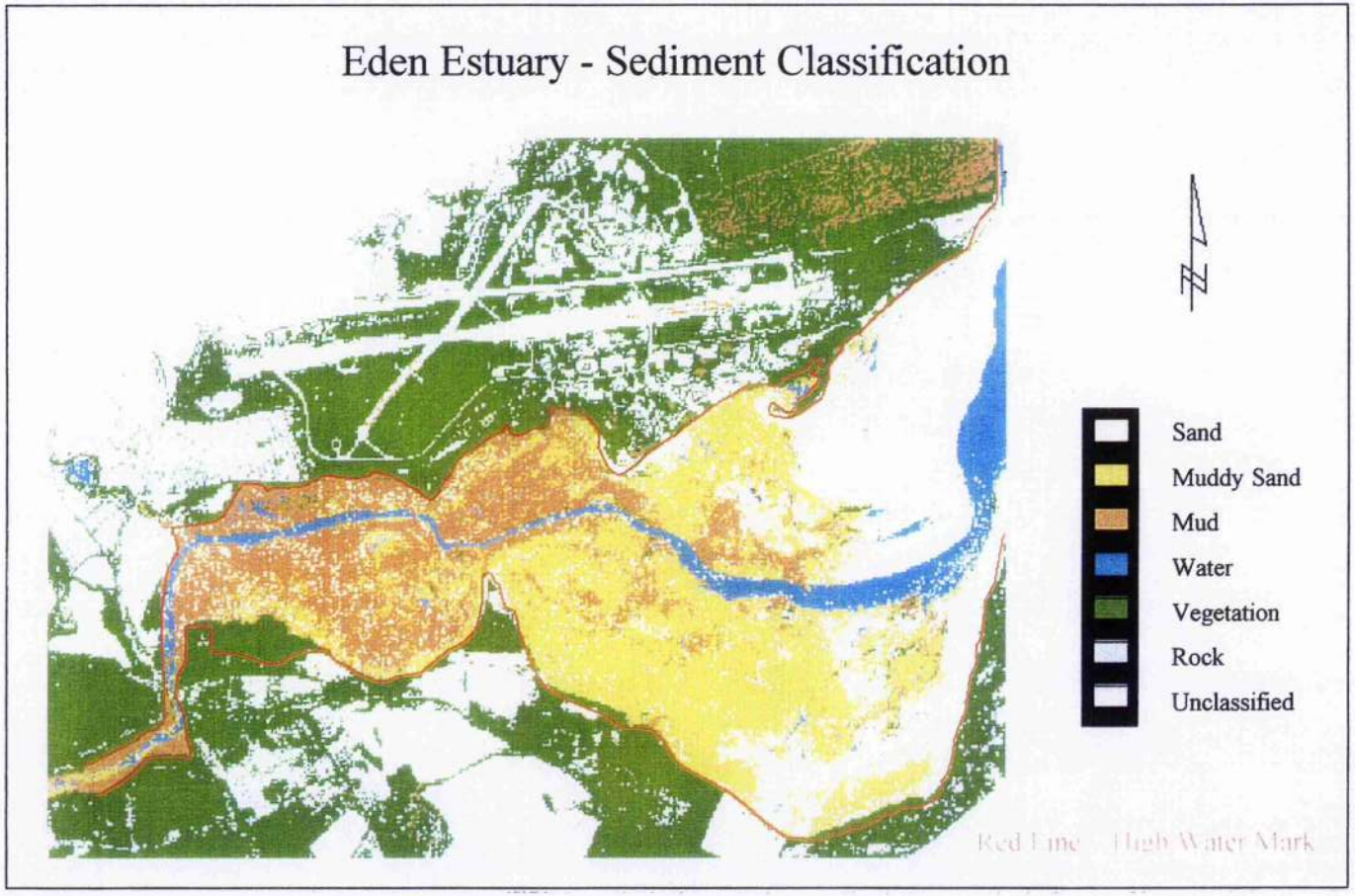


Figure 2.3 A synoptic map of sediment distribution in the Eden estuary (grateful acknowledgement to Dr Mick Yates, ITE Monkswood).

Oléron sediments was 25 μm compared with 75 μm on the Eden mud flats. There is a wide range of particle sizes at the sampling site (from < 2 μm to > 200 μm diameter) and the sediment exhibits cohesive behaviour. The sediment bed is generally flat with occasional minor drainage channels (< 0.5 m wide) in the middle and upper Estuary.

Table 2.1 *A comparison of organic content of surficial intertidal sediments (± standard deviation).*

Estuary	*% Organic content*	*Study*	*Sampling month*
Eden, Scotland	3.17 ± 0.46	Honeywill and Kelly pers. comm.	April 1997
Tay, Scotland	2.68 ± 0.02	Honeywill and Kelly pers. comm.	April 1997
Montrose Basin, Scotland	0.1-10	Ahmad 1990	1990
Humber, England	2.1 ± 0.04	Black pers. comm.	April 1995
Marennes- Oléron Bay, France	6.7 ± 0.14	Honeywill and Kelly pers. comm.	April 1997

2.1.2 The Humber Estuary

The Humber Estuary is much larger than the Eden, the width at the mouth is 8 km and according to the National Rivers Authority (1993), it is the major UK freshwater input into the North Sea. The range of a spring tide is 7 m with current speeds of 2-3 m s^{-1} (Gameson 1982). Therefore, the estuary is well mixed with little vertical stratification (Black 1996). However, there is cross-stratification between the North and South shores due to an asymmetric tidal inundation pattern. Saline flood waters enter predominately on the north shore whereas freshwater discharges mainly along the south shore. The River Humber has high concentrations of suspended sediments and supplies 200,000 tonnes of sediment to the estuary but this passes out into the North Sea as a plume layer (Pethick 1988). The sediments which form the tidal flats are believed to originate from erosion of cliffs on the Holderness Coast (north of the Humber Estuary) and be transported into the estuary during the flood tide (Black pers. comm.).

The sites on the North coast of the Humber Estuary were Hawkins Point (53° 38' N 0° 3' W) and Skeffling Clays, (53° 38.5' N 0° 4.25' E). Skeffling Clays consists of approximately 56 km^2 of intertidal mud and sand flats. Two sites were sampled, Site A (100 m from shore) and B (400 m from shore). These sites could be sampled from the

shore and were connected by an anchored wooden walkway (Figure 2.4 a, b). At site A there was an additional 9 metre transect.

2.1.2.1 Biological components

Hawkins Point and Skeffling Clays are similar in their biology, a salt marsh containing *Spartina townsendii*, *Halimione* and *Puccinellia* fringes the mud flats on the North shore (Brown 1996). The sediments are highly active and contain a variety of infaunal species including; *Nereis diversicolor*, *Hydrobia* spp., *Macoma balthica*, *Pygospio elegans* and to a lesser extent *Corophium volutator* (Morris 1994). *Macoma balthica* was the most common animal on the Skeffling mud flats (Davey and Partridge 1998). Smaller *Macoma* were found at the high and low-shore with larger sized animals at mid-shore. There was greater bioturbation at site B than at site A due to the increased size of the *Macoma* (Davey and Partridge 1998). Bioturbation was evident as feeding activity, the presence of faecal pellets and through the construction of burrows. The main primary producers on the mudflats are microphytobenthic communities containing *Euglena* spp. and several genera of diatoms (*Bacillariophyceae*). There were also patches of the macroalgae, *Enteromorpha* spp.

2.1.2.2 Sediment characteristics

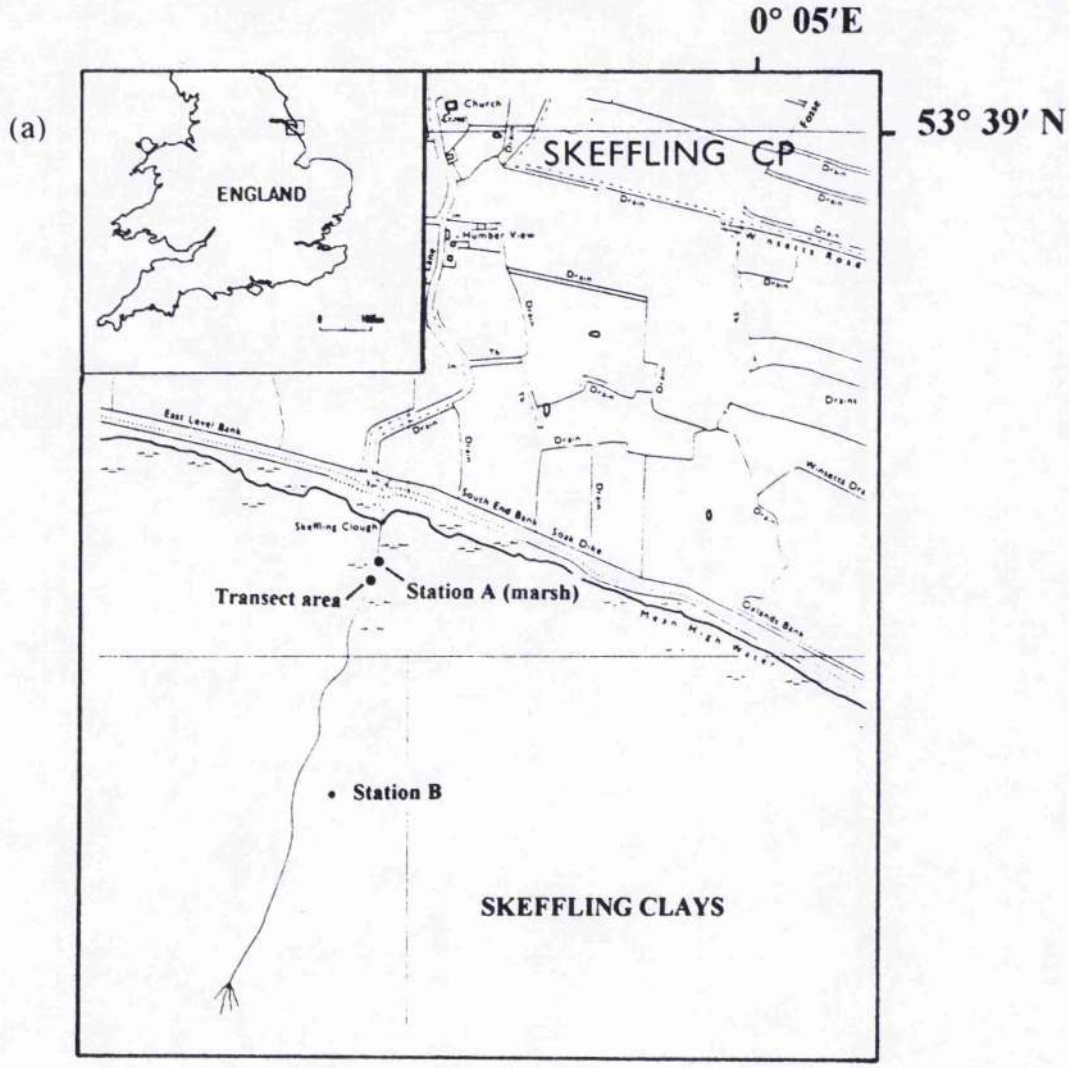
Boulder clay deposits lie underneath the tidal flats at a depth of 0.6 - 4 m and influence channel formation on the over-lying sediments. There was a gradient of particle sizes on the mud flats with finer particles at the topshore and coarser grains towards the central channel (Figure 2.5). At Skeffling site A, the sediment bed was characterised by planar areas and other areas where small ridges and runnels, reaching 10 cm in height, were found. There was a non-consolidated surface layer (approx. 2 cm) which was comprised of fine sediments. Underlying this layer were coarse, black, sandy muds and in some areas, patches of *Macoma balthica* shells. At site B, the sediment composition appeared to be homogenous within the upper 10 cm. At both sites there were many low lying areas where surface pools remained throughout the emersion period.

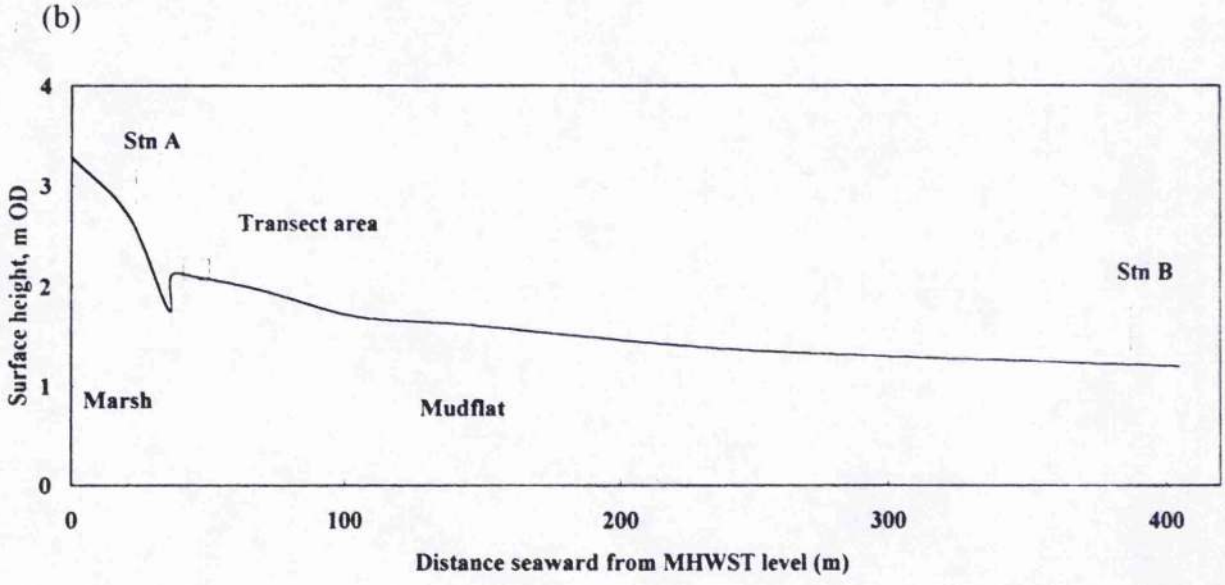
2.2 The quantification of carbohydrates

2.2.1 Introduction

Carbohydrates occurring in sediments were quantified using a spectrophotometric assay (Dubois *et al.* 1956). This was chosen above other methods (see Chapter 1) since it is least affected by other compounds and it is rapid and simple (Handa and Mizuno

Figure 2.4 Detail of the Skeffling sampling site on the Humber Estuary showing the location of a short transect in the upper-intertidal region





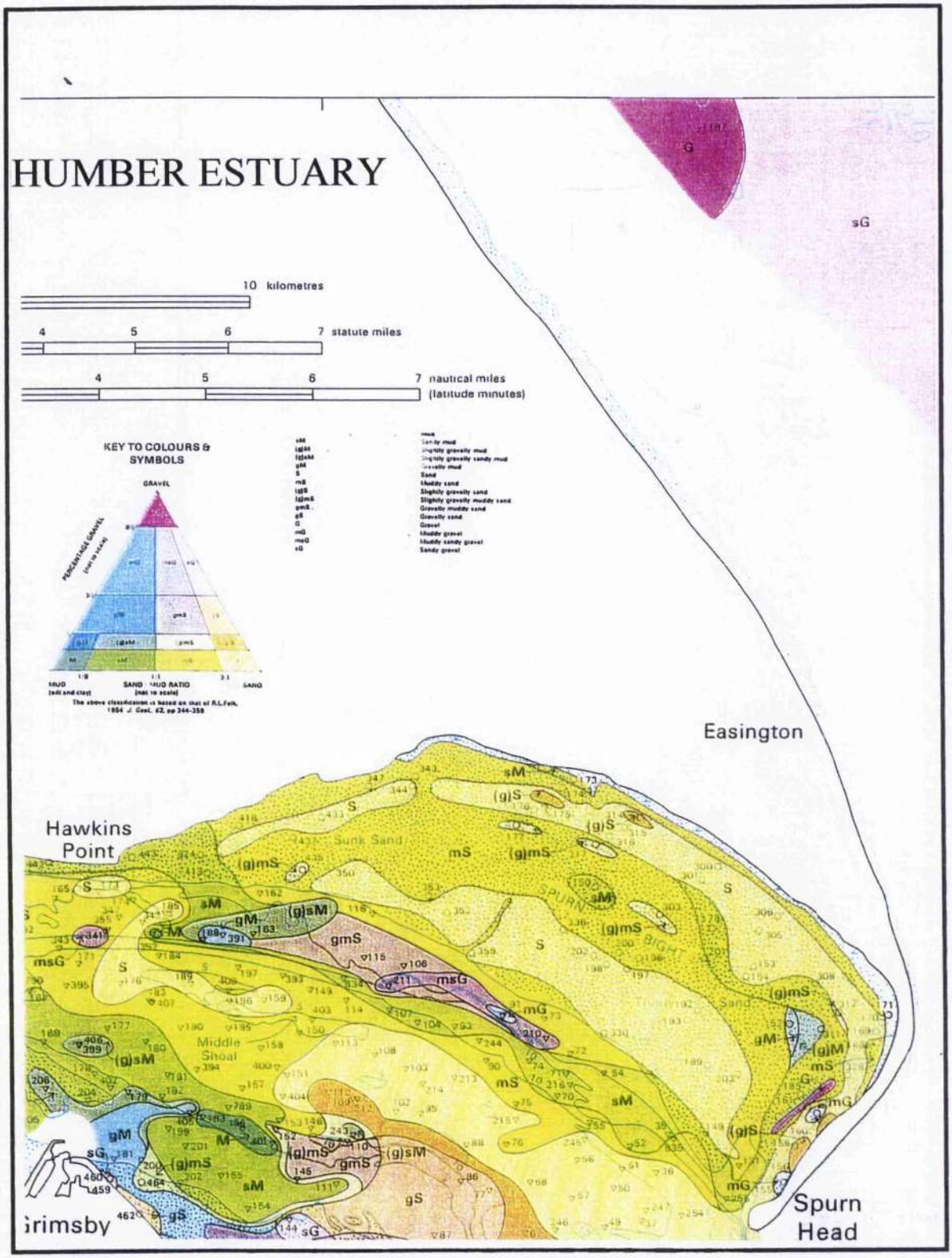


Figure 2.5 Distribution of seabed sediments for the Humber estuary with particular reference to the intertidal area within Spurn Bight (from British Geological Survey maps SPURN Sheet 53°N-00° and HUMBER-TRENT Sheet 53°N-02W°, 1:100 000).

1973). The assay is based upon the reaction of a carbohydrate-phenol mixture to the addition of concentrated sulphuric acid (H_2SO_4). The heat from this exothermic reaction, results in the production of colour compounds (furfurals and hydroxymethylfurfurals from pentoses and hexoses) which can be quantified against a series of glucose standards (Liu *et al.* 1973). Care should be taken to ensure the acid is added directly onto the carbohydrate-phenol mixture since heat required for the assay may be dissipated if the acid strikes the test-tube wall. Peak absorbancies of the coloured compounds were found to occur between 480 - 490 nm, as described in Dubois *et al.* (1956) (Figure 2.6). There was a negligible difference in the absorbance values when read between 480 and 490 and a standard wavelength of 486.5 nm was chosen for all absorbance readings.

2.2.2 Testing and modifying the assay protocol

The assay was performed on 3 sets of glucose standards (3 - 200 $\mu g\ ml^{-1}$). Although the concentration trend was clear, there was relatively high variation between replicates. Therefore, the glassware was soaked in Decon detergent for 12 - 24 h, then pre-treated with 10 % v/v HCl overnight and washed in distilled H_2O. This treatment resulted in more reproducible results.

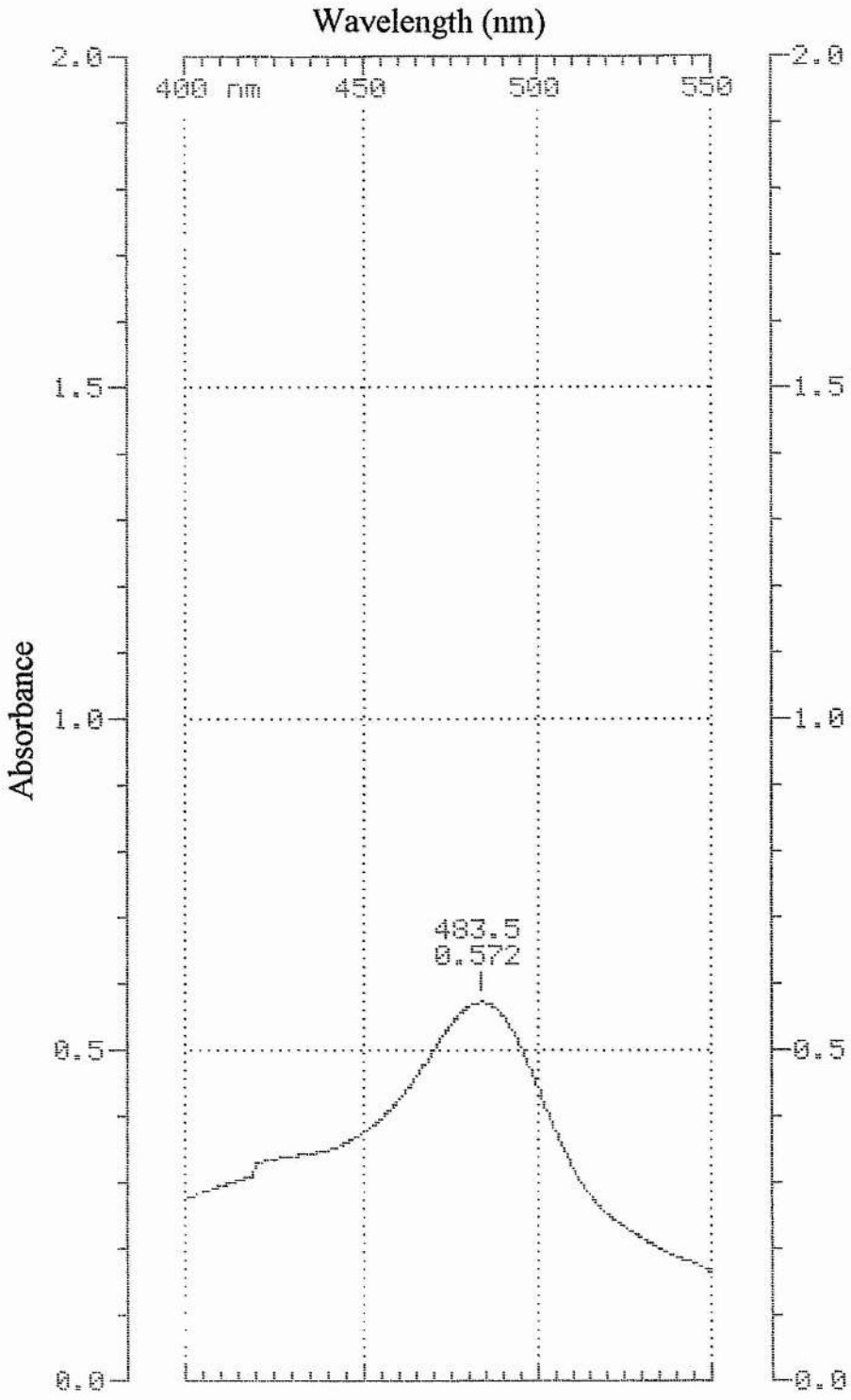
2.2.2.1 Assay development time

Dubois *et al.* (1956) suggested an incubation period of 20 - 30 minutes, following the addition of the assay reagents. However, there has been no investigation into the effect incubation time may have on colour development. Therefore spectrophotometric readings were taken at 15, 20, 35, 50, 60 and 90 minutes and significant increases in absorbance values were shown to occur over this period. (Jonckherres test for ordered alternatives, Siegel and Castellan 1988). A stepwise analysis over the 90 minute period found the increase in absorbance values to occur between 15 and 35 minutes. Thereafter, the absorbancies remained stable to the end of the experimental period (90 min). However, the assay solution may be hygroscopic and should not be kept for long periods.

2.2.2.2 Sample size

The volume of extraction solution which was recommended for carbohydrate determination was 2 ml (Dubois *et al.* 1956, Underwood *et al.* 1995).

Figure 2.6 The absorbance peak of glucose in the Dubois assay



However, epipelic diatoms live and migrate within the upper few millimetres of intertidal sediments and microspatial analytical techniques are required to understand carbohydrate production within this community (see Chapter 1). Therefore, the possibility of scaling down the carbohydrate assay to quantify small samples was examined.

Underwood *et al.* (1995) have shown a linear relationship between sample weight and the concentration of carbohydrates. However, they did not reduce the volume of extractant and reagents used. Therefore, a series of glucose standards were prepared and assayed using each of three protocols; (1) 2 ml sample, 1 ml phenol and 5 ml acid, (2) 0.4 ml sample, 0.4 ml phenol and 2 ml acid and (3) 0.2 ml sample, 0.2 ml phenol and 1 ml acid.

The absorbance values of each set of standards increased linearly with glucose concentration. However, there was a significant difference between the slopes of the large volume standards (2 ml sample) and the lower volumes (0.2 and 0.4 ml, Figure 2.7a). It was unclear whether the higher absorbances measured in the larger volume assays were due to the greater volumes used or to changes in the proportion of glucose to phenol (i.e. 2 parts glucose, 1 phenol, 5 sulphuric acid or 1 part glucose, 1 phenol and 5 parts sulphuric acid). Therefore, the comparison of different volumes was repeated using equal proportions of glucose to phenol (1 ml glucose, 1 ml phenol and 5 ml acid).

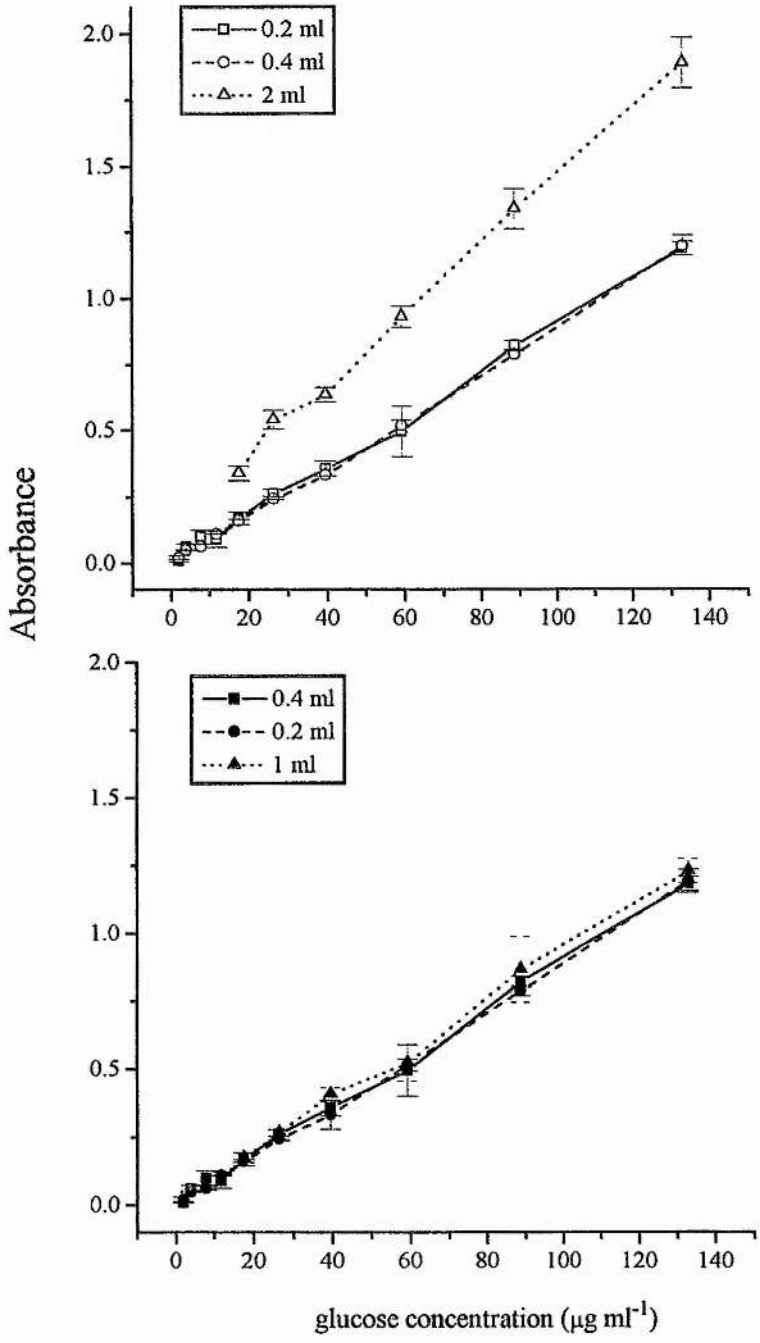
There was no difference between the standard curves using 1 ml, 0.4 or 0.2 ml of glucose (Figure 2.7b). Therefore, variability between the absorbances shown in Figure 2.7a was due to changes in the proportion of glucose to phenol. The absorbance values from 1:1:5 assays can be converted to the approximate absorbance values of the assays using 2:1:5 proportions, by multiplying by a factor of 1.8 ± 0.1 (SD, $n = 18$).

This study has demonstrated that the carbohydrate assay reagent volumes may be reduced without altering the accuracy of the assay. However, the Dubois assay is influenced by the proportion of glucose to phenol. Furthermore, absorbance values which exceed 1.1 are less reliable and therefore should not be used.

2.2.3 A protocol for the measurement of colloidal carbohydrates

From the previous results, a standard protocol was produced for the quantification of colloidal carbohydrates. 300 µl distilled H_2O was added to a known

Figure 2.7 (a) Comparison of 3 sets of glucose standards (b) comparsion of different volumes, using the same proportion of glucose to phenol (bars = St.Dev., n = 3)



volume of sediment to extract the colloidal carbohydrate fraction. After mixing, the sample was stored at 20°C for 15 min. The samples were then centrifuged at 2500 g for 15 min and 200 µl of the supernatant was recovered and placed in an acid washed glass test tube. To this, 200 µl of 5 % w/v phenol was added (the remaining 100 µl of supernatant was disposed of and the sediment pellet was frozen at -20°C for bulk carbohydrate analysis). The sample and phenol were mixed and 1 ml of concentrated H_2SO_4 was added. After 35 min at room temperature, the solutions absorbancies were read (Cecil 3000 scanning spectrophotometer at 486.5 nm).

2.2.4 A protocol for the measurement of bulk carbohydrates

After the sediment pellet (from the colloidal carbohydrate extraction) has been frozen and lyophilised, the dry weight was measured and the dry mass concentration calculated. The pellet was then homogenised and 2 mg of sediment was added to 200 µl of distilled H_2O the assay reagents were added as before. After 35 min at room temperature the solutions were centrifuged and the absorbance of the supernatant was read.

2.2.5 The calculation of carbohydrate concentration

2.2.5.1 Colloidal carbohydrate

(i) The absorbance value was converted to concentration (µg glucose equivalents ml^{-1}) using the regression equation of the glucose standard curve;

$$x_1 = (y - a) / b$$

x_1 = glucose concentration (µg ml^{-1})
y = absorbance reading
a = intercept
b = slope

(ii) The concentration was multiplied by the total extraction volume, i.e. if 200 µl of sample was taken from a 300 µl volume of sediment slurry, the concentration was multiplied by 1.5.

(iii) This value was then divided by dry weight of the sediment pellet (mg) to give a concentration of µg glu. equ. mg^{-1} sediment dry weight.

Example:

$$x_2 = (x_1 \ V) / d_w$$

x_1 = glucose concentration ($\mu g\ ml^{-1}$)
x_2 = sample concentration (glu. equ. mg^{-1} sediment dry weight).
V = total extraction volume (ml)
d_w = sediment dry weight (mg).

2.2.6 Repeated extractions

The measurement of carbohydrates in sediments is important for studies of carbon flux and sediment stability. In order to conduct comparative studies it is necessary have similar methods and accurate measurements. For this reason, the method of colloidal carbohydrate extraction was examined. Colloidal carbohydrates remain in the sediment after the extraction process. Underwood *et al.* (1995) detected colloidal carbohydrates in sediment samples after 6 H_2O extractions. However, they found that 50 % of the colloidal carbohydrates in the sample were removed in the first extraction. The purpose of this study was to repeat this experiment and determine the levels of carbohydrate extracted.

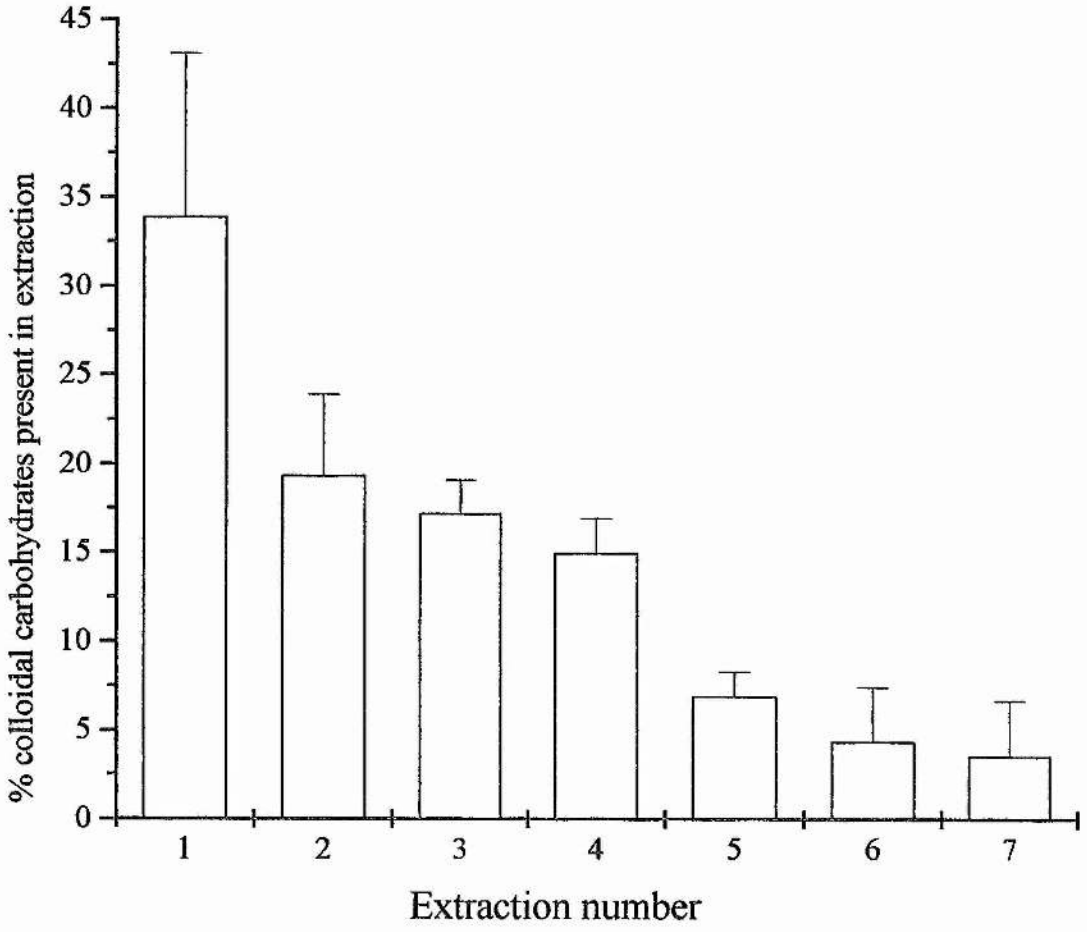
2.2.6.1 Methods

A series of 7 repeated extractions were performed on sediment cores (9 cm x 30 cm) collected from the Eden Estuary (56° 22' N 02° 51' E) 1 h after low tide. The cores were sub-sampled using a syringe core (1.8 x 0.4 cm) and the wet weight recorded. 2 ml of distilled H_2O was added to the sediment to extract colloidal carbohydrates. The sediment and water were vigorously mixed and incubated at 20°C for 15 minutes. This was followed by 10 minutes centrifugation at 2000 g after which 200 µl of the supernatant was removed for carbohydrate quantification. The remaining supernatant was discarded and another 2 ml of distilled water added and protocol continued as before. A total of 7 colloidal carbohydrate extractions were carried out on 3 sediment samples.

2.2.6.2 Results

The carbohydrate data was log transformed to achieve a normal distribution required for parametric statistics. There was a significant decrease in colloidal carbohydrate concentrations over 7 extractions (one-way ANOVA, $P = 0.00$, $n = 21$, Figure 2.8). Approximately 35 % of colloidal carbohydrates were removed in the first extraction (assuming the total of 7 colloidal carbohydrate extractions to equal 100 %).

Figure 2.8 Percentage of colloidal carbohydrates extracted (error bars = 95% confidence limits)



2.2.6.3 Discussion

One extraction removed 35 % of colloidal carbohydrate from the sediment. Colloidal carbohydrate concentrations then decreased with subsequent extractions. The total colloidal carbohydrate content may be estimated by undertaking repeat extractions and using a cumulative value. However, a small percentage was still detected in the seventh extraction suggesting that colloidal carbohydrates may never be fully extracted. This would suggest that colloidal carbohydrates detected in further extractions may not have been part of the original colloidal fraction but may have diffused into the extraction solution from particulate matter to maintain an equilibrium. In addition, the concentration of colloidal carbohydrates has been shown to increase when saline extraction solution was replaced by EDTA (Underwood *et al.* 1995). EDTA extractions may not be representative of the colloidal carbohydrate fraction but may be carbohydrates from the capsules or sheaths of microbial cells (Decho 1990, Underwood *et al.* 1995). Capsular carbohydrates are firmly attached to cells and are highly cross-linked with metal ions. The EDTA chelates metal ions and breaks the capsules apart (Decho 1990). Therefore, the first water or saline extraction is an appropriate indicator of colloidal carbohydrate concentrations present in sediments and available for microbial metabolism. The percentage of colloidal carbohydrates removed in the first extraction was approximately 15 % lower than the value previously reported (Underwood *et al.* 1995 Figure 2.9). This may be due to the hydration state of the carbohydrates measured (Decho 1990). However, the differences in these two studies suggest that the total colloidal carbohydrate content of a given sediment cannot be accurately estimated using the first value. However, the first extraction value is valid for comparative carbohydrate studies.

2.3 Collection of sediment samples

Throughout the duration of this study, the method of sediment sampling varied. Initial sediment samples were collected using 9 cm (id) cores which were sub-sampled in the laboratory using 25 mm (id) syringe cores. Alternatively 25 mm (id) syringe cores were used to collect sediment directly in the field and either frozen on site or upon return to the laboratory. Sediment samples were also collected following *in situ* freezing of the mud flat surface using liquid nitrogen (LN_2) vapour and then LN_2 (Wiltshire *et al.* 1997). The sampling method employed is described in the introduction to individual studies.

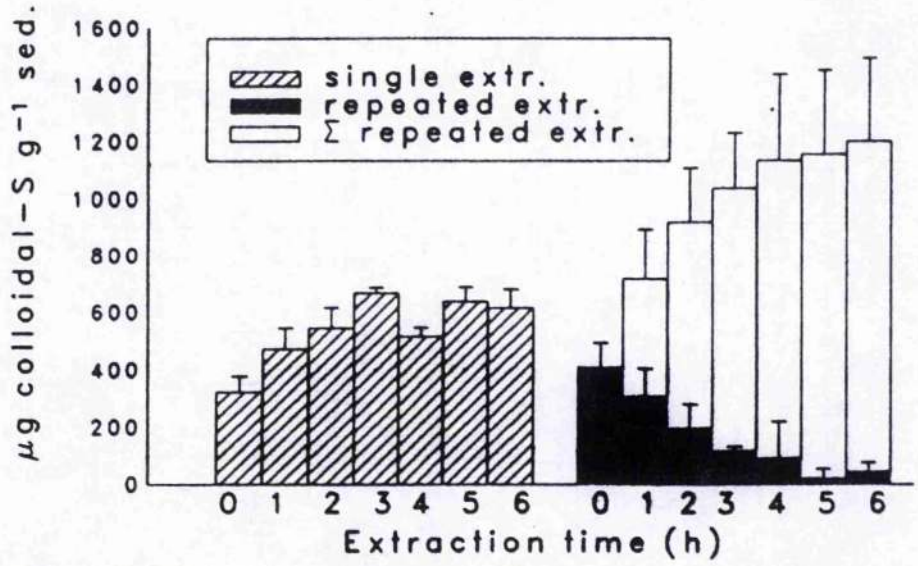


Figure 2.9 **Effect of increasing incubation time (single extractions) on colloidal-S carbohydrate concentrations compared to repeated extractions into fresh supernatant. Bars—mean + SD; n = 5. Samples—100 mg dw of sediment, incubated at 20°C** (from Underwood *et al.* 1995).

2.4 Fine scale sectioning of sediments

2.4.1 Introduction

Fine scale resolution of sediments is a crucial requirement for the study of biofilm communities. The surface of intertidal mud flats is an interface between sediment and water or air. This interface is characterised by steep chemical gradients of oxygen, sulphide and nitrite concentrations as well as light penetration. These parameters have been measured using microsensors (Revsbech *et al.* 1981, Kühl and Jørgensen 1994). However, no such device is available for microscale studies of sediment carbohydrates, chlorophyll or physical parameters, such as water content and sediment mass concentration. Therefore, to gain an insight into the microscale variation of these variables, fine scale sectioning of surface biofilms was carried out.

2.4.2 A protocol for fine scale sectioning of sediment

Sectioning was carried out using a 'Leitz Kryomat 1703' microtome at a temperature of -40°C. Frozen sediment cores (25 mm diameter) or cryolander blocks were first cut down to 20 mm in height (the maximum height accommodated under the knife) using a lapidary saw (M. L. Beach Products). This saw can also be used to allow depths greater than 20 mm to be analysed. The sediment cores were transferred in liquid nitrogen to the pre-cooled stage of the microtome and secured with distilled water.

200 μm slices were chosen since this thickness provided sufficient material for the carbohydrate assay, whilst remaining the most detailed depth analysis of carbohydrates to date. The first 200 μm section includes raised areas if the core surface is uneven. The sediment sections can then be analysed using biochemical tests. For carbohydrate and mass concentration measurements, sections were transferred to a pre-weighed Eppendorf tube using a fine brush (Miles pers. comm.). The Eppendorf tube contained 300 μl of distilled water to extract the carbohydrate. The sediment dry mass concentration, C, of the section was calculated.

$$C = M_s / V$$

C = dry mass concentration (also known as dry mass density, ρ_d)
M_s = mass of solids (dry weight, kg)
V = total section volume (m^{-3}) (Mehta 1991)

2.4.3 The use of filter paper for the transfer of sediment sections

When sediment sections are transferred from the knife to an Eppendorf tube using a brush, small amounts of sediment may be retained on the brush (Wiltshire pers. comm.) and may lead to errors in the calculation of mass concentration. Therefore, the mass concentrations from sediment sections transferred using a fine brush were compared with those transferred using filter paper. Secondly, the possibility of using the filter paper method to transfer sediment sections for carbohydrate analysis was examined.

2.4.3.1 Experimental procedure

10 sediment cores (25 mm diameter) were sectioned into 200 μm thick sections. Sections from the first 5 cores were transferred from the knife to Eppendorf tubes using a fine brush. Sections from the second 5 cores were transferred to the Eppendorf tubes using filter papers (Whatman GF/C, 47 mm dia., 0.2 μm pore size). 40 sections were transferred using each method and the wet weight of each section was measured before being frozen and lyophilised. Following this, the dry weights were recorded and the wet and dry mass concentrations were calculated.

2.4.3.2 The use of filter papers for transferring carbohydrate samples

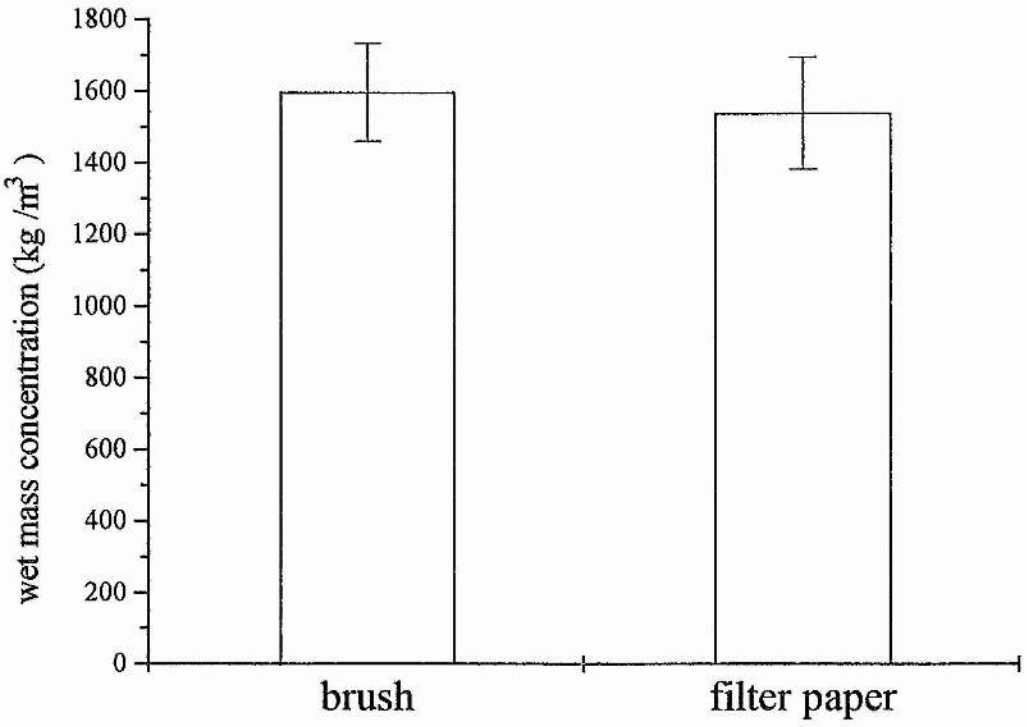
In order to use the filter papers in sediment carbohydrate assays it was necessary to determine if they contained carbohydrates. Five filter papers were added to 5 test tubes containing 200 μl of distilled water and the carbohydrate contents were determined and compared with 5 controls containing only distilled water. Positive carbohydrate readings were found (6.5 μg glu. equ. ml^{-1}) in the tubes containing filter papers. Therefore, two methods for removing sugars from the papers were examined. Firstly, 5 filter papers were treated with 30 % v/v hydrogen peroxide and secondly, 5 with 30 % v/v hydrochloric acid (HCl) and 5 controls in distilled water. The filter papers were treated for 1 hour and then dried at 100°C for 12 hours before being assayed for carbohydrates.

2.4.3.3 Results

2.4.3.3.1 The use of filter papers for mass concentration measurements

No significant difference was found between wet and dry mass concentrations of the sections removed using paint brushes or using filter paper ($P = 0.22$, one-way ANOVA, $n = 40$, Figure 2.10). There was a significant increase in wet mass

Figure 2.10 Wet mass concentration data derived using brush and filter paper (error = 95 % confidence limits)



concentration with depth in both groups using a brush or filter paper (P = 0.05 and 0.000 respectively, one-way ANOVA, n = 40, Figure 2.11). However, this increase was more clearly defined in the filter paper group. Furthermore, there was less within-group variation in the filter paper group.

2.4.3.3.2 The use of filter paper in the collection of sections for carbohydrate analysis.

The microfibre filters tested positively for carbohydrates which were not removed by pre-treatment, Figure 2.12. The filters treated with HCl had slightly lower carbohydrate readings than papers which were not treated but were still within the range of natural values. Papers treated with hydrogen peroxide had higher values than untreated papers and may have been contaminated by material which was previously in the tube. Furthermore, the use of hydrogen peroxide for the hydrolysis of organic matter in sediments has been questioned since laboratory studies on intertidal sediments found hydrogen peroxide treatment to result in higher colloidal carbohydrate extractions than untreated sediments (section 4.1.3.1, p91). This suggests that hydrogen peroxide breaks larger carbohydrate structures into colloidal carbohydrates rather than fully hydrolysing and removing them.

2.4.3.4 Discussion

Although there was no significant difference in the wet or dry mass concentration values between the brush and filter paper method, the data from sections transferred using filters showed less variation than those transferred using a brush. Furthermore, the absorbent nature of the filter paper aids the collection of material from the knife. Therefore, the filter paper method is preferable if the wet or dry mass concentration is the parameter of interest.

When the absorbance spectra of the filter paper samples were examined, a large absorption was found to cover the area between 350 - 550 nm and it is unlikely that this represents carbohydrates since they absorb within a narrow wavelength band (approximately 480-490 nm). Therefore, the filter papers may not contain sugars but react in some way to the carbohydrate assay. Therefore, the sediment sections for carbohydrate analysis should be transferred from the knife using a brush.

Figure 2.11 A comparison of wet mass concentration data from two methods (error bars = 95% confidence limits)

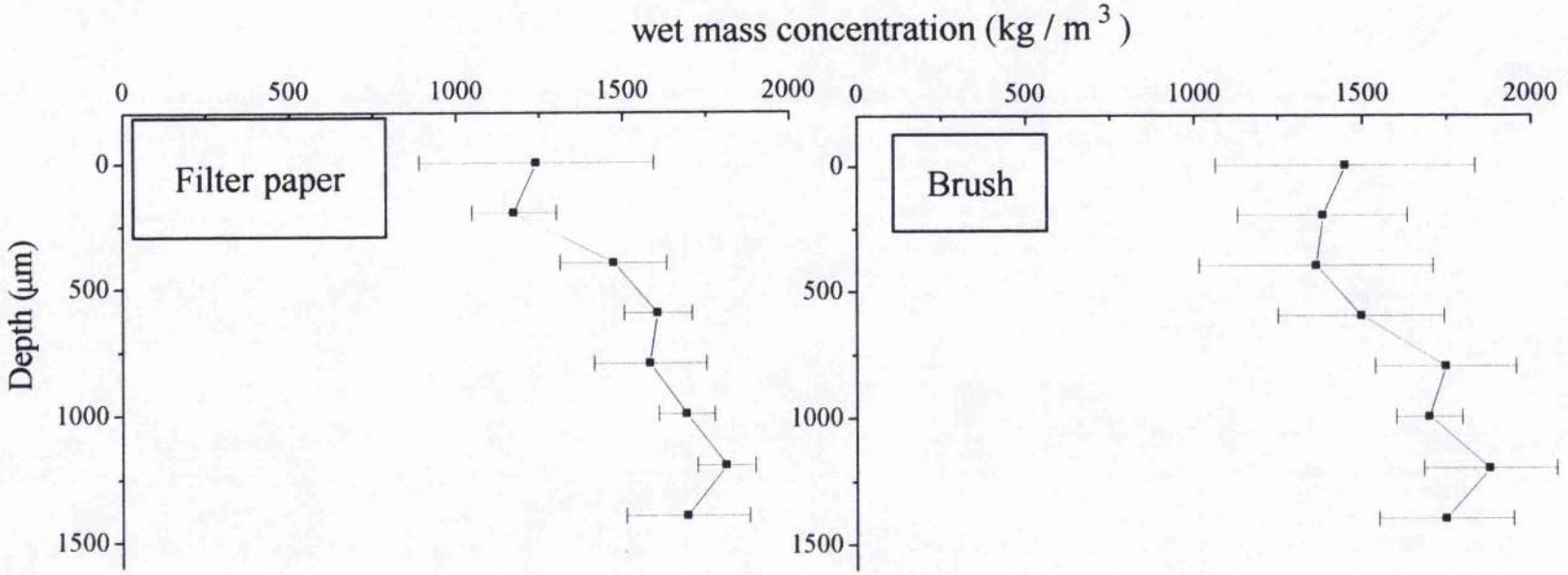
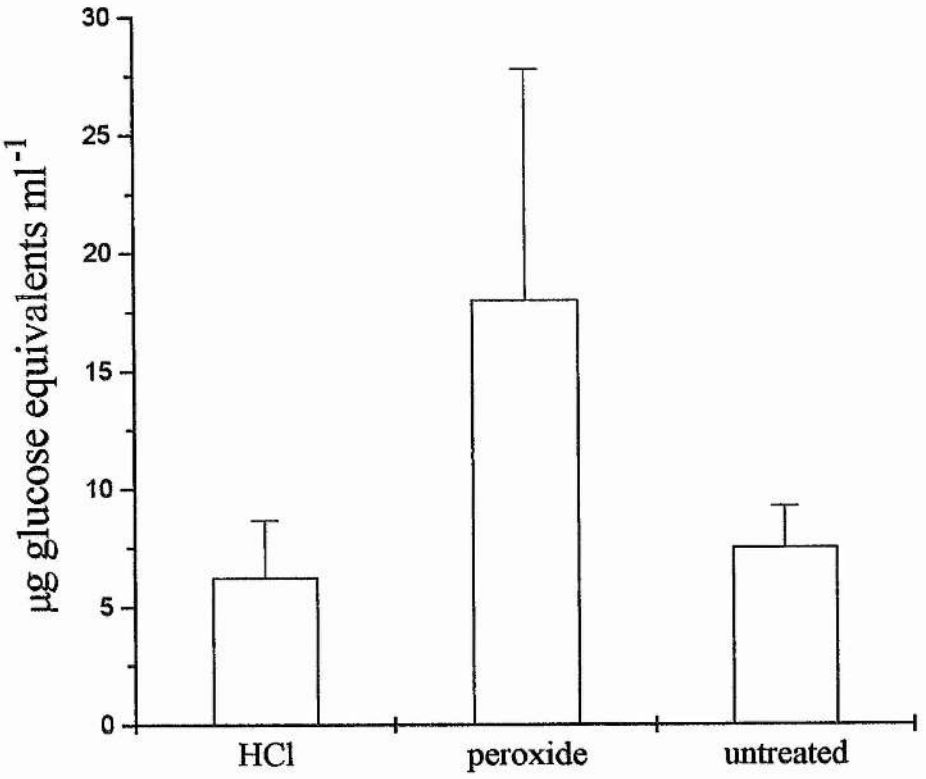


Figure 2.12 Comparison between methods to remove carbohydrates (error bars = 2 x SEM, n = 5)



2.5 A protocol for the examination of diatom assemblages using the light microscope

Diatoms are classified by size, shape, the ornate pattern of striations, pores and processes on their silica frustules. Therefore, the light microscope remains the most important tool in diatom taxonomy. The microscope used in the course of these studies was a Zeiss research microscope with facilities for phase contrast and photography (35 mm camera and videocopy processor).

2.5.1 Isolation of *in situ* diatom cells

Following the protocol of Eaton and Moss (1966) cells were isolated from surface sediment by placing a double layer of lens tissue paper (2 cm x 2 cm) onto the sediment for 1 - 2 hours. The diatom cells migrate through the tissue paper as part of their migration cycle. The upper layer of the lens tissue is peeled off and placed in vial containing 2 ml of 2.5 % v/v glutaraldehyde (with buffers 0.2 kmol m^{-3} of di-sodium hydrogen orthophosphate and sodium dihydrogen orthophosphate to preserve chlorophyll and other pigments). The lens tissue is then lacerated (using mounting needles) to dislodge the cells and filtered through a 0.1 mm filter. This separates the cell suspension from the tissue fibres and any remaining sediment. Cell counts were made using a counting chamber (Improved Neubauer, Weber Scientific).

2.5.2 Slide Preparation

In order determine the species of diatoms present, the organic components of the cells must be removed. Therefore, the cell suspension was treated with 2 ml of potassium permanganate and stored at 20°C overnight. This solution was then hydrolysed by adding 6 ml of concentrated hydrochloric acid (HCl) and heating at 70°C for 2 hours. The solution was centrifuged at 2500 g for 25 minutes and the supernatant was discarded. The pellet was re-suspended in distilled water and centrifuged again, this was repeated 6 times. The pellet was then re-suspended in distilled water and a drop placed on a polished coverslip and left to dry in a dust free environment for 48 hours. Once dried the coverslip is placed cell-side down onto a slide carrying a drop of Naphrax mountant. The slide is placed on a hotplate until the mountant begins to bubble. At this point the slide is placed on a cold surface and pressure is applied to the top of the cover slip to expel bubbles. The mountant will set after a few minutes, if this is not the case the

slide should be re-heated. Slides were then examined and 300 valves identified for accurate species composition.

2.6 Scanning electron microscopy

Low-temperature scanning electron microscopy (LTSEM) was used to provide qualitative visualisation of the sediment samples. The protocol followed that described by Paterson (1995). Samples of surface sediment were collected using 30 mm aluminium foil strips which were inserted 5 mm into the sediment bed and pushed parallel to the surface . The strips were then carefully withdrawn and plunged into liquid nitrogen and stored this way until viewed. In addition, sediment for LTSEM examination can be collected using a cryolander which quickly freezes surface sediment initially by cold nitrogen vapours, followed by liquid nitrogen (Wiltshire *et al.* 1997). The specimens were viewed using a JEOL JSM 35CF SEM, modified for cryogenic examination (OXFORD CT1500), at a temperature of -180 °C. Ambient temperature SEM normally involves drying the sample prior to viewing. Since biological material is usually associated with water, low-temperature observation of sediment samples causes less structural disruption of the sample (Paterson 1995, Défarge 1997). This is especially important in the observation of EPS since these polymers are hydrated upon secretion and have a structural role in sediment stability. The elemental composition of the sample was examined using energy dispersive x-ray analysis (LINK AN10000). Photographs were taken using an attached camera (MAMIYA). The 120 mm 125 asa, black and white negatives (Ilford FP4 plus) were printed in the laboratory.

2.7 Particle size analysis

The sediment for particle size analysis was prepared by adding 10 ml of H_2O to 1 g of sediment and then adding 25 ml of 30 % v/v hydrogen peroxide (H_2O_2) to the sediment slurry. This removes organic material thereby cleaning the sediment particles. The mixture was gently heated until approximately half the H_2O_2 evaporated then 25 ml of H_2O was added and the evaporation was repeated. Once cooled, three drops of 0.1% w/v Calgon (sodium hexametaphoshate) was added to de-flocculate the solution (Black pers. comm.). Two ml of the prepared sediment slurry was sonicated for 30 s and then analysed using a Coulter Laser Particle Sizer LS100. This provides a spectrum of particle diameters in the sample and particle size statistics such as mean and mode.

2.8 The measurement of carbohydrates in association with deployments of Gust's Microcosm

2.8.1 Introduction

Sediment erosion occurs when hydrodynamic forces exceed the strength of the sediment, causing particles to be suspended in the water column. The point at which erosion occurs will depend on many variables including the composition of the sediment and current velocity. Laboratory studies on abiotic sediments show erosion thresholds to be predictable for given particle diameters (Miller *et al.* 1977). However, natural sediments erode in a largely unpredictable manner (Paterson and Daborn 1991).

Several studies have demonstrated the influence of biological growth and activity on sediment stability. *In situ* sediments with a visible diatom biofilm require a greater eroding force than those without (Yallop *et al.* 1994, Sutherland 1996). This is due to the secretion of extracellular polymeric substances (EPS) by epipelic diatoms, which form a matrix in the surface photic zone. In addition, cyanobacterial trichomes have been shown to stabilise sediments by creating a network of filaments in the upper layer (Yallop *et al.* 1994). When sufficient force is applied to erode such sediments, large strips of sediment often tear off into suspension revealing substantially weaker sediments underneath (Sutherland 1996, Paterson and Black *in press*). Furthermore, the size of particles eroded from diatom biofilms increases from individual grains to large flakes with increasing biofilm age (Sutherland 1996). Although carbohydrate-rich polymers are known to play an important role in the biostabilisation of sediments (Yallop *et al.* 1994, Sutherland 1996, Paterson 1997), little work has been done to specify the activity of such carbohydrates in the erosion process.

The objective of this study was to conduct an *in situ* investigation to monitor the levels of carbohydrates during the process of erosion, specifically to answer the question; do carbohydrates enter the water column at a discernible point in the erosion process? The amount of carbohydrate detected during erosion will depend on the strength of the sediment and the original concentrations in the sediment (Sutherland 1996).

Sediment erosion was induced through the use of a portable erosion microcosm (Gust 1991). The cohesive strength meter (CSM, Paterson 1989, Black and Paterson 1997) is a more portable device however, it was not suitable in this study since water samples for carbohydrate analysis could not be withdrawn from the chamber during erosion. Large annular flumes have been developed for sub-tidal deployment (Amos *et al.* 1992b) and a prototype has recently been developed for intertidal use (Widdows *et al.* 1998, see a review of current technologies, Black and Paterson 1997). Two *in situ* microcosm deployments are described and the techniques used for isolating and measuring carbohydrates in the water column are discussed. The first method, where the water volume was reduced by lyophilisation, was found to be problematic. The second method, which isolated suspended polymers from the water column, was prevented from being properly tested, due to very low *in situ* diatom biomass on three field microcosm deployments. However, the processes involved in testing the methods are discussed.

2.8.2 *In situ* microcosm deployment

The microcosm was deployed at a mid-intertidal site on the Eden Estuary on 24/7/96. The microcosm consists of a perspex core (0.3 m diameter) which can be sunk into sediments and has a lid with a rotating plate (0.2 m dia., Figure 2.13). Two forces are used to create a balanced stress over the test area. A rotating force is created by a rotating disc beneath the chamber lid. The centrifugal flow is greatest at the edge of the test chamber and decreases towards the centre (Tolhurst pers. comm.). This flow is balanced by a suction force created by pumping water from the centre of the chamber into a re-circulation system. The pumping force is greatest at the centre and decreases towards the edge. Together, the two forces create a constant shear stress over the whole bed (Gust 1991, Black and Paterson 1997).

Values of shear velocity (U_*) were determined using constant temperature anemometry (Gust pers. comm.) and range between 1 and 1.7 cm s^{-1} (Table 2.2). The velocity of the rotating plate (rpm) can be increased at set increments which correspond to different U_* values. The concentration of suspended particulate matter (SPM) was measured throughout the erosion event using a nephelometer (Tolhurst). Water samples were withdrawn from the chamber at each increment and used for biochemical and sediment analysis.

Figure 2.13 An illustration of the microcosm erosion device (Gust 1991).

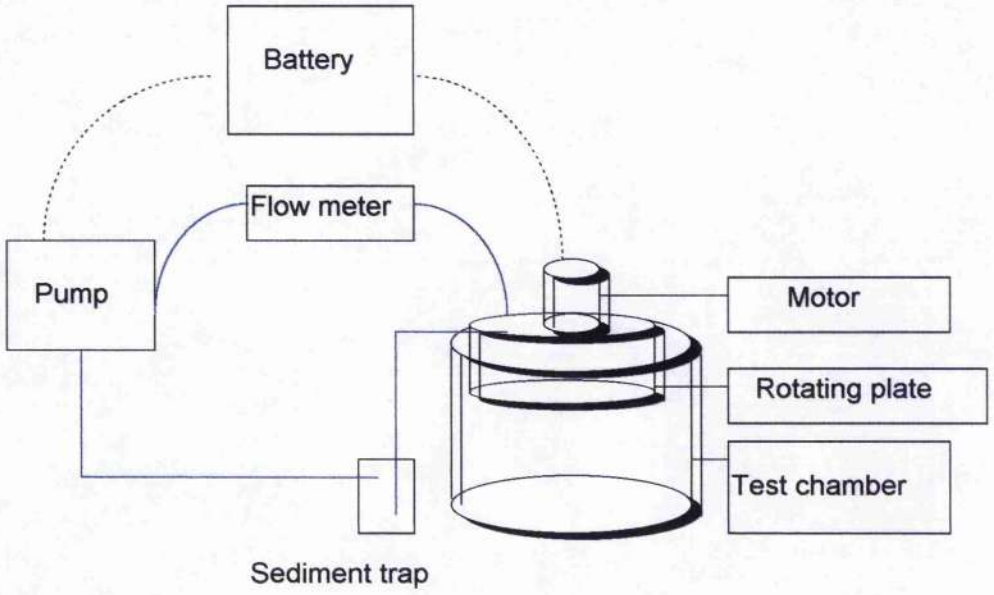


Table 2.2 The standard erosion velocity steps used with the microcosm.

Step	Voltage (V)	Rotating plate (rpm)	Shear velocity (U_*, cm s^{-1})
1	6.54	40	1.02
2	6.90	50	1.20
3	7.28	60	1.31
4	7.62	70	1.39
5	7.94	80	1.46
6	8.26	90	1.51
7	8.56	100	1.56
8	8.84	110	1.60
9	9.10	120	1.64
10	9.34	130	1.68
11	9.60	140	1.73

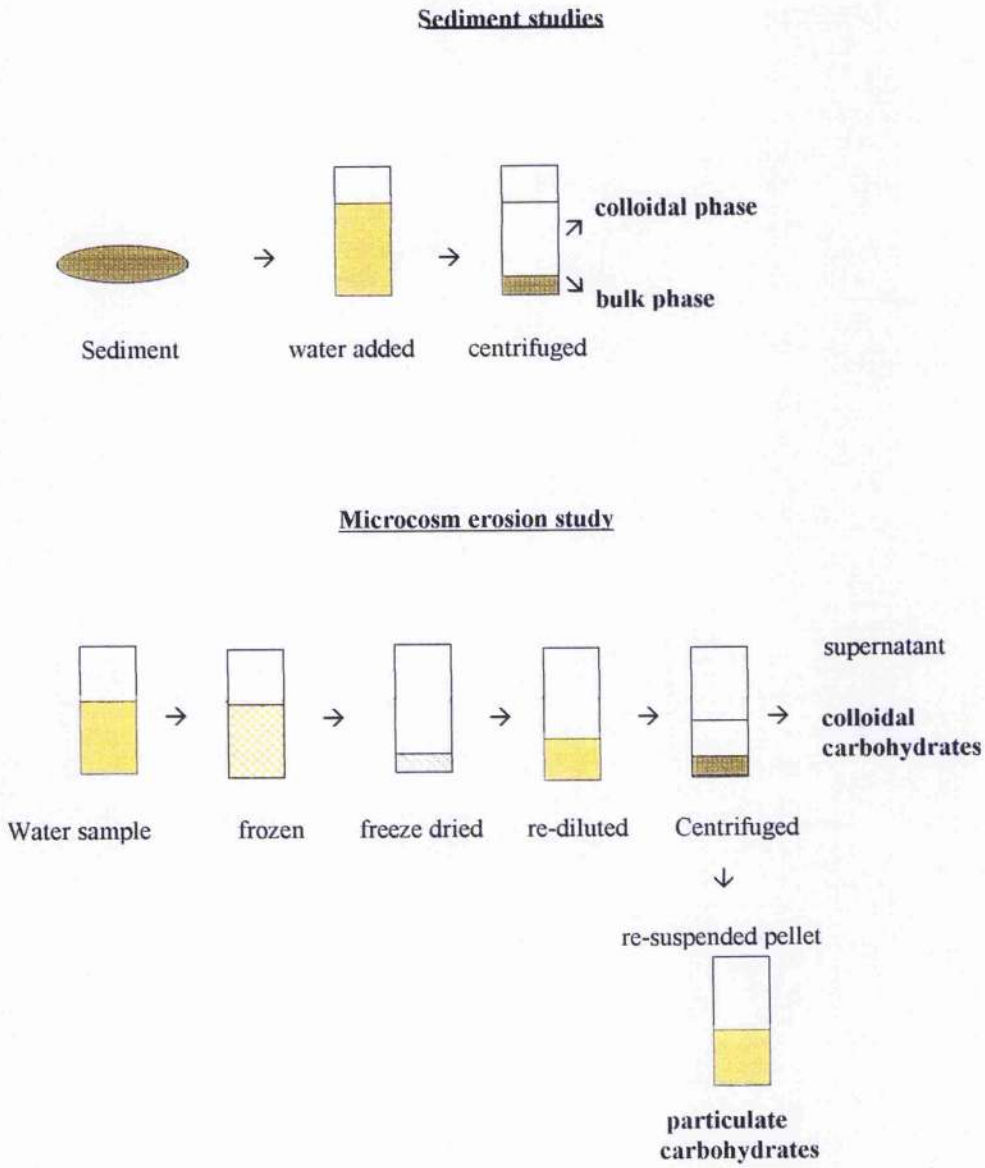
Each velocity step lasted for 10 minutes and three 20 ml samples of the water column were taken at the end of each run, resulting in 33 water samples. The water used in the microcosm was prepared by diluting seawater. The samples were withdrawn from the chamber using a 50 ml syringes with 140 mm rubber tubing attached. Care was taken to ensure that the water sample was withdrawn from the same depth in the chamber each time.

Ten sediment cores were taken in the immediate area for water content, and sediment density measurements. Lens tissue was placed onto nearby sediment to isolate the microalgae which may be producing and secreting carbohydrates.

2.8.3 The measurement of carbohydrates in sea water

An examination of low concentration glucose standards (i.e. 0.2 - 10 μg glucose ml^{-1}) found the Dubois assay was accurate to concentrations of approximately 3 μg ml^{-1} and above, however seawater carbohydrate concentrations in coastal areas are commonly 0.15 - 2 μg ml^{-1} (Johnson and Sieburth 1977, using MBTH technique). Therefore, the volume of water samples from the microcosm were reduced from 20 ml using lyophilisation (Edwards Modulyo, West Sussex, UK) and resuspended in 5 ml of distilled water (i.e. 4 x concentration). In addition, the salinity of the samples were recorded before and after freeze drying. The concentrated sample was centrifuged at 2500 g for 15 minutes and 200 μl of the supernatant analysed for colloidal carbohydrates using the Dubois assay. Particulate matter in the water samples was re-suspended and a 200 μl aliquot used to measured carbohydrates associated with suspended sediment (Figure 2.14). Since the microcosm chamber is a closed system, previously eroded matter remains in circulation and carbohydrates detected over the erosion event represent an accumulation of material rather than recently eroded carbohydrates. Therefore, carbohydrate concentrations were presented as units detected minus the concentration present at the previous time interval (Paterson pers. comm.).

Figure 2.14 Artificial separation of carbohydrate fractions



2.8.4 Results

The concentration of colloidal carbohydrates in the water samples did not increase significantly over the erosion event (Figure 2.15a, $H = 7.2$, $df = 10$, $P = 0.6$ Kruskal-Wallis Test, Minitab 10). However, at 70 min a large colloidal carbohydrate value was detected. This occurred immediately before the point of bed failure, as shown by a sudden increase in suspended particulate matter (Figure 2.16, Tolhurst pers. comm.). The concentration of SPM continued to increase until the last velocity step. Furthermore, there was no significant change in the concentration of particulate carbohydrates with increasing shear velocity (Figure 2.15b, $H = 11.96$, $df = 9$, $P = 0.2$ Kruskal-Wallis Test). The water content of the sediment was 45 ± 6.5 %, the wet bulk density and dry mass concentration were 1540 ± 208 and 857 ± 195 kg m^{-3} respectively ($n = 10$). Very few cells were present on the surface of the sediment. The number of cells present in the lens tissue samples were counted in several grids of a Neubauer Haemocytometer, however, there were insufficient numbers for the count to be reliable (normally 300 cells should be counted, instead an average of 9 cells were counted in 25 squares).

2.8.5 Discussion

The high colloidal carbohydrate value, detected directly before the point of bed failure, was isolated in the sense that it occurred at one time and was not shown in subsequent time intervals. This value supports the suggestion that some colloidal carbohydrates may enter the water column before sediment particles and could reflect the strength of the bed and signal of the onset of erosion (Sutherland 1996). However, the significance of this value must be treated with caution and the experiment should be repeated on sediments with higher diatom biomass. Although, colloidal carbohydrates were concentrated by a factor of 4, many concentrations were at the lower limits of detection and new methods for isolating carbohydrates from seawater need to be developed such as isolating the polymeric component or using a rotary evaporator to concentrate carbohydrates in seawater.

Figure 2.15 Carbohydrate concentrations with increasing erosion velocity (bars = S.E. n = 3)

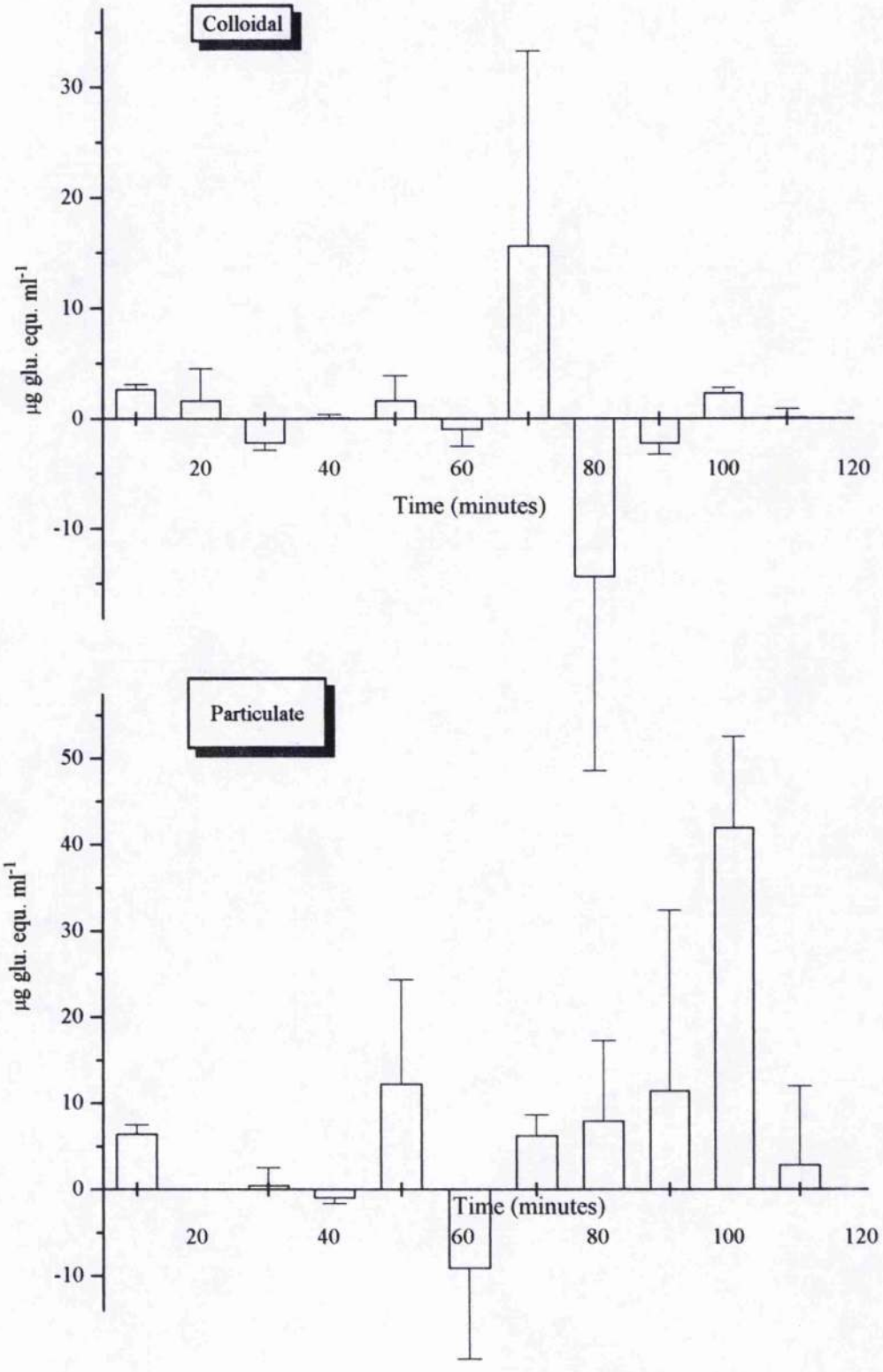
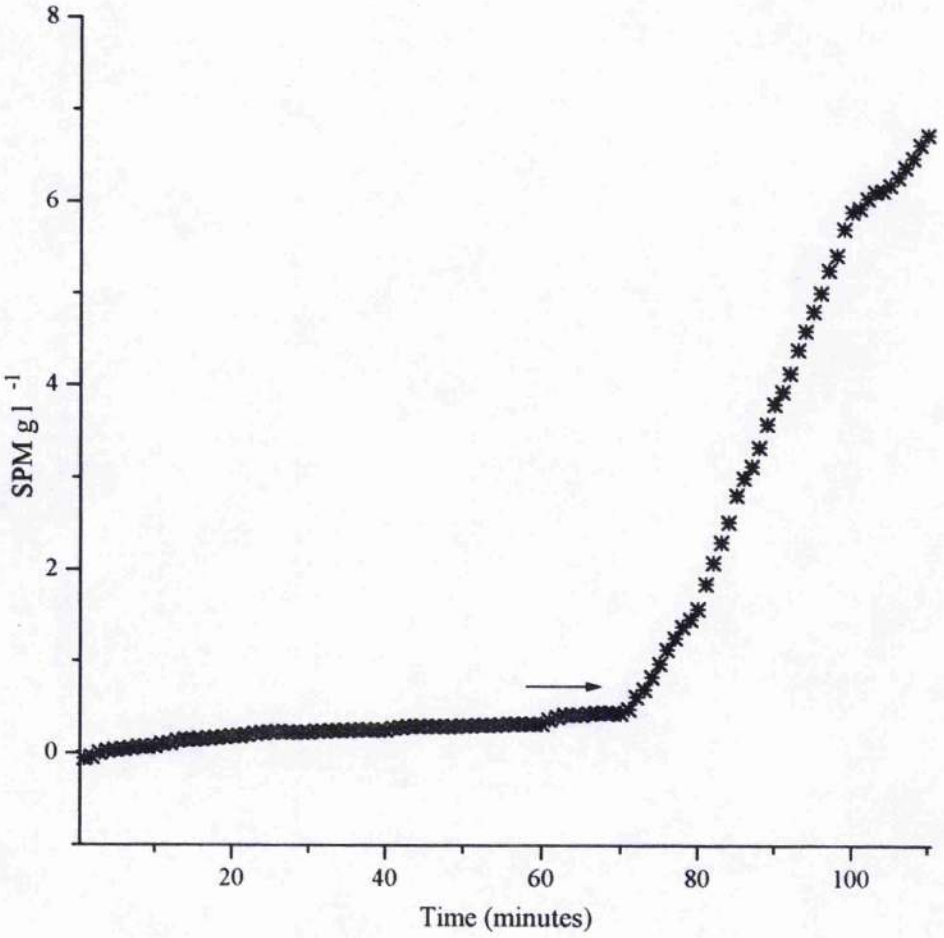


Figure 2.16 Variation in suspended particulate matter over an erosion event.

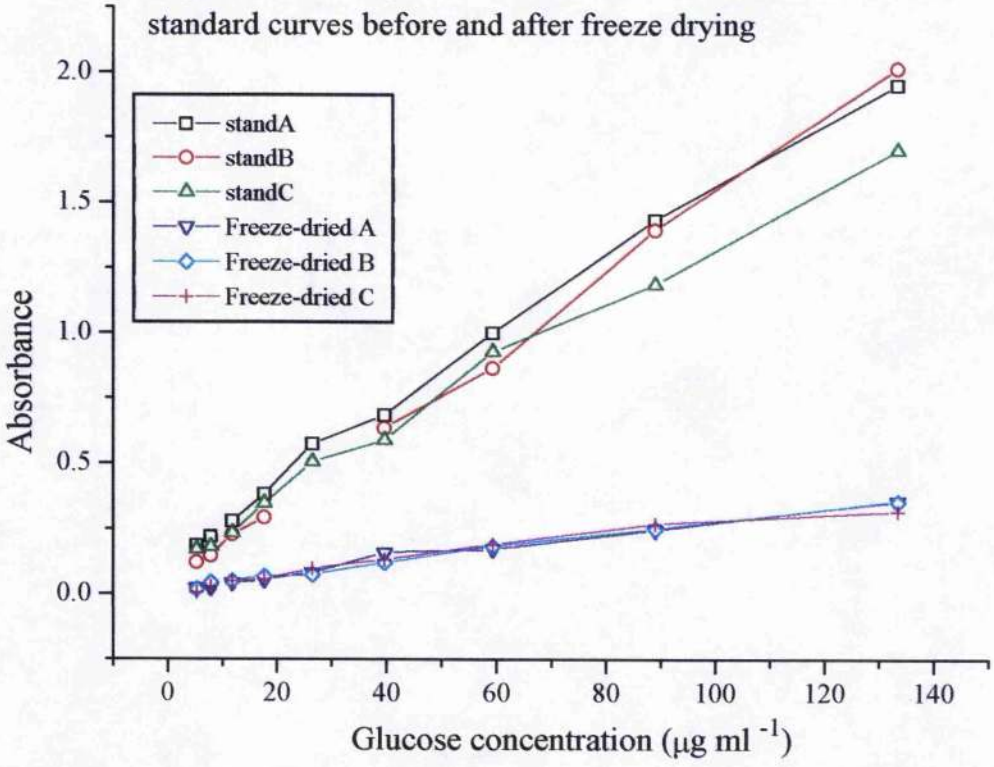


2.8.5.1 Reliability of the lyophilisation method

Laboratory tests were carried out to check the reliability of concentrating carbohydrates in water samples by lyophilisation. Three sets of glucose standards were prepared (133.33, 88.88, 59.26, 39.51, 26.34, 17.56, 11.7, 7.8, 5.2 μg ml^{-1}) and a 1 ml volume of each was assayed using the Dubois assay. A further 1 ml was lyophilised then re-dissolved in the same volume of distilled water. The absorbancies of the lyophilised standard solutions were compared to those which had not been lyophilised. Secondly, since lyophilisation of sea water increases the concentration of salts in the sample, tests were conducted to assess the possible effect of NaCl concentrations on carbohydrate determination. Glucose standards (200, 133 and 59 μg ml^{-1}) were prepared and 1 ml aliquots were set at 5 salinity levels (0, 10, 25, 50, 100) in duplicate. Absorbances were compared with the standards containing no NaCl.

The absorbancies of standards which had been lyophilised were significantly lower than the same standards which had not been lyophilised (Figure 2.17), the linear regression coefficients of the slope (*b*) were statistically different (P = >0.01, Fowler and Cohen 1990). The only significant NaCl effect was shown in the lowest glucose standard with a salinity of 100, where there was a significant increase in the absorbance values (P = 0.05, one-tailed *t*-test).

Figure 2.17 Comparison of absorbances from standard curves before and after freeze drying



2.8.5.1.1 Conclusions

The laboratory tests showed that the method for concentrating the glucose standards underestimated glucose concentrations. This may be due to the loss of the freeze-dried glucose in the vacuum of the freeze dryer. Polymeric material in seawater samples will have a higher molecular weight than glucose and less may be lost in the freeze drying process. Alternatively, glucose may not have re-dissolved with 100 % efficiency after lyophilisation, leaving some glucose behind in the Eppendorf. Since the method for concentrating carbohydrates in water samples was not accurate, the reliability of the data in the first microcosm deployment is doubtful. In addition, the lyophilisation of large water samples often took many hours. Therefore, another method for measuring low concentrations of carbohydrates was sought.

2.8.6 Second microcosm field deployment

The microcosm was deployed on the intertidal sediments of the Eden Estuary on 27/3/97, no diatom biofilms were visible. There were 11 velocity steps and 3 water samples were taken from the microcosm at 8 minutes into each 10 minute step. Due to the problems encountered with lyophilisation, carbohydrates in the water sample were isolated using cold alcohol precipitation. This technique has been used to separate the polymeric and monomeric components of colloidal carbohydrates fraction (Underwood *et al.* 1995). Polymeric material accounts for approximately 25 % of colloidal carbohydrates associated with intertidal sediments (Underwood and Smith 1998b). The water samples were centrifuged (2500 g for 15 min.) and 30 ml of the supernatant was added to 70 ml of cold alcohol (96 % v/v) and large amounts of white precipitate formed. After 24 hrs at < 5 °C, the solutions were centrifuged as before and the alcohol discarded. The precipitates were then re-suspended in 2 ml of distilled water and shaken and 200 µl was assayed for carbohydrate concentration.

2.8.6.1 Results and discussion

No carbohydrates were detected in the precipitates. When the precipitates were re-suspended in the original sample volume (30 ml) the salinity (measured by refractometer) was 25, identical to that of the water sample, suggesting that the precipitates were salts. Low molecular weight salts and sugars should not have precipitated in the alcohol. However, in Paterson *et al.* (1996b) it was suggested that

polymers were precipitated in alcohol, washed and precipitated a second time, to remove low molecular weight sugars and this may be the same for salts.

A further field deployment of the microcosm took place on 30/4/97, again there was no visible diatom biofilms. EPS was precipitated with alcohol, as before, washed and precipitated a second time. Small amounts of white precipitate formed in some samples. Unlike the previous deployment, the samples with small amounts of precipitate were found to contain very low concentrations of carbohydrates (approx. 3 $\mu g\ ml^{-1}$). However, the concentrations were so low as to be unreliable using the Dubois assay.

The EPS precipitation method for measuring carbohydrates over an erosion event, has been successful on sediments with a higher level of diatom biomass (Tolhurst pers. comm.) and is providing some results which may help to clarify the role of carbohydrates in the process of sediment erosion.

Gust's microcosm may distinguish different elements within the sediment which have not yet been studied, for example, an area of surface sediment may contain many different erosion thresholds, i.e. EPS may have a different erosion threshold to diatoms cells and laminated sediments may show distinct, successive erosion thresholds. Using smaller microcosms may increase the resolution of erosion studies and mini-flumes have recently developed to examine the rheological properties of intertidal cohesive sediments (Ruddy *et al.* 1996, Jones pers. comm.).

Chapter 3

3. Introduction

The importance of microbially-produced extracellular polymeric substances (EPS) for the ecology of marine deposits and coastal sediment transport has been described in Chapter 1. In summary, most EPS in the intertidal zone is produced through the locomotion of migratory epipelic diatoms. As cells move through the upper millimetres of surface sediments, an EPS matrix is formed which increases inter-grain binding, thus stabilising the sediment. This biogenic influence helps to account for the difference in erosion patterns of sterile sediments (Miller *et al.* 1977) and those measured in the field (Grant and Gust 1987, Paterson 1995). EPS is also an important carbon source for the heterotrophic community and is an effective binding agent for inorganic nutrients and heavy metals. Therefore, information on the abundance of EPS in natural systems is of particular importance in models of sediment erosion as well as for the general ecology of the system.

There is a need to resolve EPS distribution on a scale similar to diatom migratory movements which are usually within the upper millimetre (Hopkins 1963). The *in situ* production rates for EPS have been measured on the Humber Estuary, UK using radiolabelled ^{14}C and found to be 0.05-0.12 mg C m^{-2} h^{-1} (Underwood and Smith 1998a). However, there is very little data on the spatial variation of EPS concentration (Table 3.1). Rogers (1965) measured sedimentary sugars in the near surface and deep sediments of Clear Lake and Blue Lake, Minnesota. He compared sediment sugars to those of aquatic plants and found no similarity and therefore, suggested that microorganisms may synthesise and alter the sediment sugars. However, the scale of the core analysis (with a resolution of 10 cm) was not relevant to microbial processes. Microspatial analysis of carbohydrate concentrations in surface sediments (with a resolution of 2 mm) found concentrations to be greatest in the upper 2 mm and to co-vary with measurements of algal biomass (Paterson *et al.* 1996). Therefore, a detailed study of EPS distribution in the upper millimetres is essential for understanding diatom EPS production and stabilising effects.

The objectives of this study were to investigate *in situ* carbohydrates in the upper millimetres of intertidal sediments using new high-resolution (0.2 mm) sectioning techniques. Secondly, to measure changes in sediment fabric and assess the influence

these may have on the distribution of the carbohydrate fractions. Furthermore, to determine if there is any variation in the distribution of bulk and colloidal carbohydrate fractions with depth. In addition, macroscale spatial variation of colloidal and bulk carbohydrate concentrations were investigated at different tidal levels across the mud flats and over small scale mud flat morphological features. The implications of these results for sediment transport models are discussed.

Table 3.1. *Carbohydrate concentrations measured in natural sediments.*

Location	**Total carbohydrates** (free sugars, EPS and insoluble carbohydrates)	**Colloidal carbohydrates** (free sugars and EPS)	**EPS** (polymeric carbohydrates)	**Study**
Lake sediments (upper 1.2 m, Blue and Clear Lake, Minnesota, USA)	26.9 mg g^{-1}	—	—	Rogers 1965
Sub-tidal sediments (Kuril-Kamchatka trench, Russia)	0.4 - 1.6 mg g^{-1}	—	—	Artem'yev 1968
Sub-tidal sediments (New York Bight, USA)	1 - 20 mg g^{-1}	—	—	Hatcher and Keister 1974
Sub-tidal sediment (Dabob Bay, Washington State, USA)	3.4 mg g^{-1}	—	—	Cowie and Hedges 1984
Sub-tidal sediment (1 -2 cm, river Krka Estuary, Yugoslavia)	1.5 - 9.8 mg g^{-1}	—	—	Hadzija *et al.* 1985

Intertidal sediment (Pinellas Point, Florida, USA)	—	1.4 - 3.2 mg g^{-1}	—	Grant and Gust 1987
Intertidal sediment, 0-2 mm (Portishead, Severn Estuary UK)	max. 23 mg g^{-1}	max. 5.5 mg g^{-1}		SERG 1992
Aust, Severn	max. 16.2 mg g^{-1}	max. 1.2 mg g^{-1}		as above
Sand Bay, Severn	max. 17.5 mg g^{-1}	max. 1.5 mg g^{-1}		as above
Sub-tidal sediments (shallow bay, Strömstad, Sweden)	—	1.9 mg cm^{-2}	—	Madsen *et al.* 1993
Intertidal sediment (Severn Estuary, UK)	approx.13 mg g^{-1}	approx. 4 mg g^{-1}	—	Underwood and Paterson 1993
Intertidal sediment (Texel, Netherlands)	2.6 ± 1.2 mg g^{-1}	0.28 ± 0.09 mg g^{-1}		Yallop *et al.* 1994
Intertidal sediment (Severn Estuary, UK)	10.9 ± 8.4 mg g^{-1}	1.5 ± 0.8 mg g^{-1}		Yallop *et al.* 1994
Intertidal sediment, 0-2 mm (Portishead, Severn)	5.2 -9.1 (± 0.5 - 0.8) mg g^{-1}	0.2 - 1.5 (± 0.04 -0.4) mg g^{-1}		George 1995

Intertidal sediment (Blackwater Estuary, UK)	15.3 ± 9 mg g^{-1}	1.6 ± 0.9 mg g^{-1}	—	Underwood *et al.* 1995
Intertidal sediment, 0-2 mm (Severn)	approx. 11 mg g^{-1}	approx. 3 mg g^{-1}		Paterson *et al.* 1996
Sub-tidal estuarine sediments (Upper South Cove, Nova Scotia, Canada)	1.5 - 2.6 mg ml^{-1}	0.04 - 0.14 mg ml^{-1}	—	Sutherland 1996
Intertidal sediment (Humber Estuary, UK)	0.5 - 0.85 mg cm^{-2}	0.05 - 0.01 mg cm^{-2}	0.004 - 0.014 mg cm^{-2}	Underwood and Smith 1998a
Intertidal surface sediments (Severn and Blackwater Estuaries, UK)	—	0.01 - 10 mg g^{-1}	0.0025 - 2.5 mg g^{-1}	Underwood and Smith 1998b

3.1 Material and methods

3.1.1 Sites

Three intertidal mud flats were chosen for this study; Hawkins Point (53° 38' N 0° 3' W) and Skeffling Clays (53° 38' N 0° 4' E) on the north coast of the Humber Estuary, England and Guardbridge on the Eden Estuary, Scotland (56° 22' N 02° 51' E). Further descriptions of the sites are given in Chapter 2, section 2.1.

3.1.1.1 Sampling: Depth profiles

Sediment samples were collected from Hawkins Point in September 1994, from Skeffling in April 1995 and from Guardbridge in April 1996. At Hawkins Point, 9 sediment cores (9 cm diameter x 30 cm length) were collected from the mid-intertidal region and sub-sampled in the laboratory. Six sub-samples were taken, using 20 ml cut-off syringes (2 cm diameter x 10 cm length), and frozen at -20°C. At Skeffling, 4 sediment cores (3 cm diameter x 13 cm length) were collected upper-intertidal station and frozen on site at -20°C. The sampling regime was further improved at Guardbridge where a 5 cm area of mid-intertidal mud flat was rapidly frozen *in situ* before being removed. The mud flat surface was initially stabilised using liquid nitrogen (LN_2) vapour and further frozen by the application of LN_2, following the method of Wiltshire *et al.* (1997). 6 frozen cores (5 cm diameter x 2 cm length) were stored in liquid nitrogen until each was sectioned into six depth intervals.

3.1.1.2 Spatial variability

Two additional spatial variability experiments were conducted on surface scrapings from Skeffling. At Skeffling site A, the sediment bed was characterised by small ridges and runnels. 6 surface scrapings (upper 5 mm) were collected from an exposed sediment ridge approximately 10 mm in height and 6 scrapings from an adjacent water covered runnel. Secondly, surface scrapings were collected along a shore normal transect in the upper-intertidal station (Chapter 2, Figure 2.4 a, b). The transect contained 3 points placed at 3 m intervals and termed A1, A2 and A3. 5 samples were collected from each point and frozen at -20°C. Transects representing tidal levels (i.e. upper, mid and low-intertidal) were sampled at Hawkins Point and at Skeffling. A

minimum of three cores were collected at upper, mid and low-intertidal at Hawkins Point and 4 replicates at upper and mid-intertidal stations at Skeffling. Samples of the upper 10 mm were analysed for colloidal and bulk carbohydrates and extracellular enzyme activity was measured in the Skeffling samples (Ruddy *et al.* 1996) using fluorescently-labelled substrate analogues for α-glucosidase, β-glucosidase, leucine aminopeptidase and esterase.

3.1.1.3 Biochemical and physical analysis of the sediment

The protocols used for sediment analysis are described in Chapter 2. The frozen sediment cores were sectioned into 200 μm slices and the dry mass concentration calculated and the carbohydrate concentrations of the sections were then analysed. Surface sediments from Hawkins Point were also examined using LTSEM and particle size analysis was carried out on samples from the Skeffling transect.

3.2 Results

3.2.1 Microscale carbohydrate depth profiles

Mean colloidal carbohydrate concentrations within surface sediments (0-200 μm) ranged from 4 to 12 mg glucose equivalents g^{-1} dry weight of sediment. The highest concentrations were measured at Hawkins Point followed by Skeffling and then Guardbridge (Table 3.2). Carbohydrate values from each site were transformed (log10) to achieve a normal distribution. Significant regressions were produced showing a log-linear decrease in colloidal carbohydrates with depth in the upper 2 mm (Figure 3.1, Hawkins Point: $r^2 = 0.17$, $F_{1,44} = 9.72$, $P = 0.003$; Skeffling: $r^2 = 0.39$, $F_{1,19} = 11.59$, $P = 0.000$; Guardbridge: $r^2 = 0.5$, $F_{1,35} = 33.46$, $P = 0.000$). There was no significant difference between the regression slopes of Hawkins Point and Skeffling ($P = > 0.1$). However, both of these slopes differed from the regression slope of the Guardbridge data ($P = 0.02$), where the decrease in colloidal carbohydrates was steepest.

Figure 3.1 Regression analysis of colloidal carbohydrates (log10) with depth on 3 intertidal mud flats (linear regression with 95 % confidence limits).

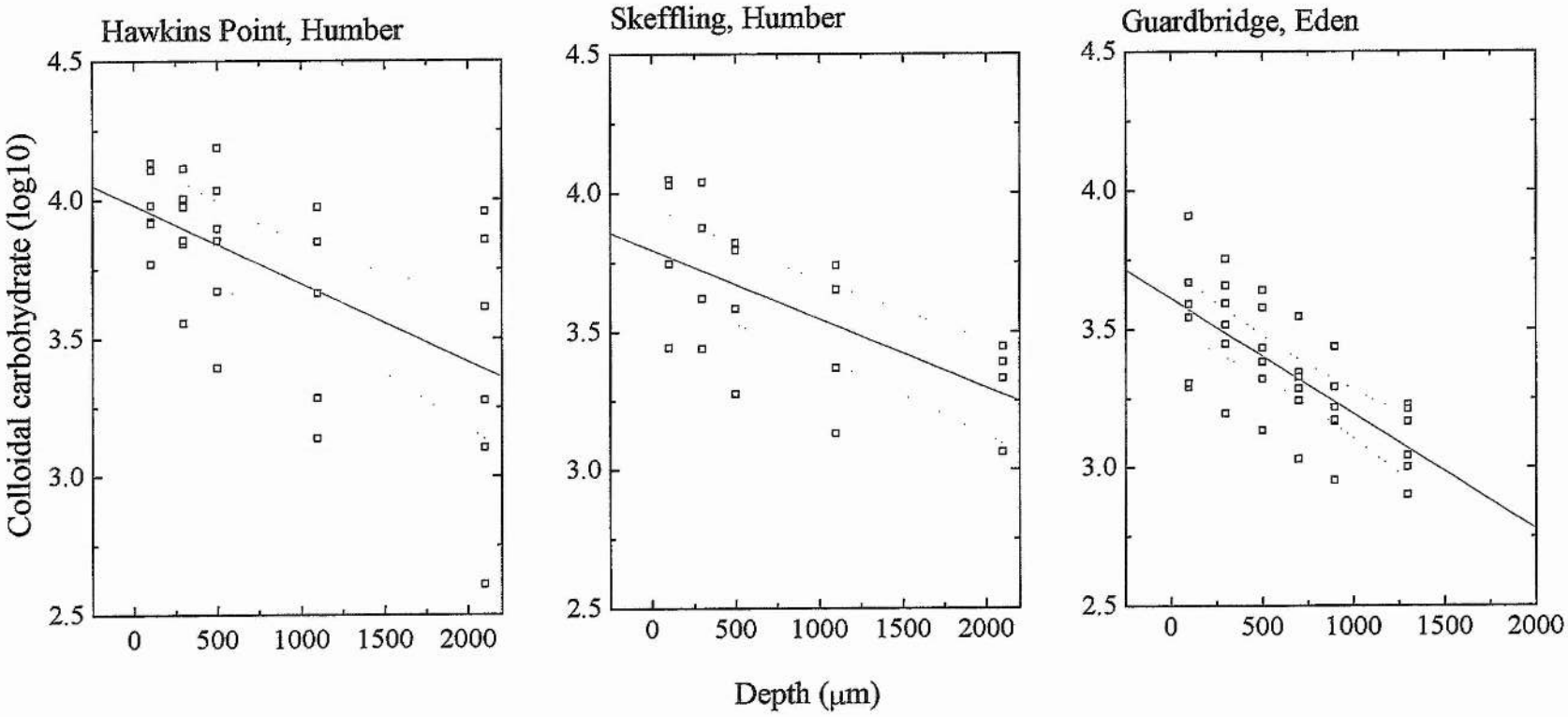


Table 3.2 *Mean concentrations of colloidal carbohydrates (± SD, n = 9, 4, 6 Hawkins Point, Skeffling and Guardbridge, respectively) expressed as mg glucose equ. g^{-1} dry sediment.*

Depth (μm)	Hawkins Point	Skeffling	Guardbridge
0-200	12 ± 11.6	7.5 ± 4	4 ± 2.2
1000-1200	5.2 ± 4	3.4 ± 1.9	1.3 ± 0.4
10000-10200	1.6 ± 1.2	0.9 ± 0.1	—

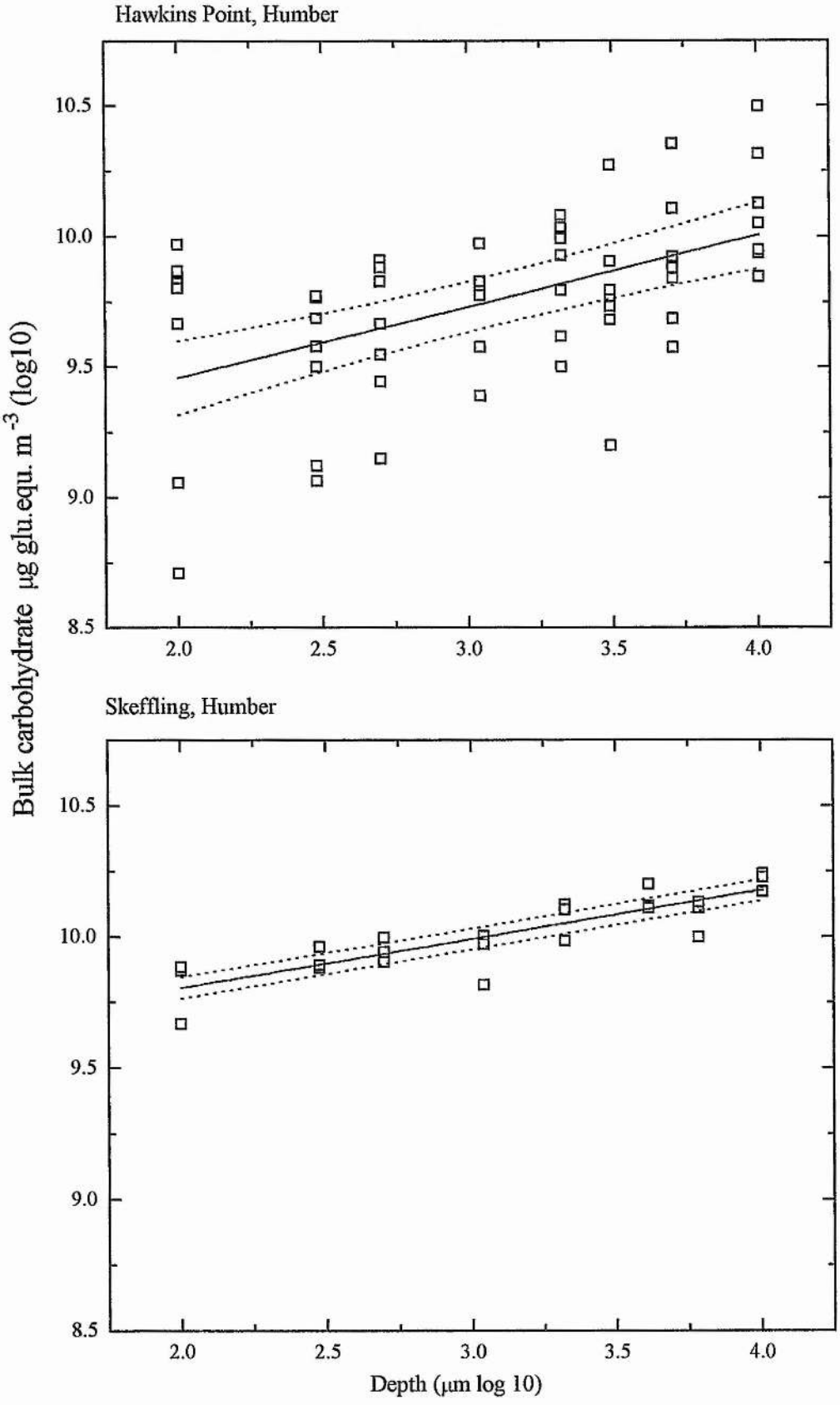
The concentration of the bulk carbohydrate fraction was consistently greater than the colloidal carbohydrate fraction. The average concentration of bulk carbohydrates on the surface (0-200 μm) of Hawkins Point and Skeffling mud flats were 37.4 and 25 mg glucose equ. g^{-1} dry weight of sediment, respectively. Bulk carbohydrates were not measured at Guardbridge. Bulk carbohydrate values (mg glucose equ. m^{-3}) from both sites were transformed (log10) and regression analysis showed a linear increase in bulk carbohydrate concentration with depth in the upper 1 cm (Figure 3.2, Table 3.3, Hawkins Point: $r^2 = 0.26$, $F_{1,54} = 19$, $P = 0.000$; Skeffling: $r^2 = 0.62$, $F_{1,23} = 35$, $P = 0.000$, two-way ANOVA without replication). Therefore, the bulk carbohydrate fraction increased with depth just as the colloidal fraction decreased. However, the slopes of the two regression analyses were different ($P = 0.01$). The regression accounted for 26 % of the variation in bulk carbohydrate at Hawkins Point and 62 % of bulk carbohydrate variation at Skeffling.

There was a highly significant inverse correlation between bulk and colloidal carbohydrates with depth, at Skeffling ($r = -0.73$, $n = 32$, $P = < 0.01$). However, the colloidal and bulk carbohydrate fractions measured at Hawkins Point were not correlated ($P = > 0.05$, $n = 40$, on log transformed data).

Table 3.3 *Mean concentrations of bulk carbohydrates (± SD, n = 9 and 3, Hawkins Point and Skeffling, respectively), expressed as kg glucose equ. m^{-3} sediment.*

Depth (μm)	Hawkins Point	Skeffling
0-200	6.4 ± 4.6	6.6 ± 1.7
1000-1200	7.3 ± 6	8.7 ± 1.9
10000-10200	14 ± 8	16 ± 1.3

Figure 3.2 Regression analysis of bulk carbohydrates with depth (log10 transformed) in the upper 10 mm on 2 intertidal sites on the Humber (linear regression with 95 % CF)



3.2.1.1 Sediment dry mass concentration profiles

Dry mass concentration of the sediment sections increased significantly with depth at Hawkins Point, Skeffling and Guardbridge (Figure 3.3, $F_{1,44} = 10$, $P = 0.000$; $F_{1,19} = 8.4$, $P = 0.002$ and $F_{1,35} = 39.2$, $P = 0.000$ respectively, two-way ANOVA without replication on transformed data). The greatest increase in dry mass concentration occurred at Guardbridge, where values increased from 288 kg m^{-3} in the upper section (0 - 0.2 mm) to 554 kg m^{-3} at 2 mm. The increases in dry mass concentration at Hawkins Point and Skeffling were less steep. A deeper profile of the upper 20 mm at Hawkins Point shows the increase of dry mass concentration within this distance to be curvilinear (Figure 3.4). This emphasises the importance of scale in the measurement of dry mass concentration in surface sediments. The results from microscale depth profiles were supported by low-temperature scanning electron micrographs of sediment from Hawkins Point (Figure 3.5). The micrographs show a clear change in the density and porosity of sediment within the upper 4 mm (Figure 3.5a). Diatom cells and cyanobacteria were present on the surface and were associated with an organic film which may have been EPS (Figure 3.5b, c). Large numbers of epipelic diatoms were situated below the film (Figure 3.5d). In addition, examination of fractured sediment revealed a matrix of diatom cells, linked together by fibres, in the upper 1 mm (Figure 3.5e).

3.2.2 Spatial variation

Physical and biological parameters were found to vary along the transect at Skeffling. The mean particle diameters were 27, 23 and 18 μm at sites A1, A2 and A3, respectively (Figure 3.6), although the range in particle sizes was similar. The moisture content of the upper 5 mm increased downshore from 30 % (A1) to 52 % (A3) ($F_{2,7} = 50$ $P= 0.000$, on log transformed data, Figure 3.7). Therefore, the transect represented a moisture gradient. Wet mass concentration decreased downshore from 912 kg m^{-3} (A1) to 756 kg m^{-3} (A3), ($F_{2,7} = 4$, $P = 0.09$). Similarly, dry mass concentration fell from 636 - 359 kg m^{-3} along the transect ($F_{2,7} = 23.5$, $P = 0.003$, Figure 3.8a). Furthermore, the concentration of colloidal carbohydrates decreased downshore (2.4 - 0.7 mg glucose equ. g^{-1}, $F_{2,13} = 22.31$, $P = 0.00$, Figure 3.8b). Bulk carbohydrate values varied significantly within the transect but with no downshore trend ($F_{2,13} = 9.5$, $P = 0.003$).

Figure 3.3. Microscale depth profiles of dry mass concentration within the upper 2 mm at Hawkins Point, Skeffling and Guardbridge mud flats (bars = S.E., n = 12, 4 and 6 respectively)

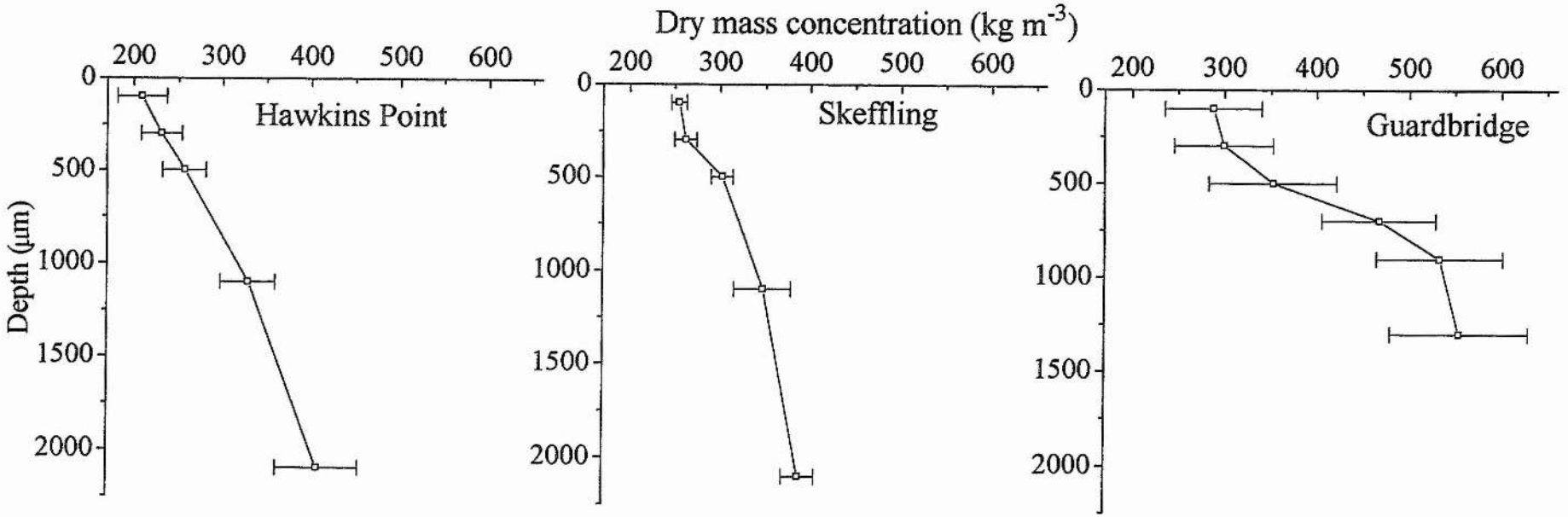


Figure 3.4 Dry mass concentration in the upper 20 mm at Hawkins Point (bars = S.E., n = 12)

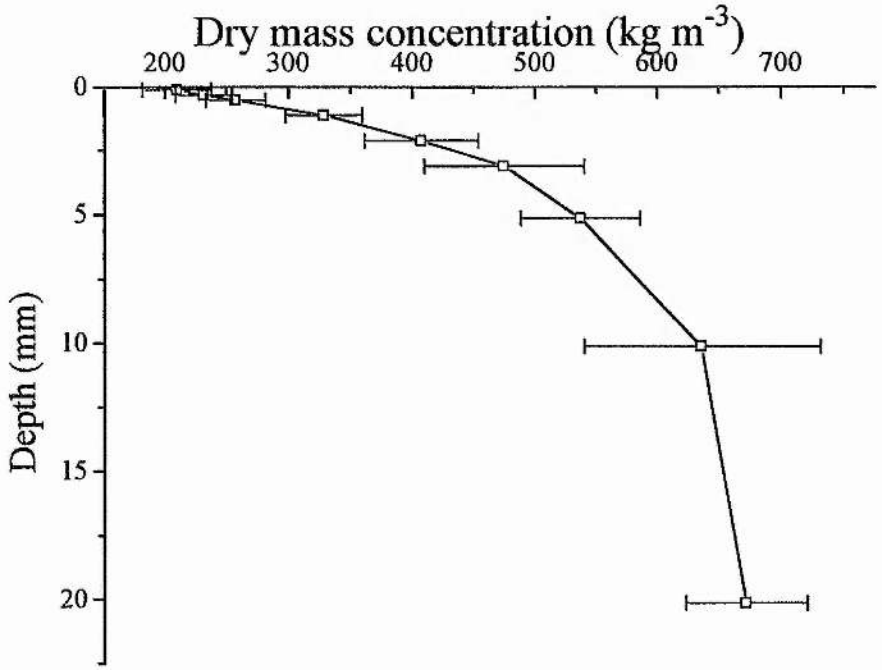


Figure 3.5

Low-temperature scanning electron micrographs of surface sediment from Hawkins Point. (a) A depth profile of the upper 4 mm showing clear changes in the porosity of sediment fabric with depth (arrow indicates surface, scale bar = 100 μm). (b) An epipelic diatom of the genus *Pleurosigma* penetrating the surface organic layer (scale bar = 10 μm). (c) A mixed assemblage of micro-organisms on the surface organic layer. Several different diatom species can be seen along side cyanobacterial trichomes (c = cyanobacteria and d = diatoms; scale bar = 100 μm). (d) Dense assemblages of epipelic diatoms can be seen through an organic film. The assemblage is dominated by small cells of the genus *Navicula* with a few large cells of the genus *Scolioneis* (scale bar = 100 μm). (e) A vertical section of surface sediment showing the arrangement of epipelic diatoms an associated bacteria in the upper 250 μm (scale bar = 10 μm).

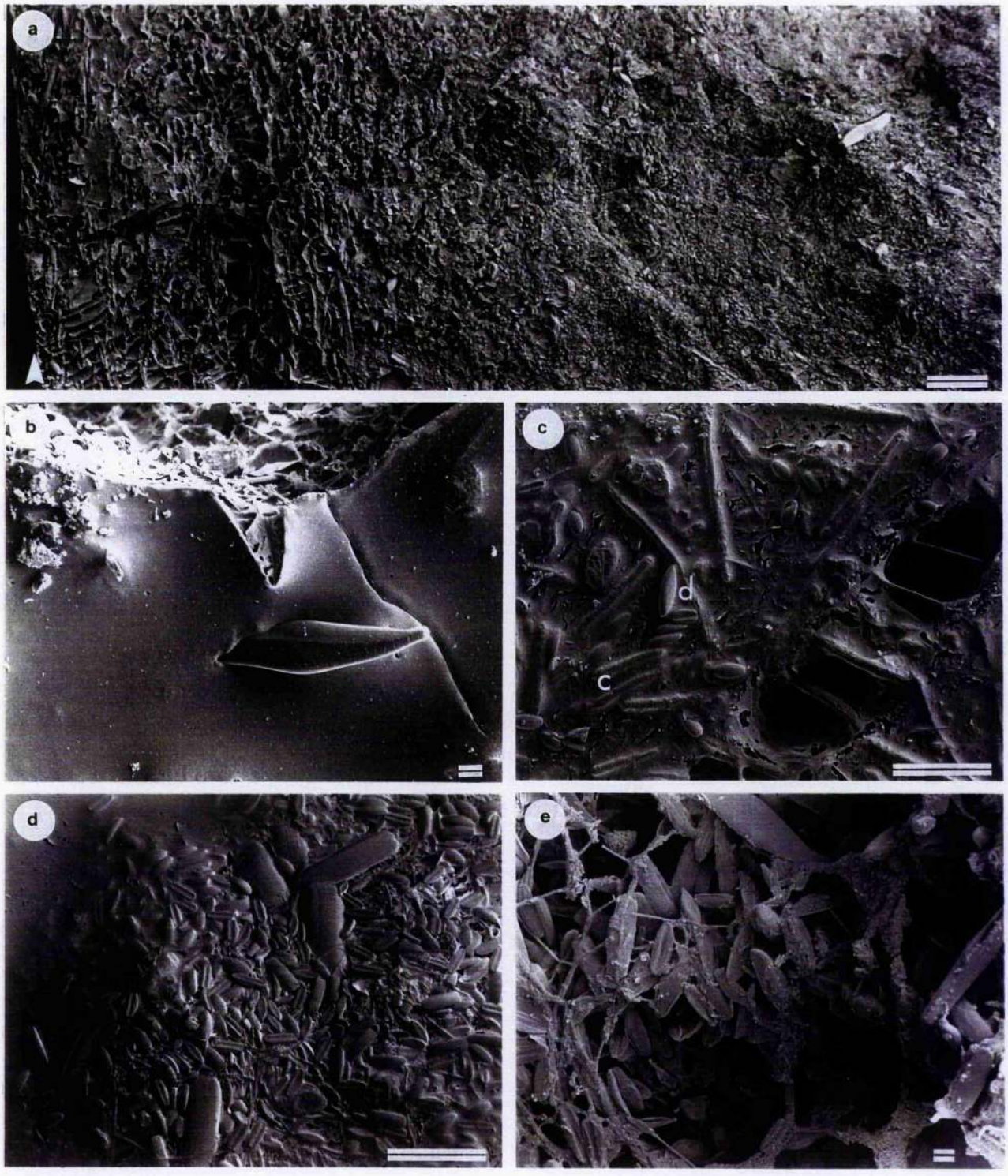

a
b
c
d
C
d
e

Figure 3.6 A spectra of particle size diameters of surface sediments along a 3 point transect at Skeffling.

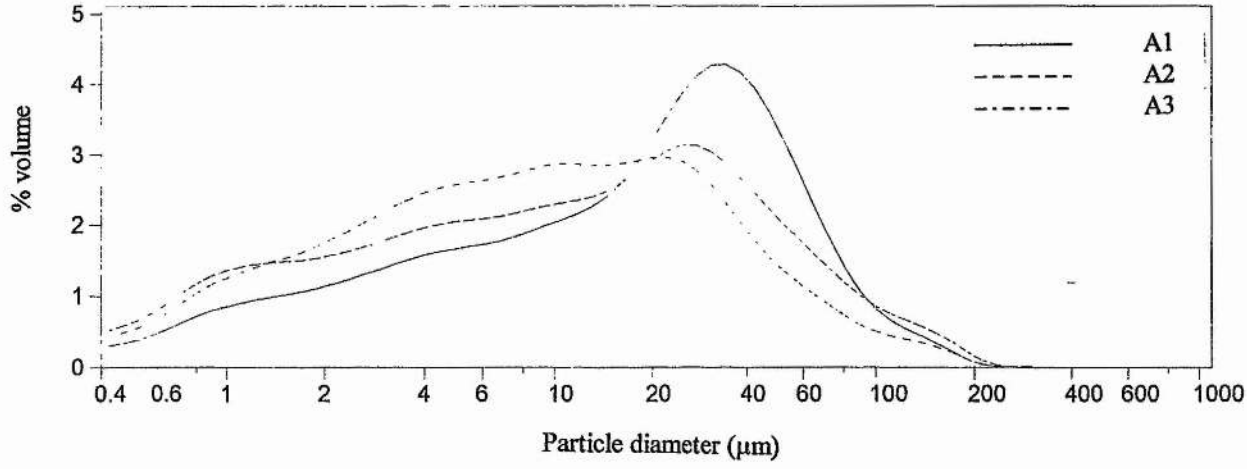


Figure 3.7 Percentage moisture content of surface sediments along a 3 point transect at Skeffling (error bars are 95 % confidence limits, n = 3)

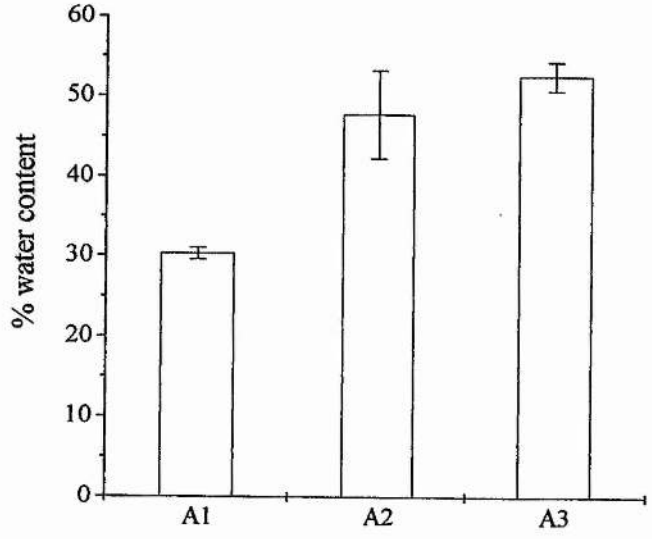
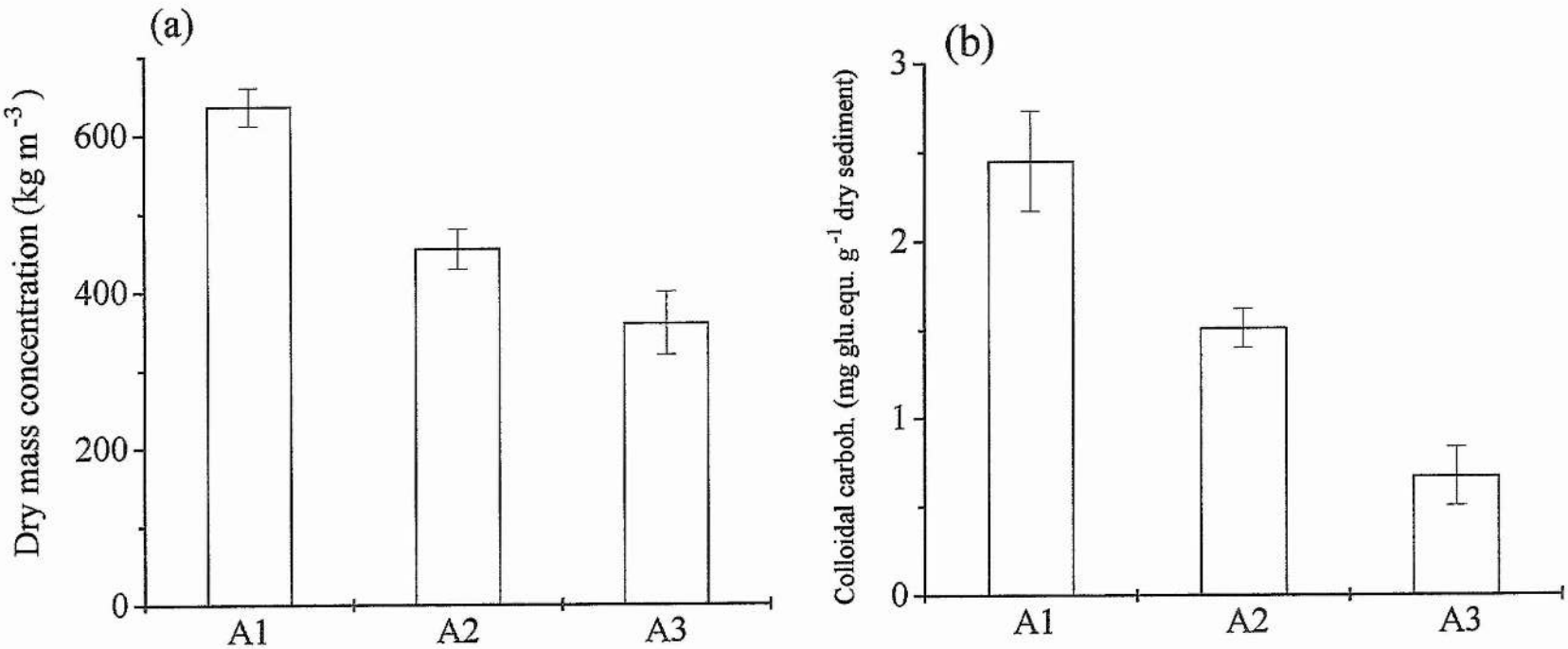


Figure 3.8 (a) Dry mass concentration of surface sediment along a shore normal transect at Skeffling (bars = S.E., n = 3) and (b) Colloidal carbohydrate concentrations along the transect (error bars are 2 x SE, n = 5)



Colloidal and bulk carbohydrate concentrations on upper, mid and low-intertidal sites at Hawkins Point increased significantly with tidal height (Table 3.4, H =28.3, P = 0.000, df 2 and H = 11, P =0.000 df 2 respectively, Kruskal-Wallis). Colloidal and bulk carbohydrate concentrations at Skeffling were significantly higher at the upper-intertidal site than at the mid-intertidal site (Table 3.4, t = 8.1, P = 0.000; t = 9.7, P = 0.000, t-test). Extracellular enzyme activity measurements made on the same sediment samples from Skeffling high-shore and mid-shore samples (Ruddy *et al.* 1996) found activity of α-glucosidase, β-glucosidase, aminopeptidase and esterase to be greatest at site A which had the greatest carbohydrate concentrations and lower enzyme activity at site B.

Table 3.4 *Carbohydrate concentration with tidal level on two intertidal mud flats (expressed as mg glu. equ. g^{-1} ± SD; Skeffling, n = 32; Hawkins Point, n = 24).*

	Colloidal carbohydrate			**Bulk carbohydrate**		
	Upper	Mid	Low	High	Mid	Low
Hawkins Point						
(9/1994)	8.2 ± 3.2	4.2 ± 3	1.1 ± 0.7	38 ± 31	21 ± 15	16.6 ± 9
Skeffling	0.7 ± 0.3	0.2 ± 0.15	—	32 ± 8.1	16.8 ± 3.1	—
(4/1995)						

Examination of a small ridge and runnel showed them to have different physical and biochemical properties. The concentration of both bulk and colloidal carbohydrates were significantly greater in the runnel than on the sediment ridge (Figure 3.9, $F_{1,11}$ = 79.89, P = 0.000; $F_{1,11}$ = 26, P = 0.000 respectively). Furthermore, the moisture content of the surface sediment (5 mm) was higher in the runnel (47 %) than on the ridge (37 %). In addition, both wet and dry mass concentrations appeared to be higher in the runnel (Figure 3.10).

Figure 3.9 Colloidal and bulk carbohydrate concentration in the upper 5 mm of a runnel and ridge at Skeffling (bars = S.E., n = 6)

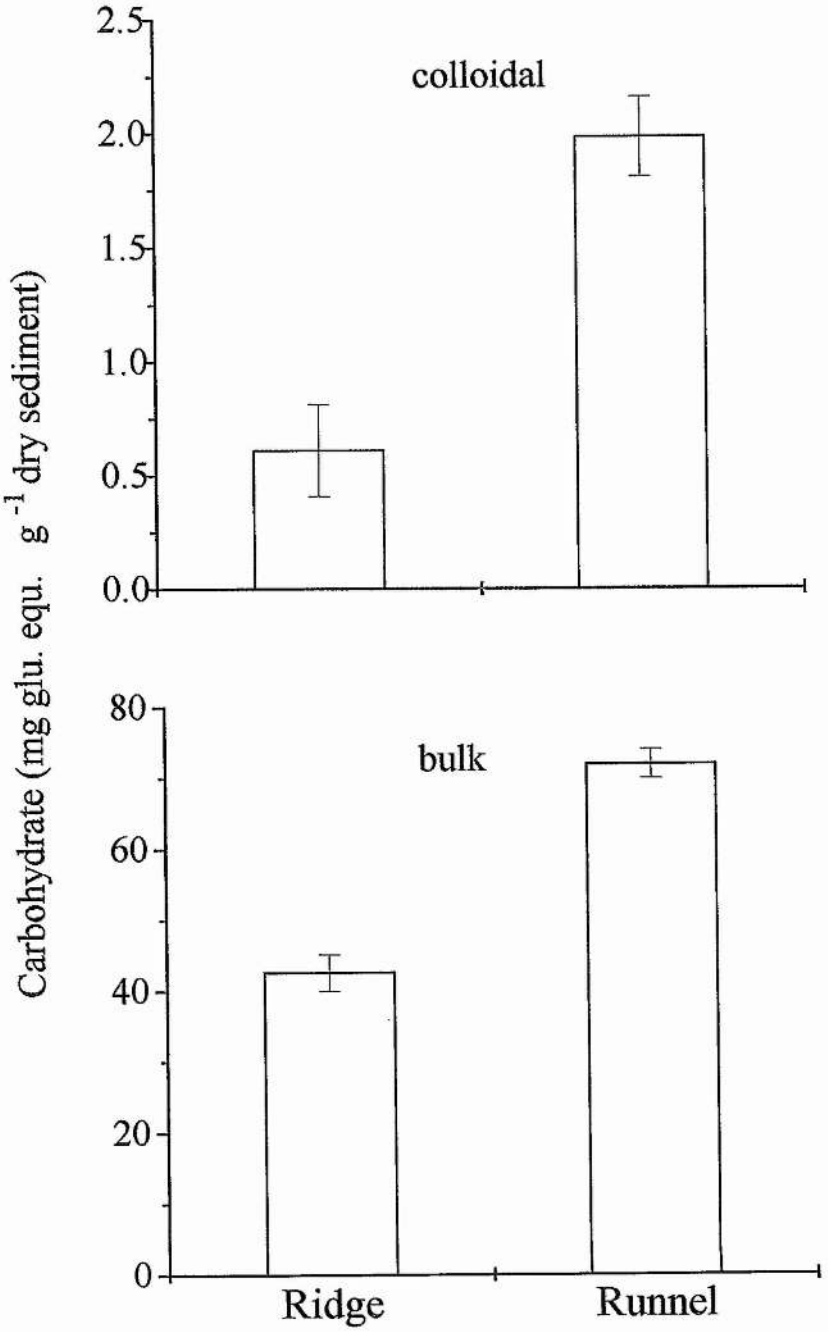
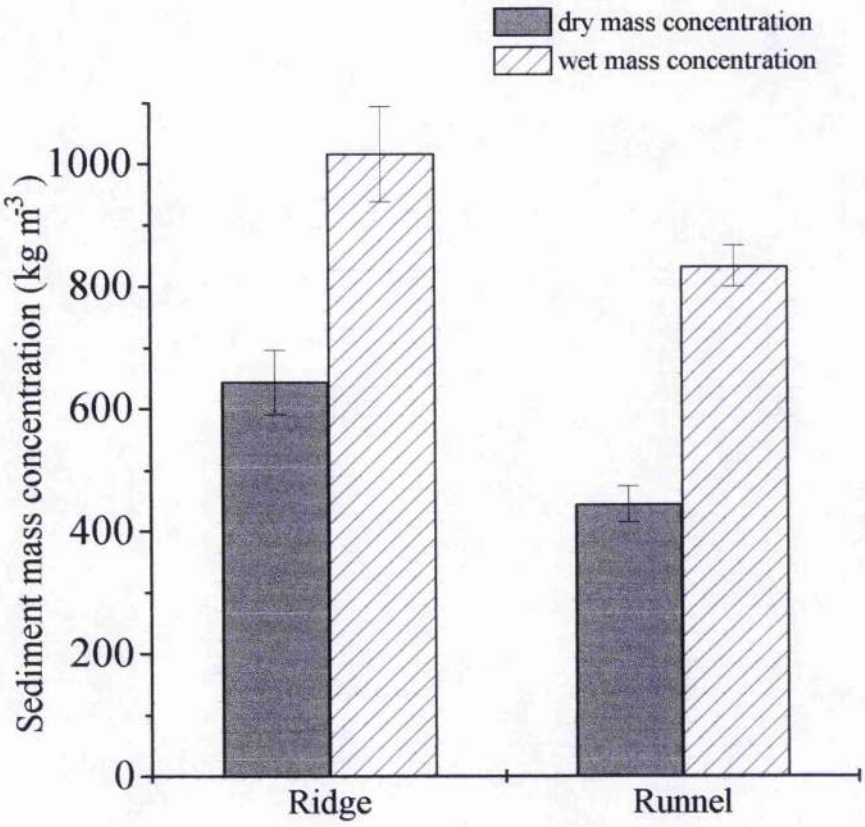


Figure 3.10 Wet and dry mass concentration of sediment in the upper 5 mm of a ridge and runnel at Skeffling (bars = S.E., n = 2)



3.3 Discussion

3.3.1 Carbohydrate fractionation

In this study the sediment carbohydrates were operationally separated into two fractions. The colloidal fraction is extractable in water and in previous studies was shown to contain approx. 80 % monosaccharides and 20 % polysaccharides (Underwood *et al.* 1995). The bulk fraction comprises carbohydrates that remain in the sediment after the colloidal fraction has been extracted and may include larger organic complexes. However, there is very little information on the spatial variation of the carbohydrate fractions. In this study significant differences were shown in the vertical distribution of bulk and colloidal carbohydrate fractions. The depth profiles of colloidal carbohydrates were expressed per unit weight but show the same distribution when expressed per unit volume (Table 7.1). Bulk carbohydrate concentrations were expressed per unit volume since they are associated with sediment material and the amount of sediment in a given volume changes dramatically within the upper 2 mm (Figure 3.3). The carbohydrate fractions could be expressed per unit biomass (Yallop pers. comm.) and studies have shown a correlation between colloidal carbohydrates and chlorophyll *a* (Underwood and Smith 1998b), however, bulk carbohydrates may not be correlated to living material since they are more refractory and can be measured below the photosynthetic zone.

3.3.2 Microscale carbohydrates depth profiles

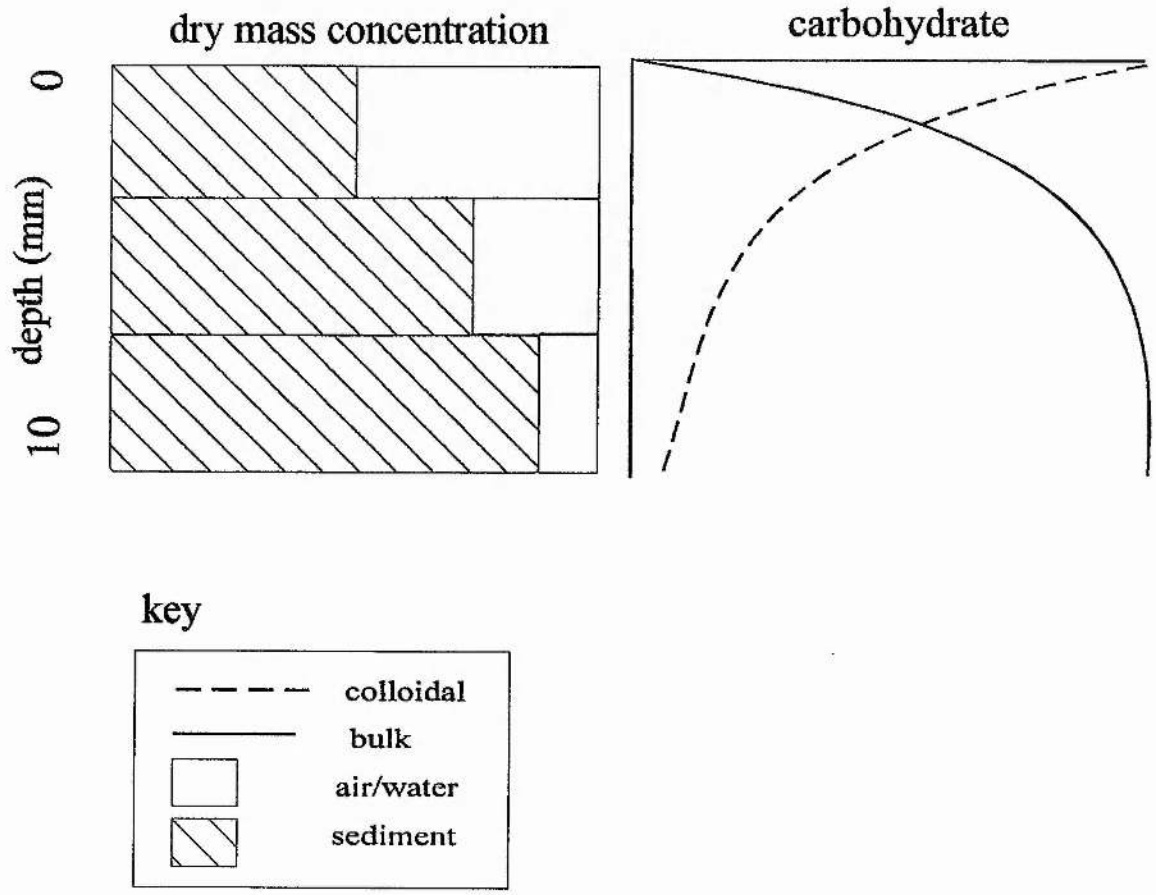
Colloidal carbohydrate concentration showed a significant linear decrease in the upper 2 mm. This may be attributed to a reduction in porosity with depth (Craig 1992) since colloidal carbohydrates are soluble and will occur in the pore waters of muds. The lowest r^2 value in the regression analysis between colloidal carbohydrates and depth was obtained from Hawkins Point data. However, large sample numbers validated the significance of this trend. There was no difference between the regression slopes of colloidal carbohydrates from Hawkins Point and Skeffling. However, at Guardbridge, where the decrease was steeper, the regression slope differed from the other two sites. The increase in dry mass concentration was also steeper at Guardbridge and this suggests that colloidal carbohydrate distribution and dry mass concentration are associated.

The bulk carbohydrate fraction may represent complex carbohydrate materials which are more refractory than monosaccharides. Since bulk carbohydrates remain

associated with sediment after the colloidal carbohydrate extraction, they may be more closely related to the particulate matter. Indeed, both bulk carbohydrates and dry mass concentration increased with depth. The increase in bulk carbohydrate concentrations was greatest at Hawkins Point. However, the r^2 value of the regression between bulk carbohydrates and depth was lowest at Hawkins Point, reflecting variability between the cores. There was less variation in bulk carbohydrate concentrations between the cores collected at Skeffling. The regression slope of bulk carbohydrates and dry mass concentration with depth was less steep at Skeffling than at Hawkins Point, suggesting a relationship between the distribution of dry mass concentration and carbohydrates. A schematic representation of the relationship between carbohydrate fractions and dry mass concentration, with depth, is shown in Figure 3.11. Since the colloidal and bulk carbohydrate fractions differ in their distribution with depth they may have different origins and turnover rates. This theory was also postulated by Sigleo (1996) who suggested that colloidal carbohydrates and those associated with particulate matter were physically distinct and affected by different processes.

Colloidal carbohydrate has been positively correlated with algal biomass (in the form of chlorophyll *a*, Sutherland 1996, Underwood and Smith 1998b). Therefore, a better understanding of the movements of diatoms in surface sediments may provide more information on colloidal carbohydrate distribution. The colloidal concentrations were significantly lower at 2 mm than in the upper 0 - 0.2 mm depth interval. Hopkins (1963) found 60 % of diatoms exist in the upper 2 mm. Diatoms may be limited to the upper 2 mm by the need to surface at each exposure (Hay *et al.* 1993), by the concentrations of toxic chemicals in the anoxic layers (Moss 1977, Admiraal 1984) and perhaps by compaction of sediment particles. More information is needed on the influence of physical parameters, such as dry mass concentration and porosity, on the distribution of carbohydrates.

Figure 3.11 Schematic diagram showing the distribution of carbohydrate fractions, dry mass concentration and porosity in the upper 10 mm, based on data from microscale profiles



The sediment/water interface is of prime importance in the study of sediment erosion and transport. Dry mass concentration is used as a parameter in a number of erosion prediction equations:

$$\tau_{cr} = 0.0045\ \rho_d^{0.9}$$

τ_{cr} = critical erosion stress

ρ_d = dry mass concentration (Ockenden and Delo 1991).

Therefore, accurate measurements of dry mass concentration in the surface layers are essential to the success of sediment transport models. There was a consistent increase in dry mass concentration in the surface 2 mm at each site. However, our depth profiles of dry mass concentration show a different pattern of increase depending on the measurement scale. The profile of the upper 2 mm shows a linear increase in dry mass concentration. However, when this profile was extended down to 20 mm, the increase in dry mass concentration was curvilinear. Therefore, it would be inadvisable to assume a linear increase in dry mass concentration unless accurate measurements are made using a high resolution (200 μm) technique. Previous dry mass concentration profiles have been conducted at a metre scale with a lower analytical resolution (Hydraulics Research Ltd. 1991), such measurements fail to detect crucial changes at the sediment/water interface. Therefore, transport models predicting the onset of particle erosion may be radically altered with the introduction high resolution techniques.

The importance of microbial changes to the bulk density of surface sediments has been shown by Sutherland (1996) using X-ray computed tomography with a resolution of 1.5 mm (Amos *et al.* 1996). This analysis showed a low-density, buoyant surface layer on marine sub-tidal sediments. The buoyant nature of this layer, which was found to contain high chlorophyll concentrations, was due to oxygen production by photosynthetic microalgae. Although the microbial associated layer was buoyant, it adhered firmly to the sea bed and was shown to stabilise these sediments.

The nature of the sediment fabric from Hawkins Point was observed using low-temperature scanning electron microscopy. A gradient of sediment porosity occurred in

the layers where diatoms were found. Sediment porosity may influence the distribution of diatoms in the upper millimetres of the sediment since it will influence light penetration and cell mobility. The microscale variation in sediment carbohydrates and dry mass concentration demonstrates the importance of microscale analysis in biofilm environments. High resolution sectioning allows several variables to be studied at a resolution of 200 μm however, there is no information on microscale temperature variability. This would be desirable since diatom motility and microbial metabolism including extracellular enzyme activity is affected by temperature (Hay *et al.* 1993, Ruddy *et al.* 1996). 99 % of light and therefore heat, is absorbed in the upper 100 μm of cohesive sediments (Miles and Paterson 1996). Therefore, temperature may be more variable on the mud flat surface than at 1000 μm in depth. In addition, the particle size of the sediment may influence the variability of temperatures, for example the temperature of sands may be more variable since the increased porosity would allow greater light penetration and moisture evaporation than in muddy sediments.

3.3.3 Spatial variation

Carbohydrate concentrations and sediment characteristics were investigated across the mud flat during a neap tidal phase using a 3 point transect. There was significant variation in particle size, moisture content, dry mass concentration and carbohydrates over the transect. Although each point had a wide range of particle sizes, the mean particle diameter decreased downshore (this value was always lower than the mode). As particle size decreased, the moisture content of the sediment increased. Carbohydrate concentrations increased landward although no diatom assemblages were observed at the upper-intertidal station, A1 (pers. obs.), however, no diatom counts were made to confirm this. Small cracks in the sediment were visible at A1 and were attributed to desiccation during the neap tidal phase. Therefore, colloidal carbohydrates extracted from sediment at A1, were probably produced by an assemblage of diatoms which had since perished through desiccation. As the cells lyse they will release intracellular carbohydrates thus enhancing the carbohydrate levels in the colloidal fraction. A comparison between carbohydrate concentrations during neap and spring tidal phases is an area requiring further research. Colloidal carbohydrate concentrations may be elevated if dry conditions result in lower levels of natural bacterial enzymatic

breakdown. In this way, carbohydrate extraction may be affected by the moisture content of the sediment. Sediment stability varies on a similar scale to the measurements made along the transect (Black and Paterson *in press*) and such variability cannot be attributed to one environmental factor alone. Transects which represent tidal zones (i.e. upper, mid and low-intertidal) vary in length depending on the slope of the shore. Colloidal and bulk carbohydrate concentrations measured at upper, mid and low-intertidal stations increase landwards, supporting the finding of the small transect and this may be attributed to longer exposure periods at upper-intertidal sites, allowing more photosynthetic production. In addition, the upper-intertidal mud flats may be more stable as they often consist of smaller, more cohesive sediment particles than lower-intertidal sediments. For example, upper-intertidal sediments at Skeffling have a mode particle diameter of 22 μm compared to 84 μm at the low-intertidal station (Black pers. comm.). Furthermore, the hydrodynamic forcing of fluvial and tidal flow is often reduced at upper-intertidal zones. The increased stability of upper- intertidal mud flats may contribute to the success of microphytobenthic biofilms and result in greater concentrations of colloidal carbohydrates than lower intertidal zones.

Most mud flats have creeks, channels and ridge-runnel features reflecting the hydrodynamics of the Estuary. The Humber Estuary is highly energetic and the low-shore at Skeffling is dominated by metre wide ridge-runnel features. In addition, microscale ridges (10 mm) and runnels occur at all tidal levels. When these were examined, carbohydrate fractions were found to occur in greater concentrations in the runnel depression than on the microscale ridge. Similarly, dry mass concentration and moisture content were greater in the runnel. The greater moisture content in the runnel is probably a drainage effect and it may be favourable for diatoms, reducing desiccation and allowing greater mobility. The greater biomass of diatoms in the runnel would explain the increased carbohydrate concentration and this should be measured in further studies. Furthermore, diatom assemblages in the runnel would benefit from the shelter provided by the ridge, reducing the effects of wind desiccation.

Information on the microscale distribution of carbohydrates is presented for the first time. A relationship between carbohydrates and dry mass concentration exists and further parallel measurements of these two variables is desirable to establish the

importance of this relationship. Measurements at a microscale are important since they focus upon the sediment/water interface where microorganisms significantly increase sediment stability (Paterson 1994). Furthermore, gradients of dry mass concentration in the upper 20 mm may have implications for sediment shear stress predictions. A greater understanding of carbohydrate distribution may be gained through the measurement of carbohydrate concentrations in surface sediments over a tidal migration cycle.

3.4 Conclusions

Sediment carbohydrate concentrations were found to vary significantly with depth in the upper millimetres of surface sediments. The carbohydrate fractions varied in their distribution with depth, the colloidal carbohydrate fraction decreased and bulk carbohydrates increased with depth. The gradient of the bulk carbohydrate concentration was mirrored by gradients of dry mass concentration and changes in the sediment porosity were observed with depth using LTSEM. It is suggested that dry mass concentrations co-vary with the distribution of carbohydrates. Colloidal carbohydrates decrease with depth due to their association with porewater. The migratory movements of diatoms may influence the distribution of carbohydrates in the upper millimetres of intertidal sediments and this was recognised as an area requiring research. The significant increases in dry mass concentration in surface sediment were measured at a high resolution and this information is of particular importance to sediment transport models.

Colloidal carbohydrates were found to increase in concentration in a landwards direction. The sediment bed morphology also influenced the distribution of carbohydrates with greater concentrations being found in runnel depression than on small sediment ridges. The physical characteristics of the ridge and runnels also varied, and it was suggested that increased moisture content of the runnel may encourage diatom growth and explain the greater concentrations of carbohydrates, although the spatial variation of carbohydrates cannot be explained by one environmental factor alone.

Chapter 4

4. Introduction

Estuarine intertidal sediments are subject to a range of temporal variations including tidal rhythms and diurnal light cycles. In addition, seasonal changes in temperature and hydrodynamic forcing overlay short-term variability. The influence of seasonal variability on the microphytobenthos was shown in a recent study in which chlorophyll *a* (as an indicator of biomass) was significantly lower in winter and summer months (2 -6 mg m^{-2} mm^{-1}) and peaked in the spring (10-12 mg m^{-2} mm^{-1}) (Cariou-Le Gaul and Blanchard 1995). The relevance of environmental variables to microphytobenthic growth can vary seasonally, for example, tidal level was shown to have a significant influence on algal biomass during winter months (when light was limiting) but was of lesser importance during the summer, where wave action affected growth (Colijn and Dijkema 1981). In addition, episodic storms can effect microphytobenthic populations as much as seasonal changes (Yallop and Paterson 1994, Brotas *et al.* 1995).

During the tidal exposure period, surface sediments may undergo extreme changes in temperature, light intensity, salinity, pH, CO_2 availability and sediment moisture content (Rasmussen *et al.* 1983, Guarini *et al.* 1997, Paterson *et al. in review*, Paterson and Black *in press*). Epipelic diatoms can respond to environmental changes very rapidly through the ability to migrate and they have been observed to emerge on the surface, minutes after the retreat of the tide (Paterson 1989, Hay *et al.* 1993, Paterson *et al. in review*).

Epipelic diatoms have been found to enhance sediment stability (Yallop *et al.* 1994, Paterson 1997, Sutherland 1996) through the production of EPS (mainly carbohydrates, Chapter 1) and the stability of intertidal surface sediments may increase over the emersion period. However, there is little information on the variability of carbohydrate concentrations on surface sediments over the emersion period (Smith and Underwood 1998b).

Intertidal mud flats are dynamic systems that represent both a source of and sink for suspended sediments. A major physical influence on intertidal sediment stability is sediment water content (Paterson 1988, Underwood and Paterson 1993). Generally, lower water content results in greater stability (Anderson 1983). The drying of sandy, non-cohesive sediments does not alter the

erosion threshold significantly (Black pers. comm.). However, when cohesive sediments (muds) de-water, the packing of sediment particles become more compact (Bennet and Hulbert 1986, Paterson 1988).

In natural sediments, changes in water content may alter the growth and activity of sediment associated diatoms (Paterson 1988). The secretion of EPS may play a vital role in protecting diatom cells against desiccating conditions (Peterson 1987, Decho 1994), which frequently occur on intertidal sediments, especially during neap tidal phases (Figure 4.1). Changes in sediment water content may change the tertiary structure of carbohydrates, as water is removed through condensation reactions leading to an increase in covalent bonding (Decho 1994). Metals usually bind to polymers through condensation reactions, causing loose EPS slime to aggregate into a polysaccharide gel (Decho 1994, Chin *et al.* 1998). Diatoms in biofilms secrete more carbohydrates when under desiccation stress (Peterson 1987) and it has been suggested that as the carbohydrates dry, they become more effective at stabilising sediments (Paterson 1988). However, this has not been tested and there have been no investigations on the possibility that sediment water content may affect the extraction of carbohydrate fractions. This is important since *in situ* carbohydrate measurements taken over the emersion period may coincide with changing water conditions. This study describes measurements of colloidal and bulk carbohydrates on a microscale, investigating variation over the emersion period on the Eden and Humber Estuaries. Complementary measurements of sediment properties were taken to obtain a greater understanding of the physical changes which occur on intertidal mud flats during the emersion period.

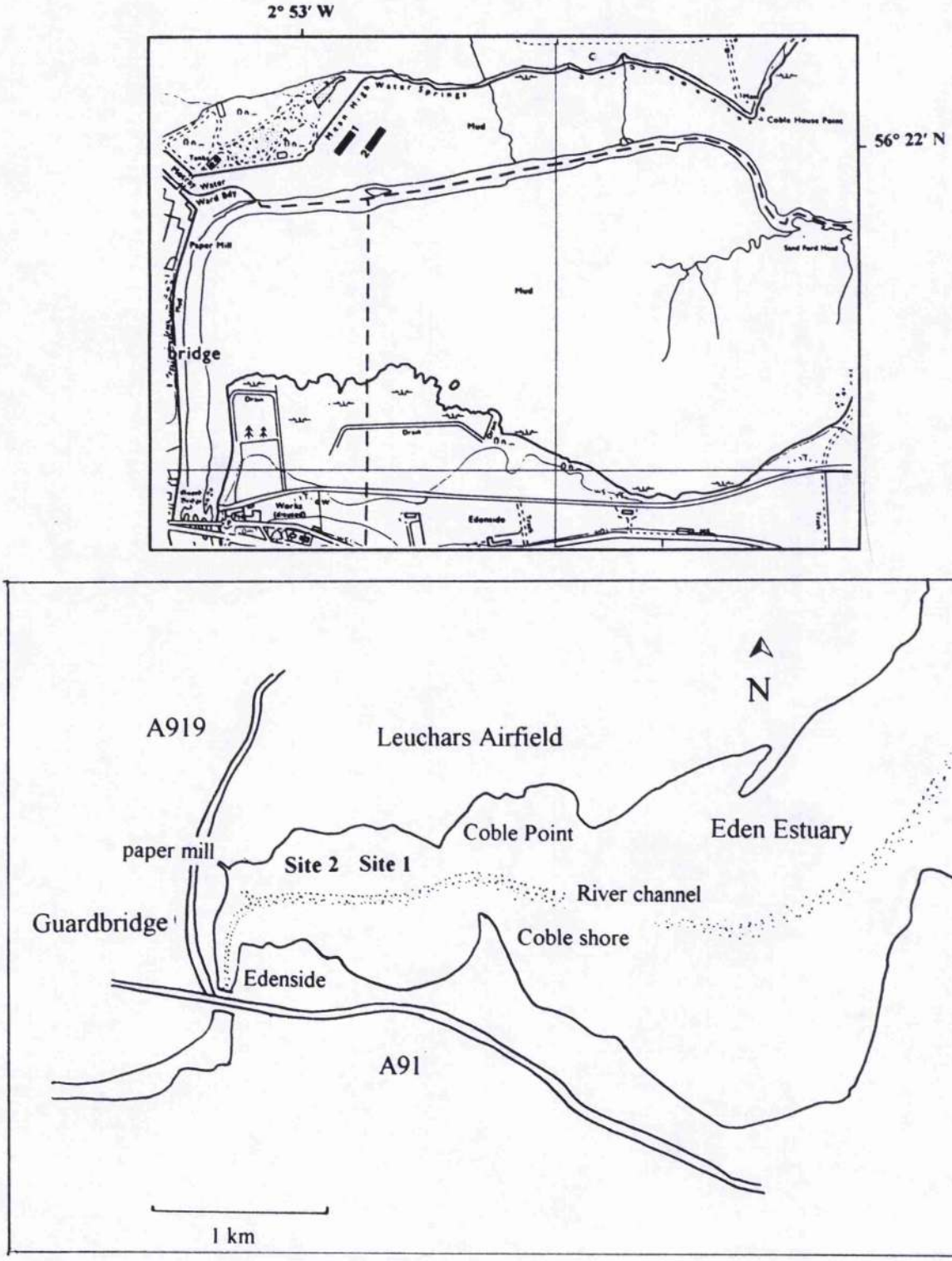
Figure 4.1 Orthogonal cracks on upper-intertidal sediments at Skeffling Clays, Humber Estuary, during a neap tidal phase

4.1 Materials and methods

Surface carbohydrate concentrations were examined at Skeffling Clays on the Humber Estuary, UK (53° 38' N 0° 4' E) over a spring tidal emersion period in April 1995, commencing at 08:45 hrs, at the beginning of the ebb, until 16:00 hrs when the upper mud flat became water covered. Samples were collected hourly at upper and mid-intertidal stations (100 m and 400 m from shore, Chapter 2, Figure 2.4b). For the examination of carbohydrate concentrations over the emersion period, sediment core samples (20 mm diameter x 10 mm depth) were added to 3.5 ml of 0.2 μm filtered seawater. 1 ml of this slurry was used to measure bulk and colloidal carbohydrate concentrations. The remaining sample was analysed for extracellular enzyme activity (Ruddy *et al.* 1996). Further sediment core samples (30 mm x 130 mm) were taken at the upper-intertidal station (08:45, 10:45 and 12:30 hrs) for high resolution depth profile analysis. These cores were frozen on site (-20°C) and the upper 10 mm of each core was finely sectioned into 200 μm thick slices using a freezing microtome (Leitz Kryomat 1703 microtome) at a temperature of -40°C. Eight depth intervals (0-200, 200-400, 400-600, 1000-1200, 2000-200, 4000-4200, 6000-6200, 10000-10200 μm) were analysed for bulk and colloidal carbohydrate concentrations. In addition to short-term studies, surface sediment samples (10 mm) were collected daily, one hour after low tide, from upper and mid-intertidal sites for nine days. The tidal stage was moving towards neap tides at the beginning of this measurement period and was returning to a spring tide on the 9th measurement day.

Colloidal carbohydrates concentrations were also measured on the Eden Estuary, Fife (56° 22' N 02° 51' E). In this study, the surface sediments were sampled over the emersion period at two upper-intertidal sites (site 1 on 20th and site 2 on 22nd of April 1996, Figure 4.2). At site 1, the sediment was smooth and many burrowing amphipods (*Corophium volutator)* were observed. The sediment surface of site 2 appeared rough and high densities of *Arenicola marina* and *C. volutator* were observed. Sediment samples were collected by rapidly freezing the mud flat surface using liquid nitrogen vapour (LN_2) followed by LN_2, (liquid) using the cryolander method of Wiltshire *et al.* (1997). Four replicate

Figure 4.2 Location of the sampling sites on the Eden Estuary, Fife.



cryolanded-samples were taken at 90 min intervals and 5 intervals were sampled during the emersion period. One sample from each time interval was thin-sectioned to a depth of 1400 μm in a pre-determined series (0-200, 200-400, 400-600, 600-800, 1200-1400 μm). The upper 600 μm of the other 3 replicates were analysed for colloidal carbohydrates, water content, sediment wet density and dry mass concentration. Measurements of critical erosion threshold were made using the Cohesive Strength Meter on adjacent sediments (work carried out by D.M. Paterson).

4.1.1 Low-temperature scanning electron microscopy (LTSEM)

Low-temperature scanning electron microscopy (LTSEM) was used to visualise surface sediment samples from the Eden Estuary. LTSEM examinations were carried out on the same cryolanded sediment samples as carbohydrate analyses. All samples were stored under LN_2 until examined (Paterson 1986) using a SEM (Jeol JSM 35CF), modified for cryogenic examination (Oxford Instruments CT1500). LTSEM of samples prevents water loss and preserves the structure of hydrated material (Paterson 1995, Défarge 1997).

4.1.2 Protocol for carbohydrate assays

The frozen sediment sections were collected in pre-weighed Eppendorfs containing 300 μl of distilled water and the wet weight of each was recorded. The sample was mixed and centrifuged at 2500 g for 15 minutes and 200 μl of the supernatant was removed for estimation of carbohydrate concentration using the phenol-sulphuric acid assay (Dubois *et al.* 1956, see Chapter 2). The pellet was then lyophilised and the dry weight taken, to allow dry mass concentration and carbohydrate concentration (g^{-1} dry weight) to be calculated.

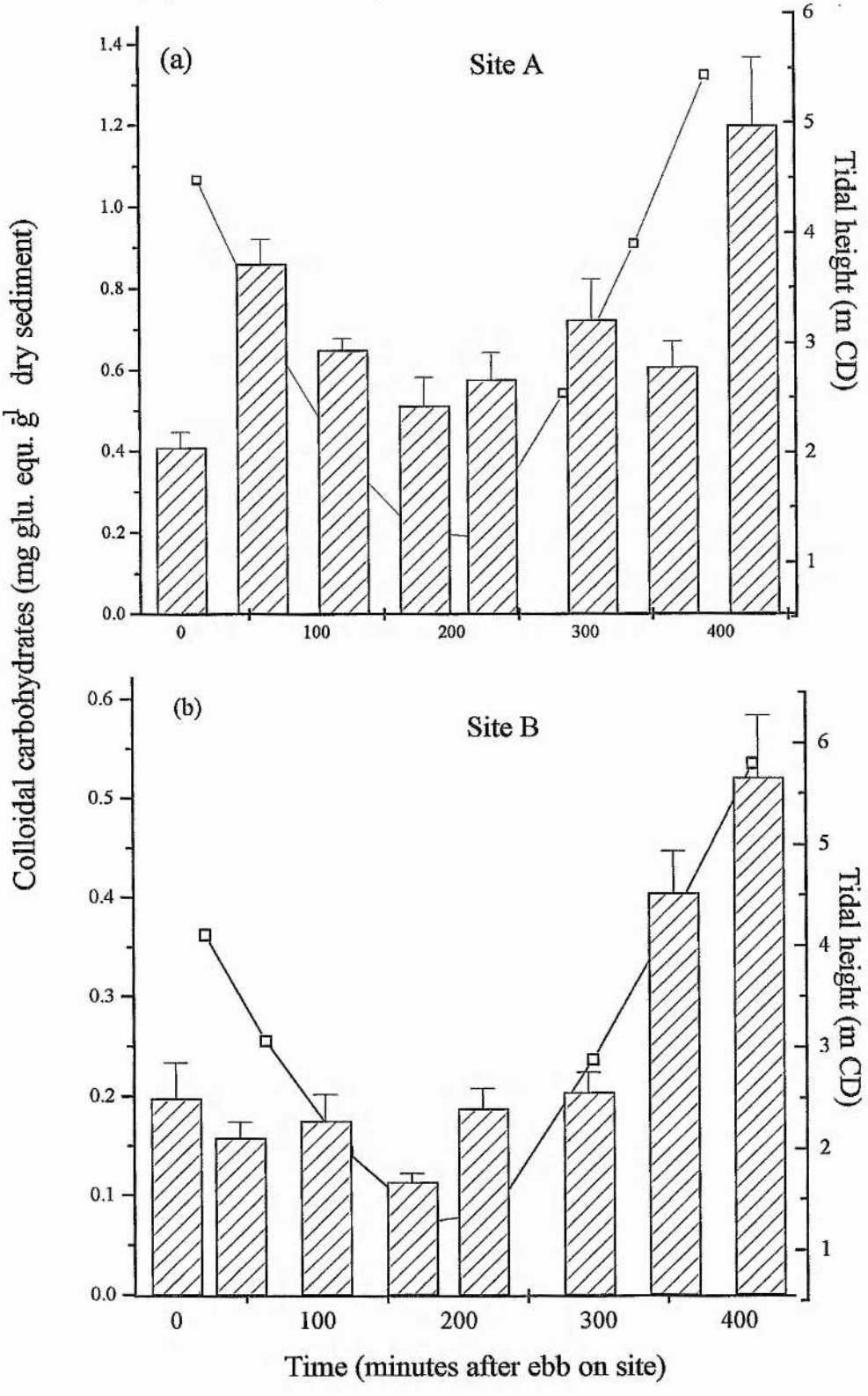
4.2 Results

4.2.1 Variation of carbohydrate concentrations over the emersion period

4.2.1.1 Humber Estuary

Hourly measurements of surface colloidal carbohydrate concentrations revealed significant increases over the sampling period at the upper-intertidal station from a mean of 0.4 to 1.2 mg glu. equ. g^{-1} dry sediment ($F_{7,24} = 8.3$, $P = 0.000$, one-way ANOVA, Figure 4.3a) and similar increases were observed at

Figure 4.3 Colloidal carbohydrate concentrations (columns) and tidal height (line) over the emersion period on the Humber Estuary (bars = S.E. n = 4)



the mid-intertidal station ($F_{7,24} = 17.95$, $P = 0.000$, Figure 4.3b). The colloidal carbohydrate concentrations increased sharply towards the end of the emersion period at both upper and mid-intertidal stations. High-resolution depth profiles of colloidal carbohydrates in the upper 10 mm, at the upper intertidal station, showed a concentration gradient to exist, with highest concentrations at the surface (200 μm) (Figure 4.4). A single depth profile was taken at 3 time intervals and carbohydrate concentrations increased with time at 0 - 200, 200 - 400, 400 - 600, 1000 - 1200, 2000 - 2200, 4000 - 4200 but not at 6000 - 6200 or 10000 - 10200 μm. The increases with time were greatest on the surface and decreased with depth.

The concentration of bulk carbohydrates did not significantly change over the tidal emersion period at the upper or mid-intertidal stations (Figure 4.5, $F_{7,24} = 1.38$, $P = 0.3$; $F_{7,24} = 2.15$, $P = 0.07$ respectively).

4.2.1.2 Eden Estuary

Sediment samples of the upper 600 μm collected over the emersion period at the Eden Estuary showed colloidal carbohydrate concentrations to increase as the 7 h exposure period progressed (Figure 4.6). This increase was found to be statistically significant at both sites (20/4/96, $F_{4,8} = 10.41$, $P = 0.003$; 22/4/95, $F_{4,10} = 7.17$, $P = 0.005$). However, at site 1, there was an obvious decrease in concentration at the last measurement and colloidal carbohydrate concentrations did not show a continual increase but showed some variation throughout the emersion period. The increase in concentrations at site 2 was more gradual than at site 1 and at the sites on the Humber.

A depth profile of colloidal carbohydrate concentrations was measured at 6 time intervals at site 1 and 5 time intervals at site 2 and are shown in Figure 4.7. At site 1 (4.7a), the lowest colloidal carbohydrate values were in the depth profile taken at 08:25 hrs and the highest concentrations were measured in the depth profile at 13:00 hrs. This was also shown in the colloidal carbohydrate measurements of the upper 600 μm (Figure 4.6). Values were most variable in the upper 200 μm and least variable at the lowest depth interval (1200 μm). At site 2 (4.7b), colloidal carbohydrate concentrations were generally lower than at site 1, in common with the measurements of the upper 600 μm (Figure 4.6).

Figure 4.4 Temporal and spatial study of colloidal carbohydrates on the Humber Estuary

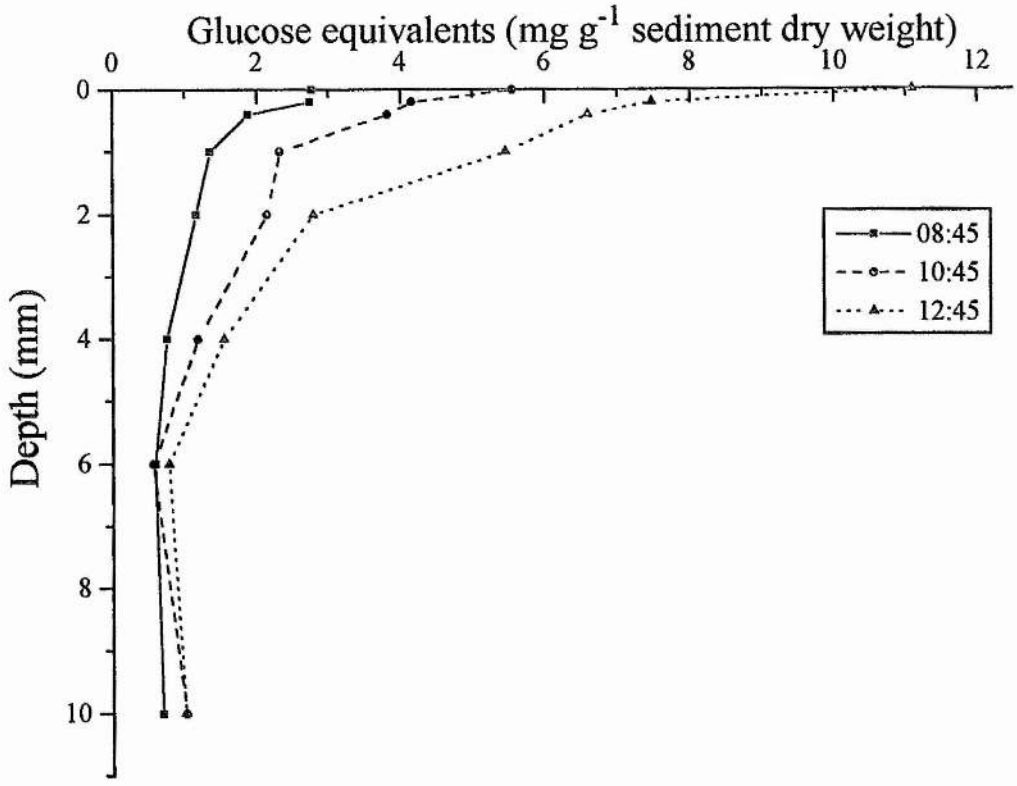


Figure 4.5 Concentrations of bulk carbohydrate (columns) and tidal height (line) over the emersion period on the Humber Estuary (bars = S.E. n = 3)

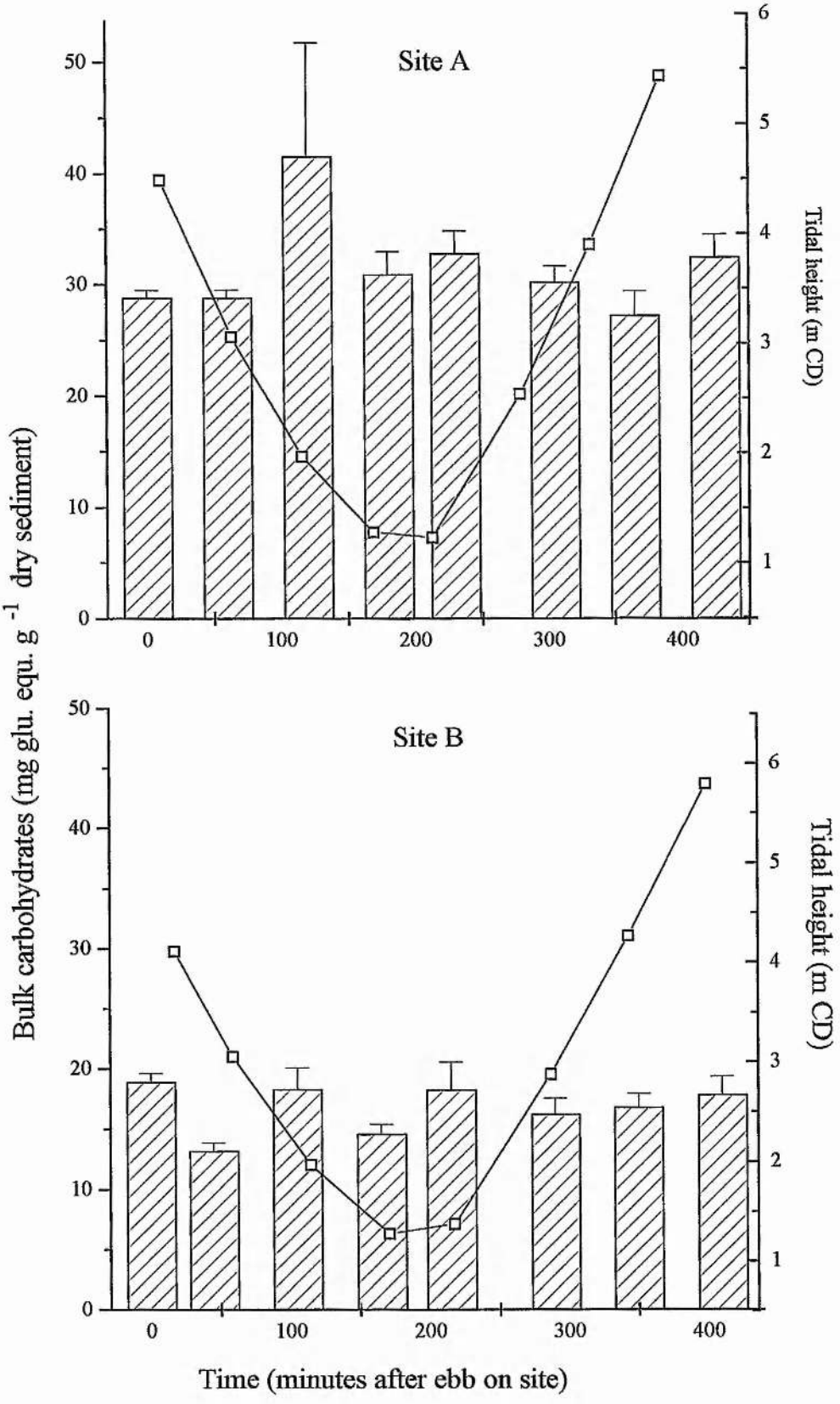


Figure 4.6 Colloidal carbohydrate concentrations (upper 600 μm) over emersion period on the Eden Estuary (bars = S.E. n = 3)

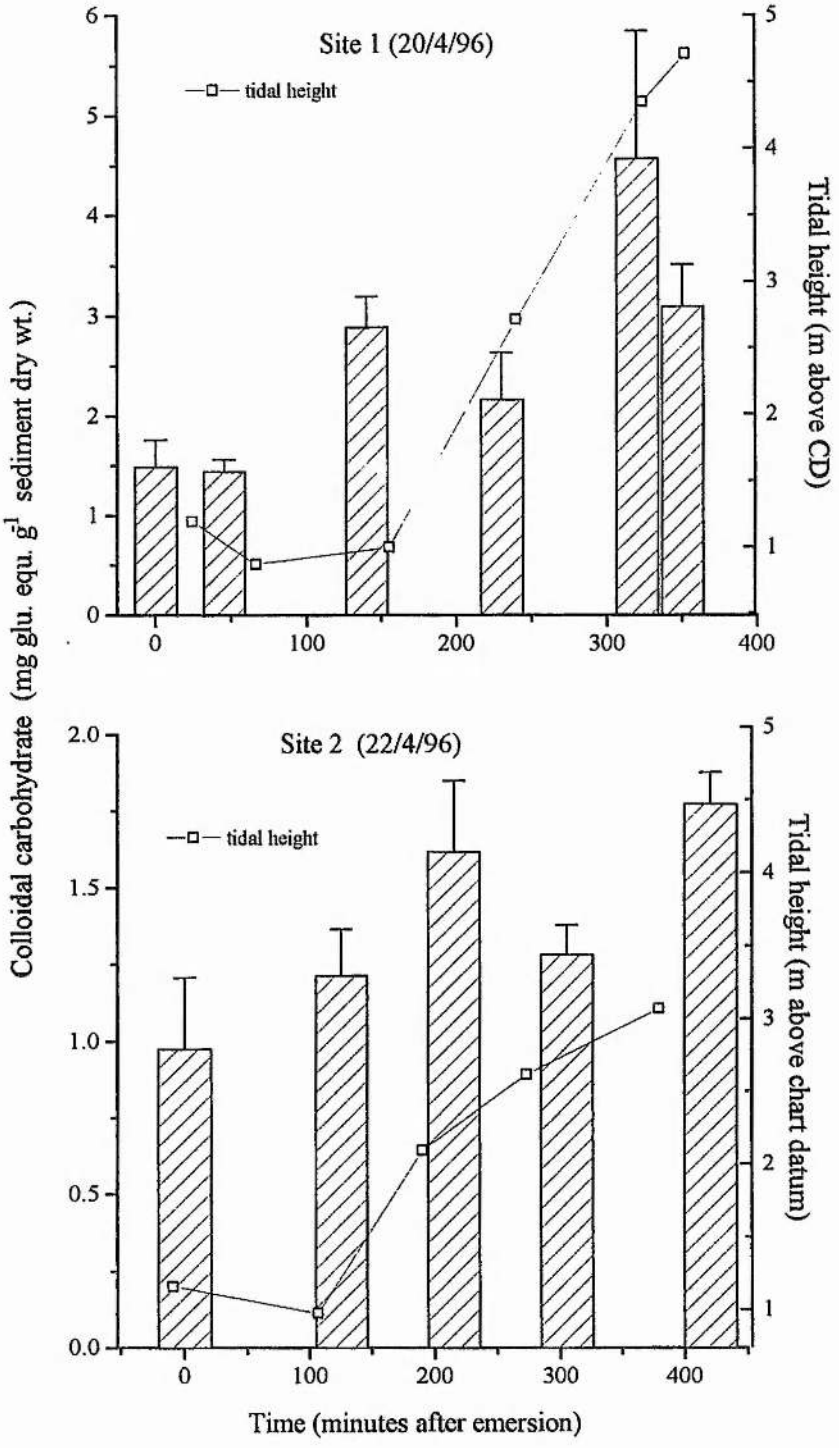
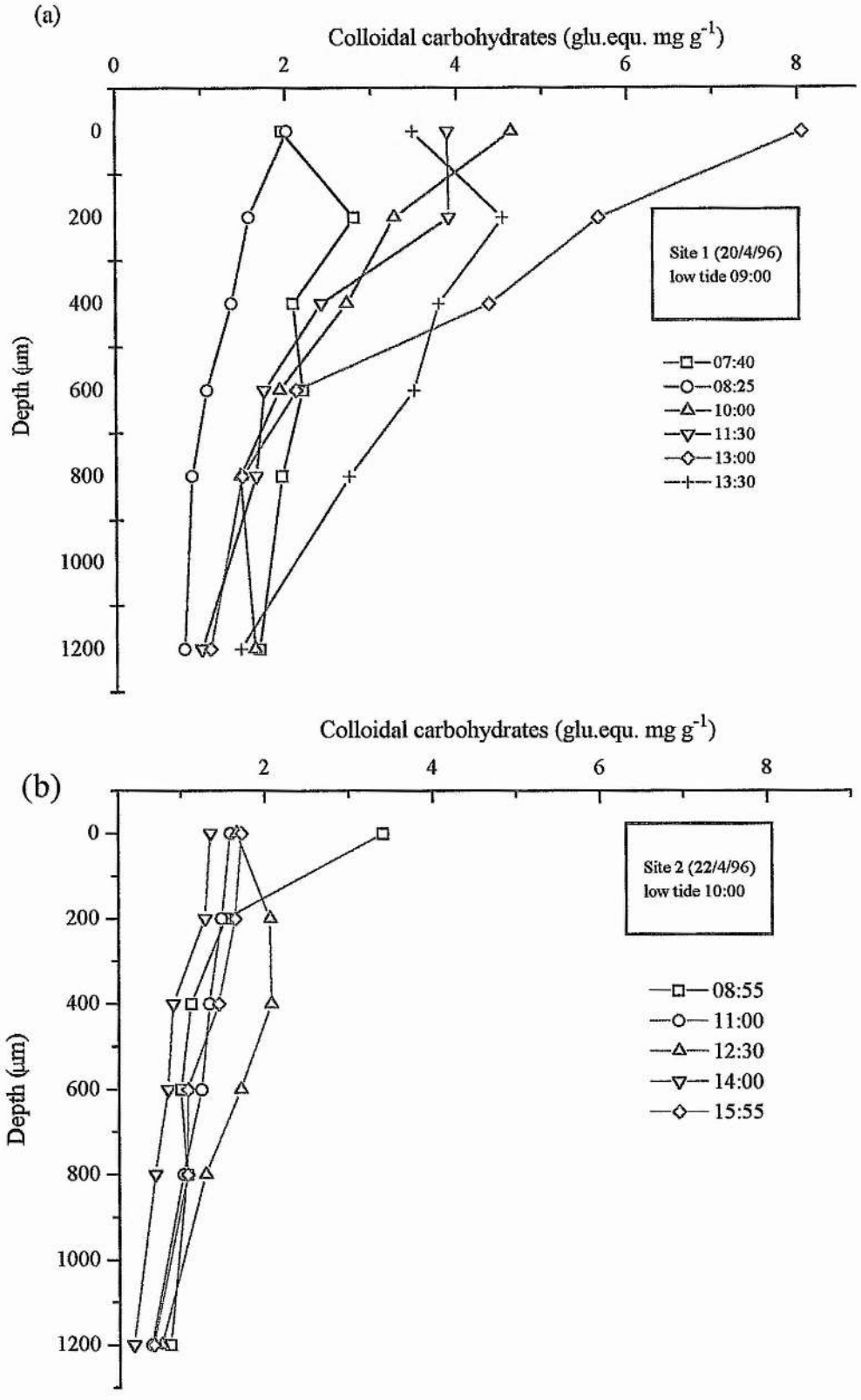


Figure 4.7 Microscale depth profiles of colloidal carbohydrate distribution over tidal emersion period



In addition, there was less variability in colloidal carbohydrate concentrations between time intervals. The highest concentration was measured at the first time interval (08:55 hrs), contrary to measurements of the upper 600 μm, where the highest concentrations were recorded at the last time interval (15:55 hrs). The variation in colloidal carbohydrate concentrations with time were more accurately recorded in measurements of the upper 600 μm since 3 replicates were analysed.

4.2.2 Measurements of physico-chemical factors over the emersion period

Measurements of sediment temperature and light (photosynthetically active radiation, P.A.R.) over the emersion period are summarised in Table 4.1. Primary production on the Humber mud flats (measured as ^{14}C incorporation) was correlated to light intensity (Underwood and Smith 1998a, Table 4.2).

Table 4.1 *Variations in light and temperature over the emersion period.*

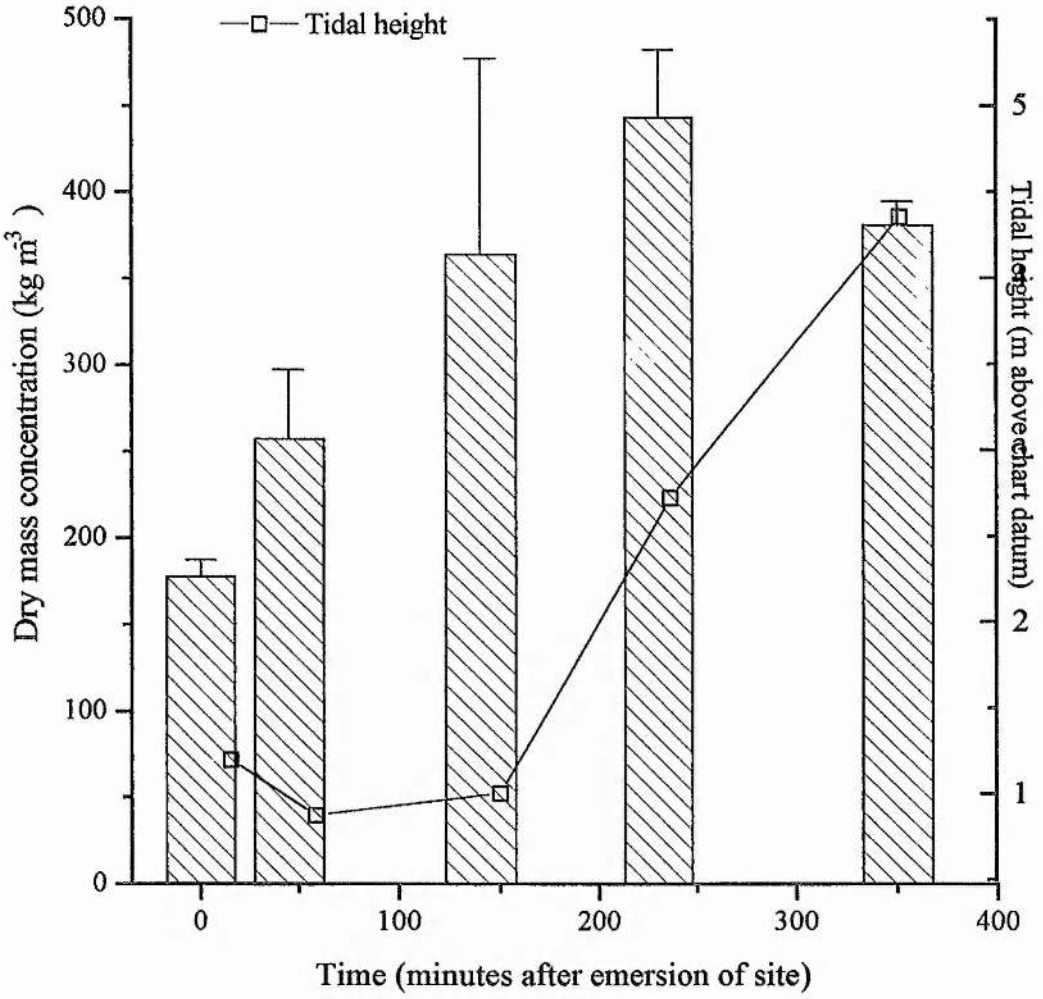
Humber (15.4.95)			Eden Estuary (20.4.96)		
Time	Light PAR μmol m^{-2} s^{-1}	Sed. Temp.(°C)	Time	Light PAR μmol m^{-2} s^{-1}	Sed. Temp.(°C)
08:45	1170	8	07:40	100	7
09:45	845	9	08:25	300	9
10:45	1080	9	10:00	200	11
11:45	2240	9	11:30	800	13
12:35	1910	10	13:30	300	12
13:50	1390	12	-	-	-
14:50	2060	12	-	-	-
15:50	787	12	-	-	-

Table 4.2 *Measurements of primary productivity and light intensity on the Humber Estuary (from Underwood and Smith 1998a).*

Time (hh:mm)	*Light (W m^{-2})*	*Primary production (mg C m^{-2} h^{-1})*
13:30	210	80
14:30	140	40
15:30	90	20
16:30	40	20
17:30	20	7

Wet sediment density did not vary significantly over the tidal cycle on the Humber (Wiltshire *et al.* 1996), or at sites 1 and 2 on the Eden Estuary ($F_{4,8} = 0.9$, $P = 0.5$; $F_{4,10} = 0.5$, $P = 0.7$). However, dry mass concentration values increased significantly at site 1 (Figure 4.8, $F_{4,8} = 5.7$, $P = 0.02$) but decreased again at the last time interval.

Figure 4.8 Increase in dry mass concentration over emersion period at Site 1 (20/4/96) on the Eden Estuary (bars = S.E. n = 3)



Dry mass concentration did not vary significantly at site 2 ($F_{4,10} = 0.3$, $P = 0.9$). There was a significant decrease in sediment water content over the emersion period at sites 1 and 2 on the Eden Estuary (Figure 4.9, $F_{5,14} = 6.7$, $P = 0.002$; $F_{4,15} = 3.87$, $P = 0.02$ respectively, one way ANOVA on log10 transformed data). Concentrations of Chlorophyll *a* did not vary significantly over the emersion period on the Humber Estuary (Underwood and Smith 1998a) or Eden Estuary (Sevecke and Krumbein 1996).

4.2.3 Extracellular enzyme activity

The activities of extracellular enzymes (α and β-glucosidase, esterase and leucine aminopeptidase) measured in the slurry samples from Skeffling during the tidal emersion period of 15/4/96 were below detection limits. However, enzyme activities were measured in sediment samples collected during the tidal cycle of 18/4/96 and were found to increase over the emersion period following a temperature-dependant response (Figure 4.10, Ruddy *et al.* 1996).

4.2.4 Sediment stability

During the emersion period of 15/4/96, an increase in surface stability was recorded within the first block of measurements on the Humber Estuary (Figure 4.11a). The sediment was more stable in the second block of measurements but stability decreased rapidly coinciding with a period of rain. The third block of measurements shows the stability recovering to the level recorded at the end of the first period. This data suggests that intertidal sediment stability increases over the emersion period but can be greatly affected by precipitation during exposure. For comparison, stability measurements recorded at the same site during an earlier emersion period (11/4/96) with no precipitation are shown in Figure 4.11b. These measurements were taken 3 days earlier, and show sediment stability to increase dramatically at the beginning of the emersion period and continued to increase, in the absence of rain, until the onset of the flood tide.

On the Eden Estuary, some variability in sediment strength was measured but there was no overall change in stability over the emersion period (Paterson pers. comm.).

Figure 4.9 Variation in % water content of the upper 600 μm of intertidal sediments of the Eden Estuary over the emersion period (bars = S.E. n = 3)

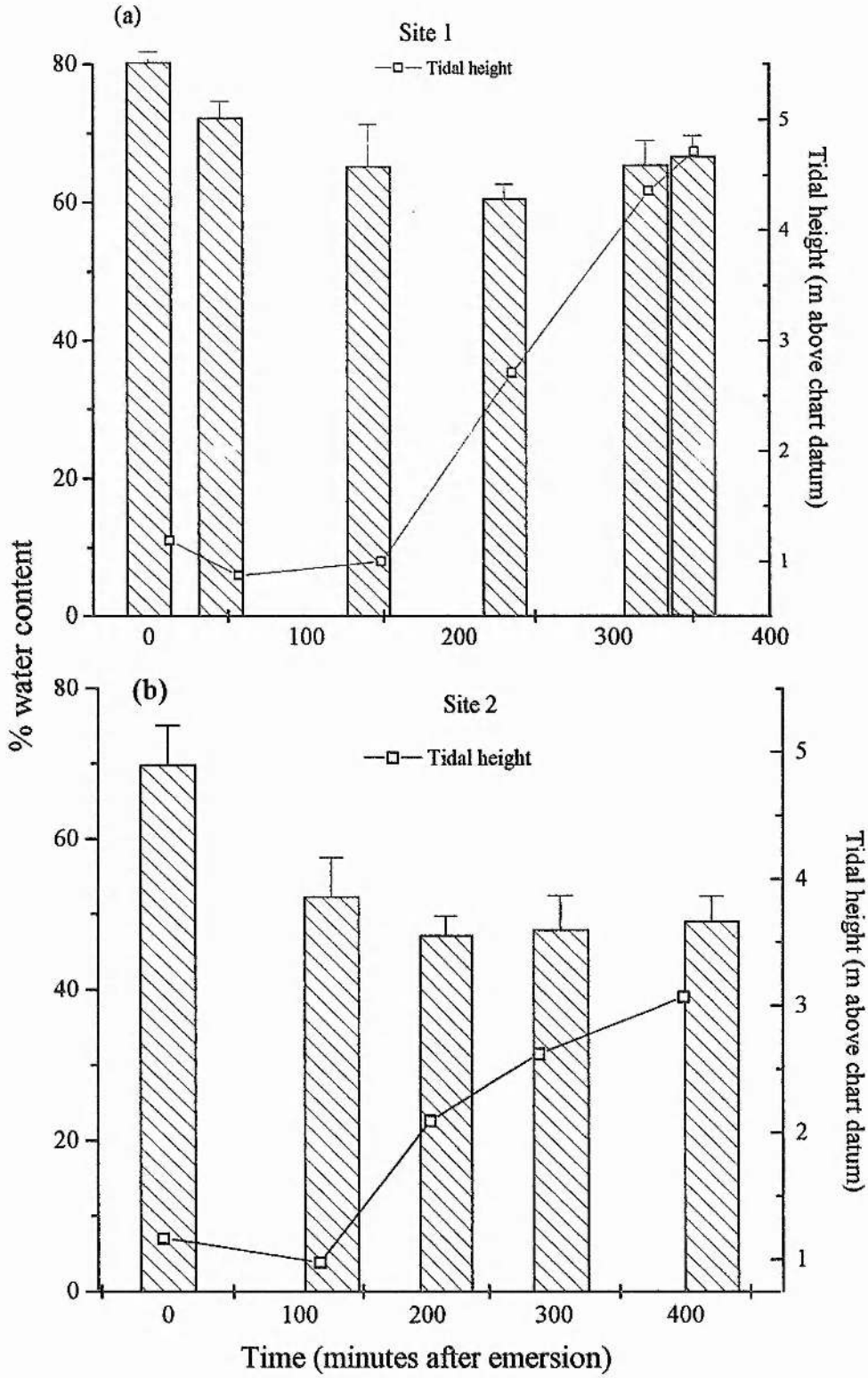


Figure 4.10 Temporal variation in extracellular enzyme activity and temperature over emersion period (Humber Estuary 18.4.95, T^0 = 11:00 hrs data from Ruddy and Turley 1996)

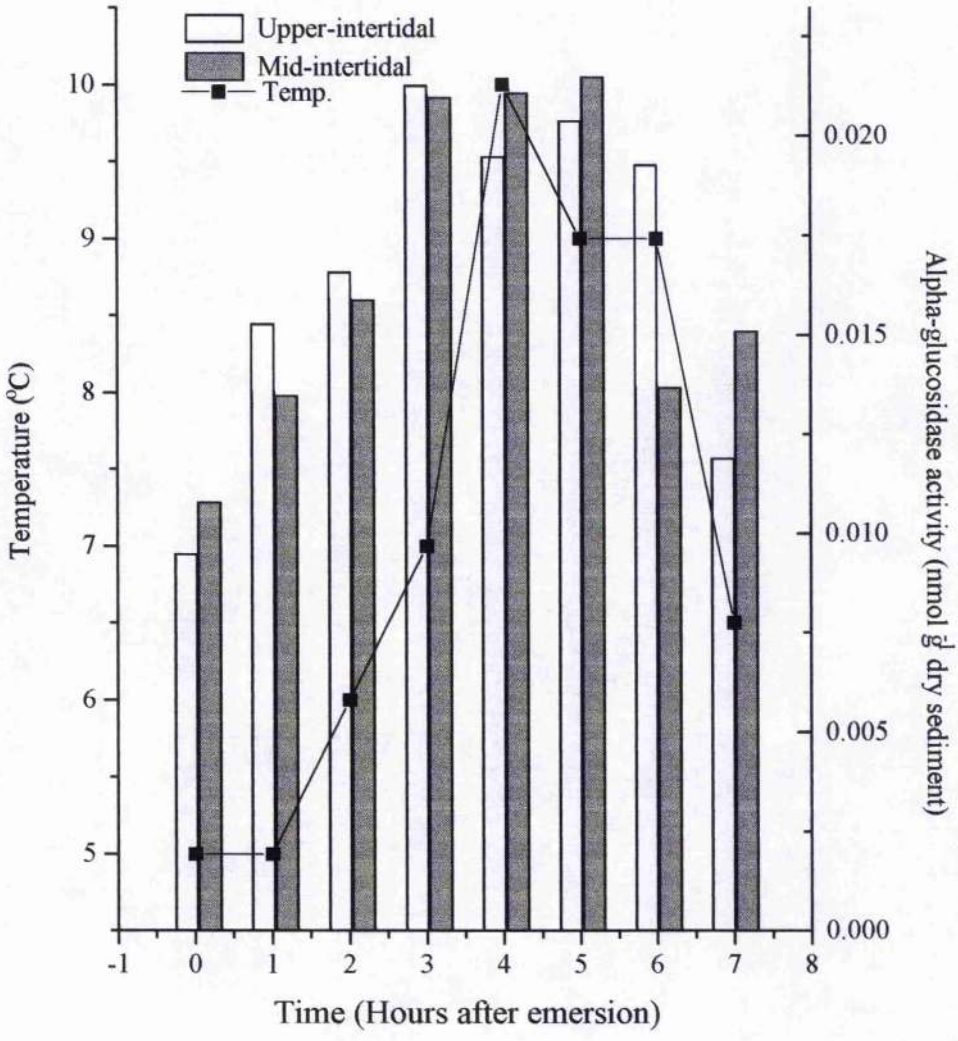
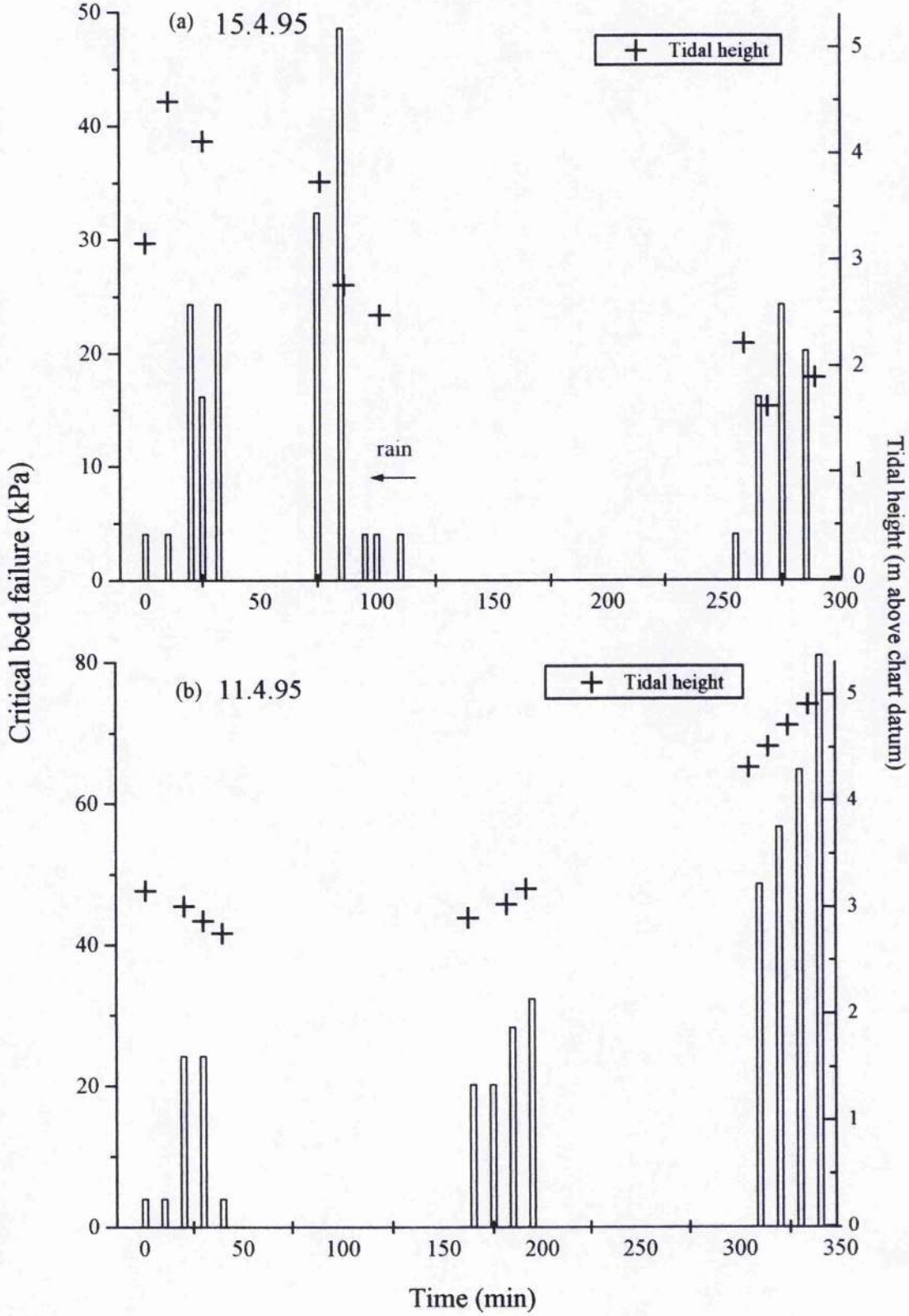


Figure 4.11 Sediment stability over the emersion period on the Humber Estuary



Sediment samples from the Eden Estuary were examined using LTSEM and several epipelic diatoms were observed associated with EPS (Figure 4.12). Light microscopy found the assemblage was dominated by *Diploneis crabro* (Droop pers. comm.) with *Navicula* sp., *Nitzschia* sp., *Surirella* sp. and *Rhoicosphenia curvata* also present.

4.2.5 Measurements of daily variation

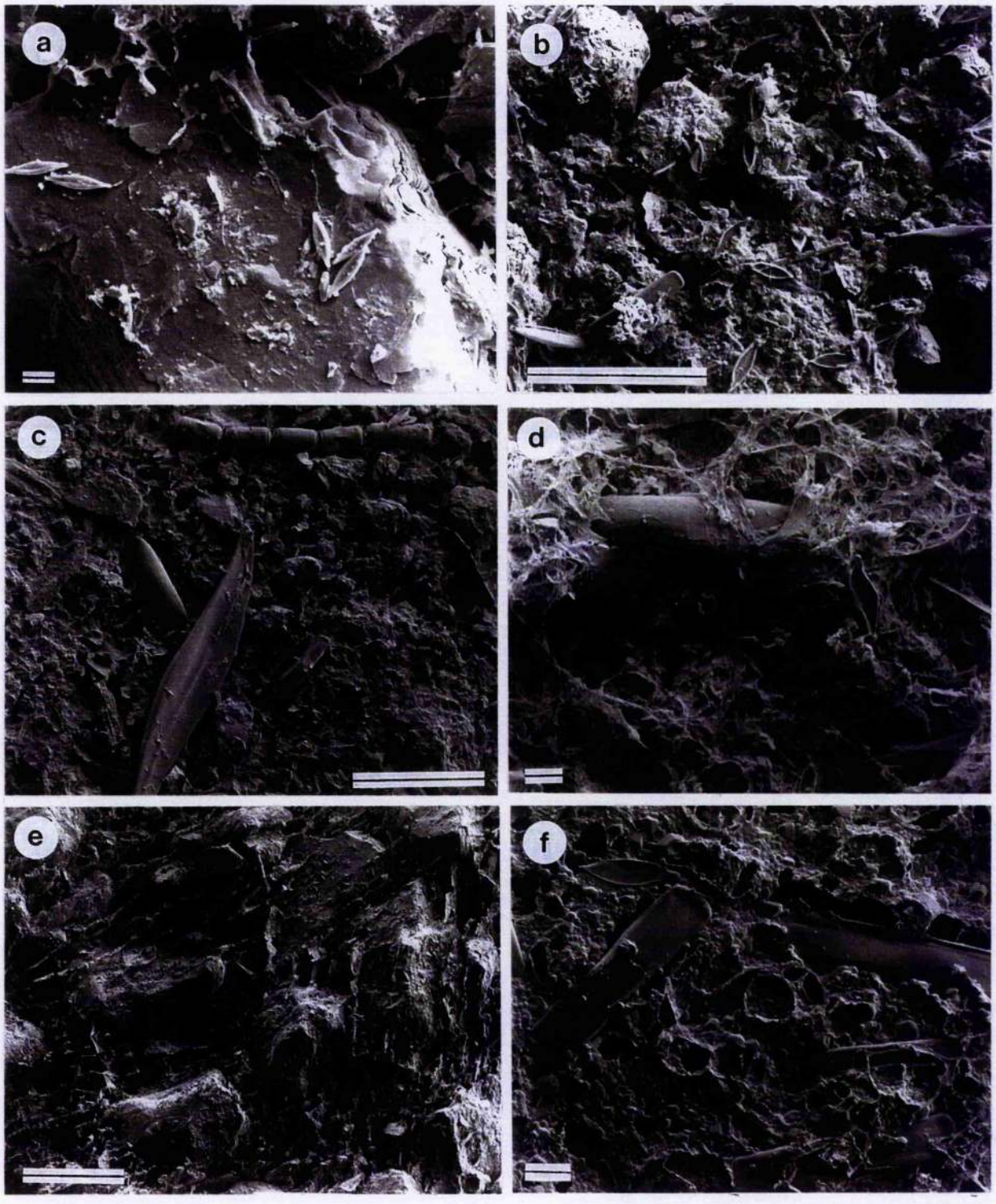
Sediment samples were collected daily for nine days from the upper and mid-intertidal sites on the Humber Estuary. There was a dramatic drop in colloidal carbohydrates from values of 1.9 ± 0.4 (SD) and 0.3 ± 0.04 mg glu. equ. g^{-1} dry sediment (upper and mid-intertidal respectively, $n = 5$) on day 1 to undetectable levels the following day. Furthermore, on the second day it was noticed that microphytobenthic populations failed to migrate to the surface during the emersion period. Colloidal carbohydrates were not detectable again until the final sampling day (day 9, upper -intertidal; 0.7 ± 0.2 (S.D.) and mid-intertidal; 0.24 ± 0.14 mg glu. equ. g^{-1} dry sediment, $n = 5$).

4.3 Discussion

With each tidal exposure, surface water drains off intertidal mud flats and sediment moisture content often decreases. During emersion, the mud flats and organisms dwelling within them are exposed to climatic changes such as rain and temperature fluctuations (Rasmussen *et al.* 1983). The migration of diatoms onto the surface occurs very shortly after the tide recedes (Paterson *et al. in review*). Photosynthesis increases with greater light intensities and temperatures towards mid-day (Underwood and Smith 1998a, Miles and Paterson 1996). Likewise, extracellular enzyme activities vary directly with temperature (Ruddy *et al.* 1996).

During the daylight emersion periods, there were significant increases in colloidal carbohydrate concentrations in the upper 10 mm at upper and mid-intertidal sites on the Humber Estuary. Similar increases in colloidal carbohydrates with time were shown to occur over the emersion period in the upper 600 μm of the Eden Estuary sediments. However, these increases were not continuous but varied within the emersion period. The variation in colloidal concentrations over the emersion period may be due to several factors. Firstly,

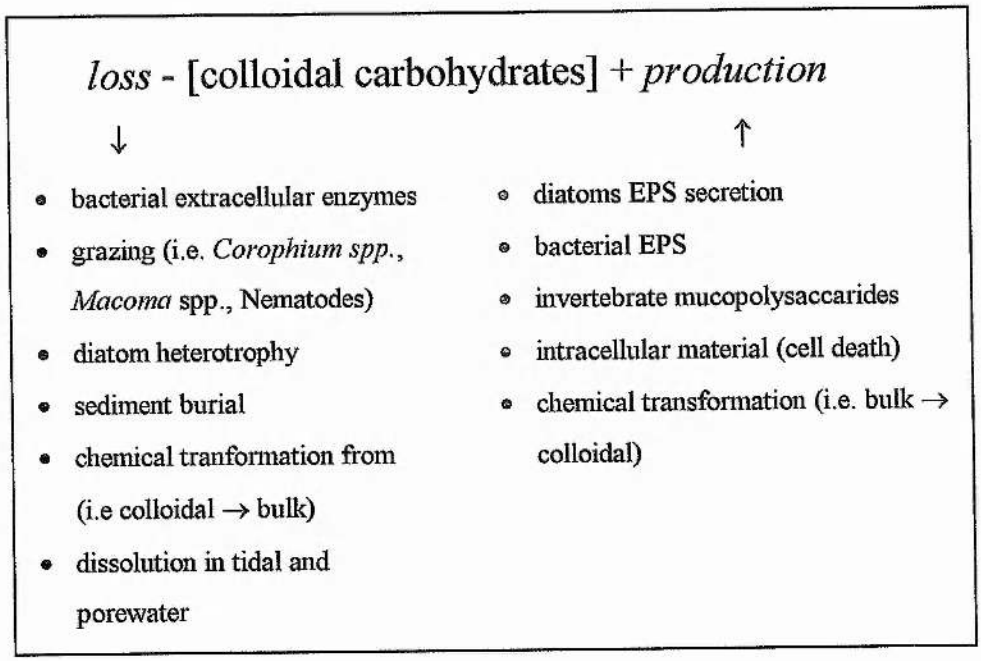
Figure 4.12 Low-temperature scanning electron micrographs of surficial sediments from the Eden Estuary. (a) Epipelic diatoms were associated with surface clay minerals and the sediment matrix (scale bar 10 μm). (b) A mixed assemblage of epipelic diatoms were present between sediment particles and organic debris (scale bar 100 μm). (c) *Gyrosigma sp.*, a chain of *Melosira sp.*, and other smaller epipelic forms connected to smaller particles by a thin fibrous matrix (scale bar 100 μm, negative courtesy of Dr Karen Wiltshire). An organic matrix was visualised in several different forms including thin fibrils (c) and (d) thick extensive sheets surrounding the diatom cells (scale bar 10 μm). EPS may also have been held within thin sheets (e) of pore water and between sediment particles (scale bar 100μm) and (f) as a globular matrix in which diatom cells were embedded (scale bar 10 μm).


a
b
c
d
e
f

more extracellular carbohydrates may be released as a result of increases in the photosynthetic rate of diatoms over daytime emersion (Rasmussen *et al.* 1983, Miles and Paterson 1996, Smith and Underwood 1998b). This is balanced by loss processes (Figure 4.13) such as extracellular breakdown of polysaccharides and the uptake of small sugars by bacteria and, at low light levels by diatoms themselves (Paterson *et al* 1996b).

Fluctuations in the concentration of colloidal carbohydrates over the emersion period may also occur due to changes in cell numbers on the surface (Palmer and Round 1967). Happey-Wood and Jones (1988) found cells migrate towards the surface after tidal emersion and increase to a peak at mid-emersion. In addition, they recorded higher diatom velocities corresponding with the period of tidal emersion, suggesting that there may be differential EPS production with migration. Sediment cores removed from the tidal influence showed higher EPS production during the time corresponding with emersion than during immersion (Smith and Underwood 1998b). However, it seems unlikely that the increase in carbohydrates concentrations measured at Skeffling are entirely due to the peak in cell numbers, since surface cell numbers generally decrease rapidly as diatoms move downwards into the sediment 1-2 hours before the onset of the tide (Happey-Wood and Jones 1988) and carbohydrates were shown to continue to increase until the end of emersion at Skeffling and at site 2 at the Eden Estuary. However, colloidal carbohydrates were shown to decline at the last time interval at site 1 on the Eden Estuary. Smith and Underwood (1996) and Sevecke and Krumbein (1996) found no increase in chlorophyll *a* concentrations (as a measure of biomass) over the emersion period. The high concentrations in colloidal carbohydrates measured at the end of emersion at Skeffling and at site 2 at the Eden Estuary could represent a time lag between peak periods of photosynthetic activity and carbohydrate secretion. Alternatively, elevated carbohydrate concentrations may have occurred as a result of changes in physico-chemical conditions of surface sediments, for example, salinity and pH increase and CO_2 availability is known to decrease significantly towards the end of the emersion period (Rasmussen *et al.* 1983). Such changes may induce an increase in secretion of EPS from diatom cells prior to downward migration (de Winder *et al. submitted*).

Figure 4.13 Processes which influence the transient pool of colloidal carbohydrates on surface sediments.



In addition, carbohydrates may concentrate on the surface layer through physical evapo-transpiration processes of surface waters and therefore may not represent a real increase (Paterson pers comm.). However, for this to happen colloidal carbohydrates would have to be drawn from lower depths and this was not seen to be the case (Figure 4.4). Increased salinity and pH, combined with a decline in temperature, may cause a reduction in the rate of loss processes and explain elevated colloidal carbohydrate concentrations at the end of the emersion period at Skeffling and at site 2 at the Eden Estuary.

There was no change in water content or sediment density over the exposure period on the Humber Estuary, yet, there were significant increases in the critical erosion threshold. This suggests that stabilisation was not the result of physical drying processes. However, further studies are needed to determine if there is a correlation between colloidal carbohydrate concentration and short-term changes in sediment stability.

The sediment density increased over the tidal exposure period at site 1 on the Eden Estuary but remained unchanged at site 2. This may reflect re-working of the upper millimetres of the sediment by *Corophium volutator* since numbers recorded at site 2 (9300 m^{-2}) were almost double those of site 1 (5000 m^{-2}, Ford pers. comm.). Furthermore, concentrations of colloidal carbohydrates were much lower in the sediments with higher *C. volutator* numbers, this is probably due to predation of the diatoms by *C. volutator*. Individual animals were estimated to consume between 2150 and 3767 diatom cells per day (Smith *et al.* 1996). This level of predation would obviously effect diatom primary productivity and the abundance of colloidal carbohydrates on surface sediments.

There was no change in bulk carbohydrate concentration over the same period and measurements of the carbohydrate fractions over the emersion period suggest that colloidal and bulk carbohydrates have different origins and turnover rates since the changes which occur over the short-term (i.e. the emersion period) are entirely in the colloidal carbohydrate fraction. Colloidal carbohydrates may represent a more dynamic fraction and stronger indicator of epipelic diatom metabolism than the bulk fraction. The bulk material is associated with the sediment particles rather than with pore water and is more refractory. When

colloidal carbohydrates are available, they will be preferentially broken down by bacteria (and diatoms, Paterson *et al.* 1996b) leaving the more complex organic materials including bulk carbohydrates to accumulate.

Daily measurements of colloidal carbohydrate found a dramatic decrease after the first day, which coincided with a visual reduction in diatom numbers on the mud flat. It is likely that meteorological conditions were the reason for the crash. Air temperatures on the afternoon of the first day (7/4/95) reached 18 °C however, air temperatures fell to 2 °C, corresponding with low tide at night. Studies have shown that epipelic diatoms migrate into the sediment surface and do not re-surface during night emersion periods however, such an extreme drop in temperature may have affected buried diatoms.

4.3.1 Conclusions

Colloidal carbohydrate concentrations were shown to increase significantly towards the end of the emersion period at Skeffling and at site 2 at the Eden Estuary. However, at site 1 on the Eden Estuary, colloidal carbohydrate concentrations did not increase but fluctuated over the emersion period. The increase in carbohydrates may reflect a time lag following peaks in photosynthetic activity or changes of pH, salinity, CO_2 availability and sediment density. Bulk carbohydrates did not vary over the same time period at Skeffling.

Sediment critical erosion velocity increased at the end of the emersion period at Skeffling and may reflect increased carbohydrate concentrations and subsequent inter-grain binding, since there was little change in the sediment density with time at Skeffling. Colloidal carbohydrates were shown to vary significantly on a time-scale of hours in response to meteorological conditions. Episodic storms or rapid temperature changes obviously have a greater effect on colloidal carbohydrates than short-term changes over the emersion period and their effects may even exceed seasonal variation.

Chapter 5

5. Introduction

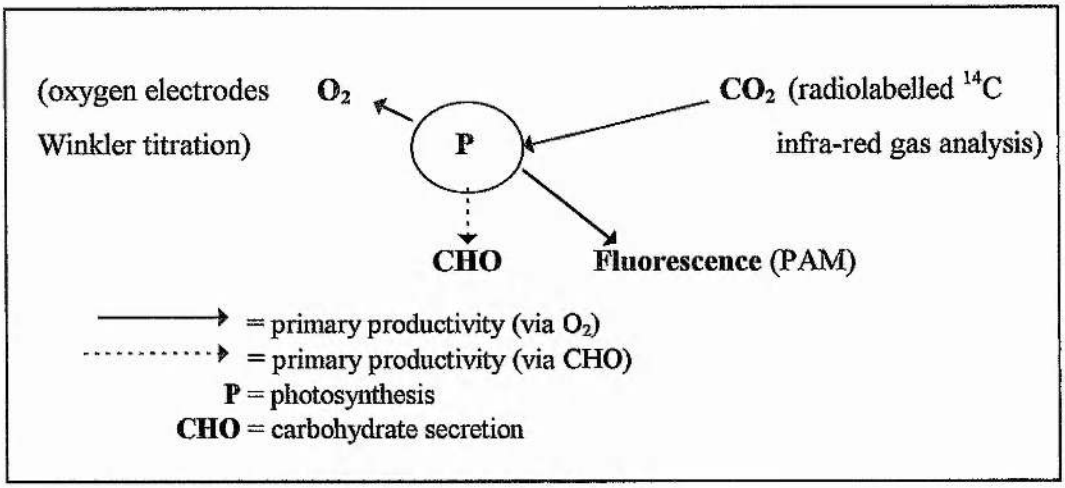
Epipelic diatoms contribute significantly to the annual primary productivity of estuarine ecosystems (Pinckney and Zingmark 1991). In addition, the fixation of CO_2 and the subsequent production of biomass will determine the type and abundance of heterotrophic organisms present in the ecosystem.

In Chapter 4, colloidal carbohydrates were shown to increase over the intertidal emersion period and it was suggested that this may be partly due to a midday peak in photosynthetic productivity. This Chapter describes laboratory experiments in which measurements of colloidal carbohydrate and biomass (as chlorophyll *a*) were made from sediment cores placed under selected light intensities. Complementary microsensor analysis of oxygen concentrations and gross primary productivity was conducted and net primary productivity was modelled.

The laboratory experiment was followed by a field study, where *in situ* measurements of colloidal carbohydrates and chlorophyll *a* were made over the emersion period on the intertidal mud flats of Baie de Marennes-Oléron, France. In the field studies described in Chapter 4, algal biomass values were provided by colleagues and were often obtained at inappropriate scales and therefore, were of less direct relevance . However in this Chapter, chlorophyll *a* measurements were coupled with fine-scale depth profiles of colloidal carbohydrates to provide a greater understanding of the relationship between primary productivity and colloidal carbohydrate concentrations on a relevant scale.

Primary productivity can be measured in several ways, usually through the production of oxygen molecules or the consumption of CO_2 (Figure 5.1). Micro-techniques with a high spatial resolution are required to measure the fluxes of these gases in the environments which microbial autotrophs live. High resolution techniques are especially important since microorganisms with different metabolic requirements can co-exist in biofilms and stratified assemblages less than a millimetre thick (Pinckney *et al.* 1995).

Figure 5.1 *Methods used for measuring primary productivity*



Benthic photosynthesis was investigated using microelectrodes developed at the Institute of Microbial Ecology, Aarhus, Denmark. Electrodes capable of measuring oxygen at a micro-scale were first introduced into the field of microbial ecology in the late 1970's, although they have been used in the field of human neurophysiology since the 1950's (Revsbech and Jørgensen 1986).

The application of microelectrodes has provided new information on oxygen production and consumption by micro-organisms (Revsbech 1989a, Jørgensen and Des Marais 1990, Lorenzen *et al.* 1995). Sediment oxygen concentrations have been shown to be positively related to light intensity due to increased photosynthetic productivity (Revsbech and Jørgensen 1981). Therefore, the depth of the oxic layer in sediments varies between light and dark conditions (Revsbech 1989a). Other microsensors have been developed to measure fine-scale changes in nitrate, nitrous oxide, sulphide and methane concentrations (Revsbech 1989a, Larsen *et al.* 1997) and light microsensors, utilising fibre optic technology, provide valuable information on the penetration depth of light and spectral quality into the surface of intertidal sediments (Kühl and Jørgensen 1994).

The relationship between light intensity and photosynthesis can be measured by constructing a photosynthetic-irradiance (*P-I*) curve (MacIntyre *et al.* 1996, Kühl *et al.* 1997) which shows an exponential increase in photosynthetic rate corresponding to an increase in light intensity until a saturation point is

reached whereby the photosynthetic mechanism is limited by factors other than light energy. There is a need for more information on the influence of light intensity on the production of extracellular carbohydrates by micro-algae (Smith and Underwood 1998b). The migration of epipelic diatoms occurs, partly, in response to light levels and the secretion of EPS for locomotion is therefore, dependant to a certain extent on light intensity (Palmer and Round 1967). However, diatoms can retain locomotive ability despite several days in darkness (Paterson 1986, Hay *et al.* 1993). This suggests that energy from primary productivity must be stored as a cellular reserve (probably glucan) and can be exploited for a considerable period after the cessation of carbon fixation (Smith and Underwood 1998b). Therefore, a direct relationship between EPS (or colloidal carbohydrates as an index of EPS) and light may be unlikely. However, under conditions where light is in excess, there may be "luxury" production of EPS as a way of removing excess photosynthate, resulting in higher rates of secretion and possibly locomotion. This implies that daily and seasonal variations in light intensity would influence the production of colloidal carbohydrates and in turn affect heterotrophic metabolism and sediment stability.

The primary objectives of this study were, therefore, to become competent in the use of microsensors and to gather technical knowledge in their operation. Secondly, conduct laboratory experiments under controlled conditions which would provide a greater understanding of primary productivity, oxygen and colloidal carbohydrate concentrations in relation to light intensity. Finally, to measure temporal and microspatial relationships between colloidal carbohydrates and chlorophyll *a* in natural sediments.

5.1 Methods

5.1.1 Construction of microelectrode systems

The techniques of oxygen microelectrode construction involved lengthy micro-manipulation of molten glass, platinum and silver wire to achieve a final tip diameter of 1-10 μm (see Revsbech 1989b). The oxygen electrodes consisted of a

gold cathode, a sliver guard cathode and an anode (Figure 5.2, Revsbech 1989b). The gold cathode was surrounded by a glass casing and had a charge of 0.8 volts, enabling it to reduce O_2 forming a small charge (picoamps). The tip of the glass casing was sealed with an electrically insulating membrane of silicone rubber which is extremely permeable to oxygen (Appendix 1). This apparatus was placed inside an outer glass casing and an electrolyte solution of potassium chloride was added. The silver guard cathode and anode were inserted into this casing and the cathode was polarised by 0.8 volts, thereby reducing oxygen in the electrolyte and preventing diffusion to the measuring tip. Therefore, the current in the measuring circuit is dependant only on the diffusional supply of O_2 to the surface of the sensor membrane. Upon completion, the dimensions of the electrode were noted and the sensor was calibrated in oxygen saturated and anoxic solutions to determine a residual (zero) reading. The effect of advective oxygen transport (stirring) was tested and the response time of the electrode measured. All electrodes produced are required to pass minimum response criteria (Table 5.1).

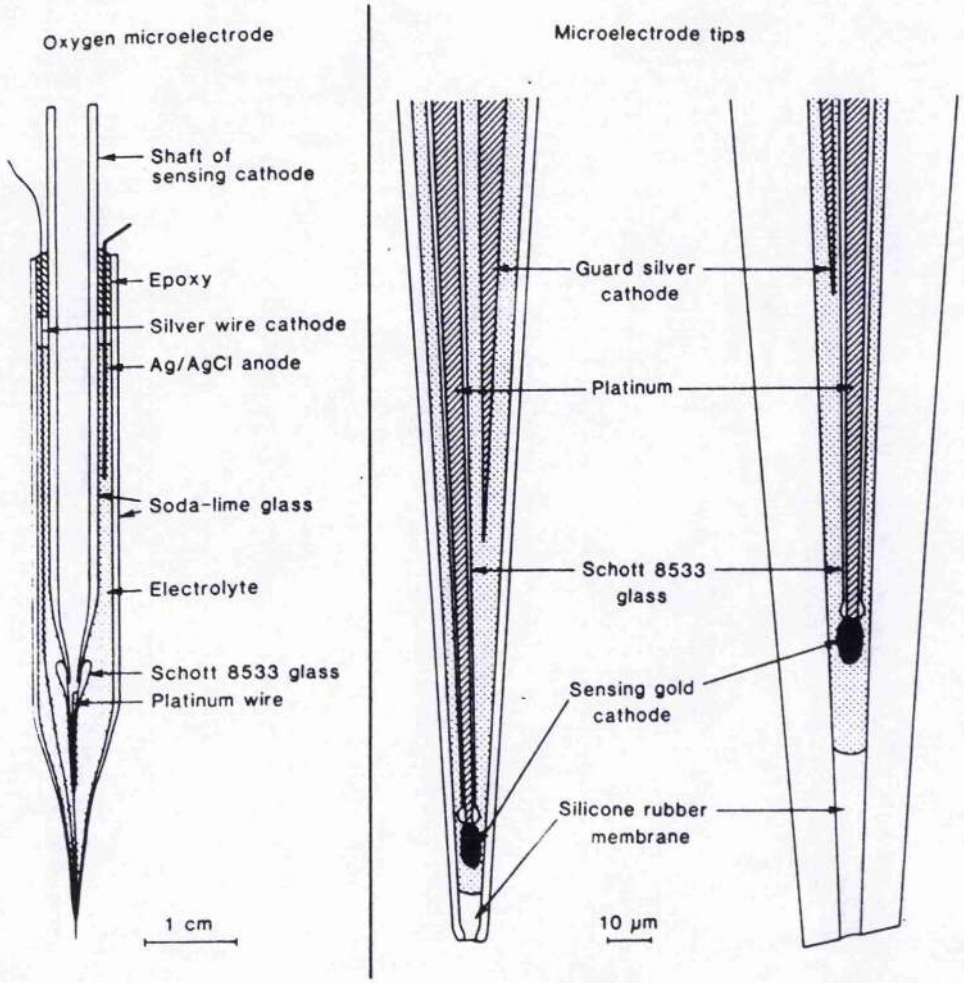
Table 5.1 Oxygen microelectrode dimensions and performance

Tip diameter (mid point of silicone)	9 µm
Length of silicone	10 µm
Distance between tip and gold cathode	50 µm
Gold cathode to silver guard cathode	60 µm
Internal diameter of tip	4 µm
Response time	2-3 s
Stirring effect	4 %

5.1.2 Laboratory experiment

Following the construction of a microelectrode, a laboratory experiment was carried out to investigate the relationship between microalgal photosynthesis and carbohydrate concentrations. Sediment cores were incubated at a range of light intensities and measurements of oxygen concentration, photosynthetic rate, carbohydrate and chlorophyll *a* concentrations were taken on 4 sampling days

Figure 5.2 Left; components of an oxygen microsensor with guard cathode. Right; thin tip, fast response electrode and a thick tip, sturdy but slow response microsensor (reproduced from Revsbech 1989b).



over an 8 day period. A simple model was used to estimate net average productivity rate. The relationship between chlorophyll *a* and colloidal carbohydrates was investigated, the bulk carbohydrate fraction was not measured since spatial and temporal studies (Chapter 3 and 4) have shown this fraction to be less representative of microphytobenthic activity.

5.1.2.1 Sampling site

Eight sediment cores (i.d. 8 cm, 12 cm deep) were collected from an exposed mud flat at Norsminde Fjord (56° 1.25' N 10° 15.9' E), 16 km south of Aarhus on the east coast of Jutland, Denmark on 28/11/95. The site is commonly used by the Microbial Ecology Research Institute. The salinity at the site was 13. After 20 min, the cores were placed in a tank, containing water from the sampling site so that water covered the sediment to a depth of approximately 30 mm. All microelectrode profiles of sediment cores at the institute are conducted on water covered cores. In a comparison of exposed and submerged cores, exposed cores were shown to have lower oxygen production rates than submerged cores (140 and 350 μmol O_2 cm^{-3} s^{-1} respectively, Paterson *et al.* 1996a). The tank water was aerated continuously. In addition, water movement was maintained within each core by adding a small magnetic stirrer suspended from a perspex ring inside the core, approximately 20 mm above the sediment surface. The tank was stored in a controlled temperature room, maintained at the ambient temperature of the sampling site (6°C). White fluorescent strip lights, above the tank, were operated by a timer switch to produce a 9 : 15 h light/dark regime. Variation in light intensities was achieved by placing neutral filters of increasing density on top of duplicate sediment cores. The light intensities in each condition were approximately 0, 50, 100 and 150 μmol photons m^{-2} s^{-1}, the midday light intensity at the site was 170 μmol photons m^{-2} s^{-1}. A light microscope examination of the biofilm found some cyanobacterial filaments and many epipelic diatoms including *Navicula salinarum*, various other *Navicula* spp., *Cylindrotheca closterium*, *Surirella ovata*, *Gyrosigma fasciola*, *Nitzschia sigma* var., various *Nitzschia* spp. and the centric chain-former *Melosira* sp.

5.1.2.2 Oxygen and photosynthesis measurements

After a 24 h equilibrium period in the dark, light conditions were set up and oxygen concentrations and photosynthetic rates were measured. Several profiles were taken within each core on each sampling day. Measurements of gross oxygenic photosynthesis were made by darkening the sediment core for 1-2

s and measuring the rate of oxygen decrease (Revsbech *et al.* 1981). This corresponds to the rate of gross photosynthesis since oxygen concentrations reach a steady state where productivity is equal to O_2 loss by respiration and diffusion. When the sediment core is shaded, photosynthesis stops but the processes which remove oxygen continue at the same rate so the rate of decrease equals the rate at which oxygen was produced in the light (Revsbech 1989a, Glud *et al.* 1992). Three depth profiles of oxygen concentration were taken randomly from the duplicate cores of each light condition. During each oxygen profile, approximately 4 gross photosynthetic rates were recorded. Microsensor readings were taken at 100 µm depth intervals using an automatic stepping motor. Estimates of net photosynthetic rates were made using a 1-dimensional flux model (Lorenzen *et al.* 1995) which estimates the combined value of oxygen flux from the photosynthetic zone into the water column and into deeper layers of the sediment, following Ficks 1st law of diffusion (Wiltshire *et al.* 1996b). The difference between net and gross photosynthetic rates will equal the respiration rate within the photosynthetic zone.

5.1.2.3 Oxygen flux model:

$$J = D\Phi(\delta C/\delta x)$$

J = oxygen flux (nmol cm^{-2} s^{-1}, Lorenzen *et al.* 1995, Wiltshire *et al.* 1996b)
D = diffusion coefficient (cm^{-2} s^{-1})
Φ = porosity $(\rho_g - \rho_b)/(\rho_g - \rho_{sw})$
ρ_g = grain density
ρ_b = sediment bulk density
ρ_{sw} = seawater density (Vogel 1994, Black pers. comm.)
$\delta C/\delta x$ = oxygen concentration gradient, above and below photosynthetic zone (nmol cm^{-4}).

Oxygen flux into the water column (J_w):

$J_w = D_{water}\ 1\ (\delta C/\delta x)$
$J_w = (2.01 \times 10^{-5})\ 14666.67$ (Wiltshire *et al.* 1996b)
$J_w = 0.295$ nmol cm^{-2} s^{-1}

Oxygen flux into the sediment (J_s):

$J_s = D_{sediment}\ 0.56\ (\delta C/\delta x)$
$J_s = (1.41 \times 10^{-5}\ 0.56)\ 9400$ (Glud *et al.* 1995)
$J_s = 0.074$ nmol $cm^{-2}\ s^{-1}$

5.1.2.4 Net average oxygen productivity rate (NAPR, from Lorenzen *et al.* 1995)

$$NAPR = (J_w + J_s) / X_{ph.}$$

$X_{ph.}$ = depth of photosynthetic zone
NAPR = (0.295 + 0.074) / 0.5
NAPR = 0.74 nmol $cm^{-3}\ s^{-1}$

5.1.2.5 Carbohydrate and chlorophyll *a* measurements

For each measurement time, 5 sediment sub-cores (1.2 cm id) were taken of the upper 2 mm from each light condition and frozen for colloidal carbohydrate and chlorophyll *a* analysis.

Sediment chlorophyll *a* was quantified using reverse phase high performance liquid chromatography (HPLC) against the response of standards of known concentration (*Anacystis nidulans*, Sigma). The sediment pellet, remaining after the extraction of colloidal carbohydrates was used for chlorophyll *a* determination (the supernatant containing extracted colloidal carbohydrates was found to contain no pigments). The sediment pellet was lyophilised and the individual weights were recorded. One millilitre of 100 % acetone was added to the pellets, they were vigorously shaken and stored in darkness at -70°C overnight. The liquid, containing extracted pigments, was then drawn up by a hypodermic needle into a 1 ml syringe. The needle was replaced by a syringe filter (13 mm, 0.2 µm pore size, Whatman™) and the filtrate was added to 1 ml, amber, glass vials (Waters™) sealed with a cap. A volume of 60 µl was injected into the HPLC (Waters 996, with photodiode array detector, column type, C18-Lb5 20452, held in a column oven at 25 °C). Two solvents were used, A; 80 % methanol, 10 % H_2O and 10 % buffer (1.5 g tetrabutylammonium and 7.7 g

ammonium acetate in 100 ml H_2O) and B; 90 % methanol and 10 % acetone. The solvent gradient used to separate the pigments is shown in Table 5.2. The eluted pigment peak areas were analysed using Millennium software.

The temperature, salinity, water content and sediment wet bulk density was recorded in each light condition.

Table 5.2 *The solvent gradient used for pigment analysis.*

Step	*Time (min.)*	*Time (cumulative)*	*Flow (ml/min)*	*A %*	*B %*	*Curve*
0	0	0	0.8	80	20	-
1	0.3	0.3	0.8	80	20	-
2	3.7	4	0.8	55	45	linear
3	31	35	0.8	0	100	linear
4	10	45	0.8	0	100	-
5	10	55	0.8	80	20	linear

5.1.3 Field study

Field studies were carried out at Baie de Marennes-Oléron in order to investigate the relationship between carbohydrate and chlorophyll *a in situ*.

5.1.3.1 Sites

Baie de Marennes-Oléron is situated near La Rochelle, on the Atlantic coast of France (45° 55' N 1° 10' W). The area of the bay is 170 km^2 and 60 % of this area is intertidal mud flats (Guarini *et al.* 1997). Samples were collected over a time series from a mid-intertidal site (approximately 2.5 km from shore) on 10/4/97. The mid-intertidal sediments were smooth, with occasional water-filled pools and the sediments appeared green/brown in colour from the presence of microphytobenthos (Figure 5.3a). An upper-intertidal site, approximately 500 m from shore, was sampled over a time series on 11/4/97. This site was dominated by shore-normal ridges (20-50 cm height and width), with metre wide, water-filled runnels between them, so that most of the sediment was submerged. The sediment on the ridges had high numbers of the gastropod, *Hydrobia* sp. and unlike the mid-intertidal site, the sediment appeared a reddish grey colour (Figure 5.3b).

Figure 5.3 Photographs of the sampling sites at Baie de Marennes-Oléron. (a) mid-intertidal and (b) upper-intertidal, showing differences in sediment bed morphology and colouration.

(a)

(b)

5.1.3.2 Sampling

Three sediment cores (50 mm diameter x 20 mm height) were collected using the cryolander method (Wiltshire *et al.* 1997) at 13:00, 13:40 and 15:30 hrs on 10/4/97 and at 12:00, 13:00, 13:40, 14:00 15:00 and 15:30 on 11/4/97. The frozen cores were cut into 3 - 4 blocks using a lapidary saw and each block was finely sectioned using a freezing microtome (Chapter 2). Depth intervals analysed were 0-200, 200-400, 400-600, 1000-1200 and 1400-1600 μm. Colloidal carbohydrate and chlorophyll *a* were quantified in each depth section. In addition, frozen sediment samples from each site were examined using low-temperature scanning electron microscopy (LTSEM).

5.2 Results - Laboratory experiment: The effects of light intensity on sediment carbohydrate, chlorophyll *a* , oxygen concentrations and benthic photosynthetic rates.

On day 1, chlorophyll *a* and estimated oxygen production rates varied significantly between conditions ($F_{3,6} = 6.93$, $P = 0.02$; $F_{3,14} = 4.37$, $P = 0.018$ respectively, one-way ANOVA, Figure 5.4), with the highest concentrations and rates being detected in the 150 μmol m^{-2} s^{-1} light condition. There was no significant variation in colloidal carbohydrate concentrations under different light intensities ($F_{3,8} = 3.68$, $P = 0.06$).

On day 4, highest chlorophyll *a* and carbohydrate concentrations occurred in the 50 μmol m^{-2} s^{-1} condition and there was significant variation between light conditions ($F_{3,14} = 4.37$, $P = 0.023$, $n = 3$, Figure 5.5). However, there had been little change in chlorophyll *a* concentrations in the dark or 100 μmol m^{-2} s^{-1} light conditions. There was variation in oxygen productivity between light regimes ($F_{2,6} = 6.41$, $P = 0.03$) with most occurring in the 150 μmol m^{-2} s^{-1} light condition.

On day six, sediment under the 150 μmol m^{-2} s^{-1} condition contained greatest chlorophyll *a* and colloidal carbohydrate concentrations and there had been an increase in chlorophyll *a* in the 100 μmol m^{-2} s^{-1} cores (Figure 5.6).

Figure 5.4 Chlorophyll *a*, colloidal carbohydrate and NAPR in 4 light climates on Day 1 (bars = S.E., n = 3)

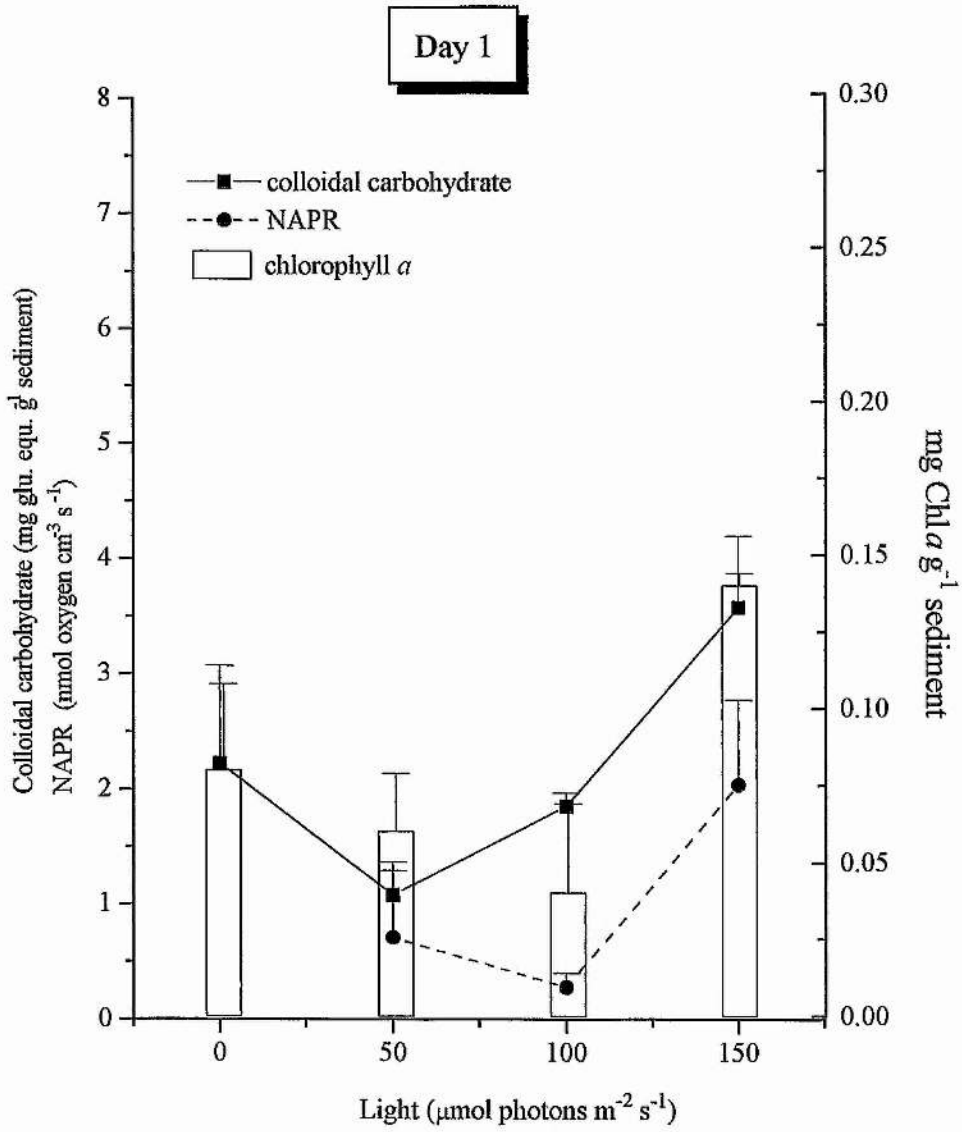


Figure 5.5 Chlorophyll *a*, colloidal carbohydrate (n = 5) and NAPR (n = 3) in 4 light climates on Day 4 (bars = S.D.)

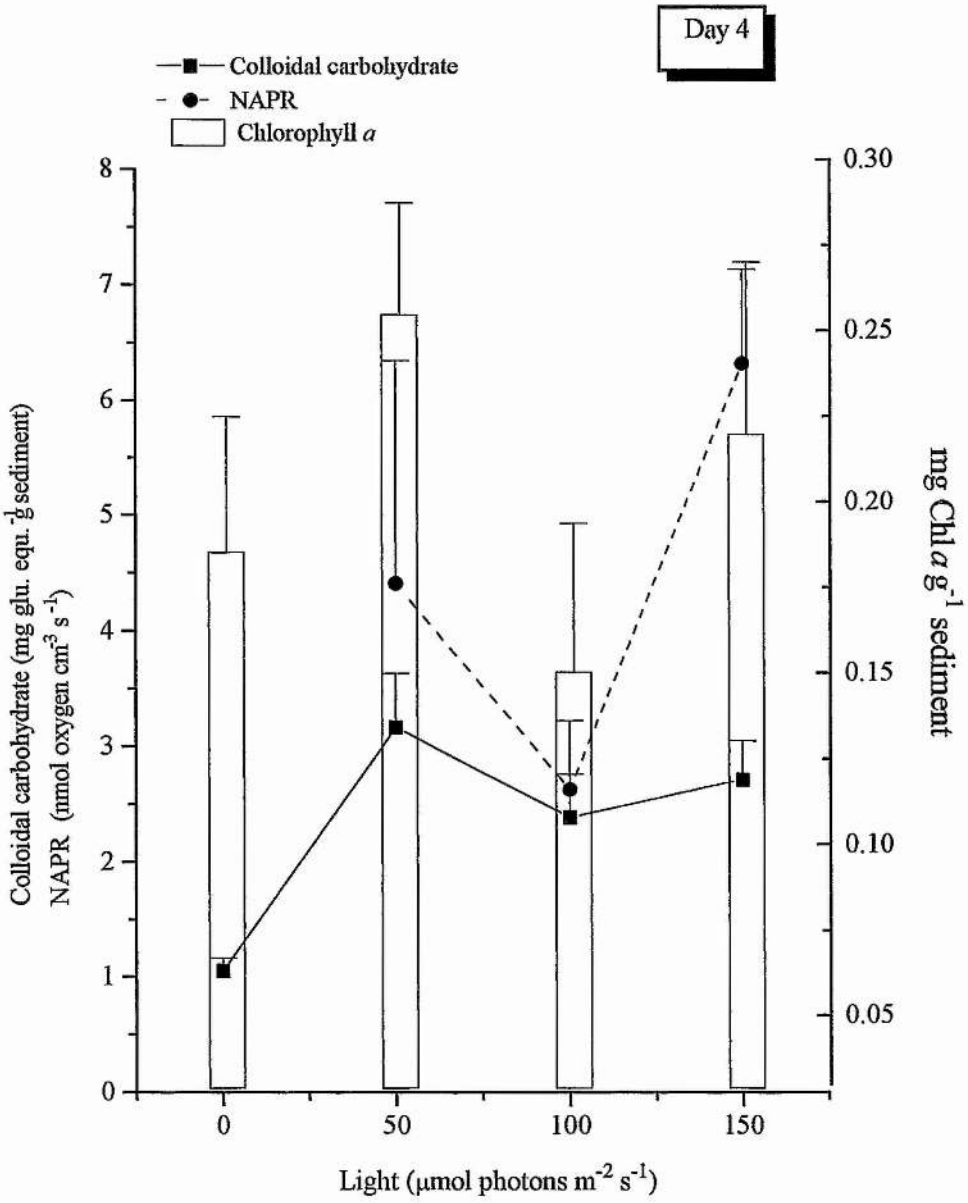
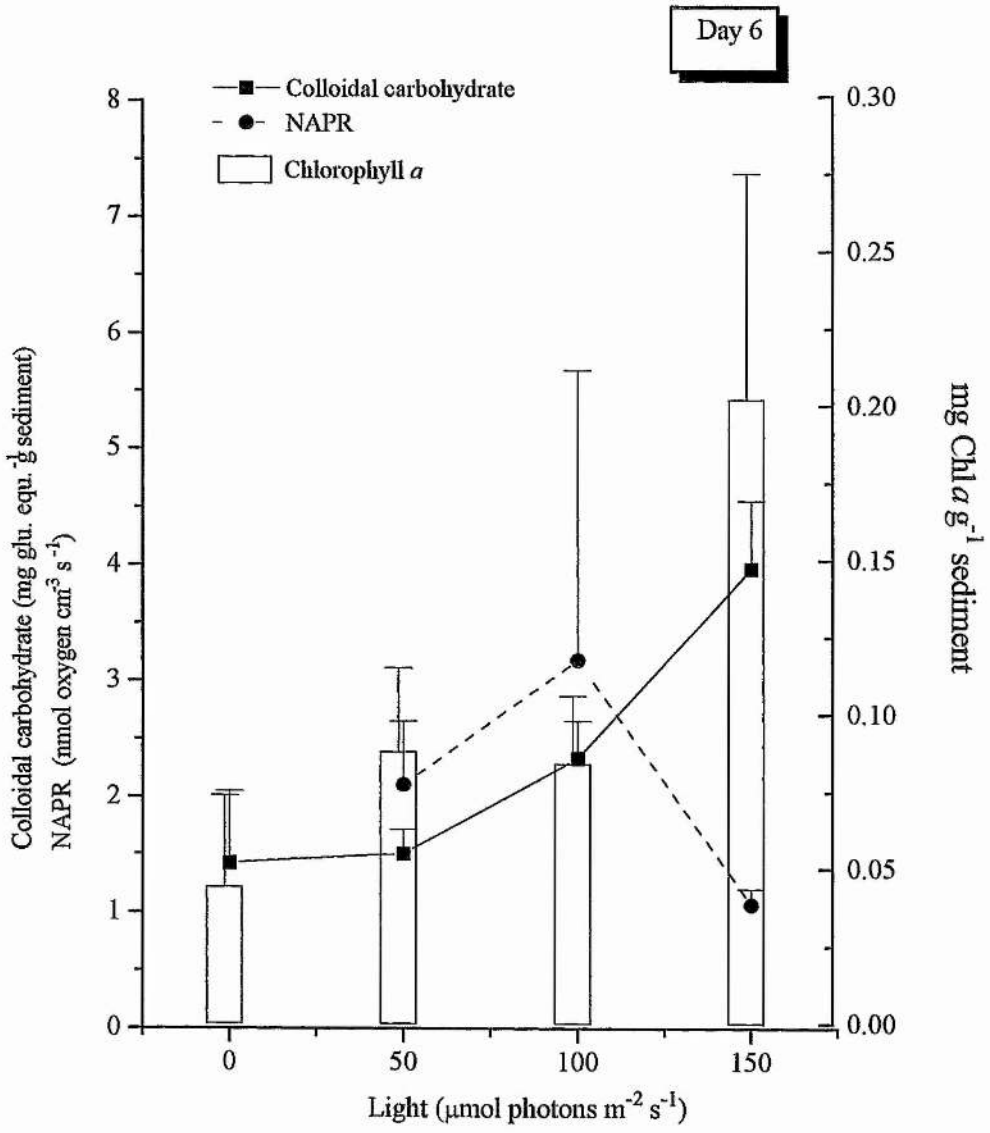


Figure 5.6 Chlorophyll *a*, colloidal carbohydrate (n = 5) and NAPR (n = 3) in 4 light climates on Day 6 (bars = S.D.)



There was no significant variation in oxygen productivity, but values were lowest in the 150 μmol m^{-2} s^{-1} light condition ($F_{2,7} = 2.05$, $P = 0.2$, Figure 5.6).

On day 8, the lowest chlorophyll and carbohydrate concentrations were measured in the dark cores. Greatest carbohydrate concentrations occurred at 150 μmol m^{-2} s^{-1}, however concentrations of colloidal carbohydrates and chlorophyll *a* were lower in this condition than on Day 6. Oxygen productivity varied significantly over light regimes and was highest in the 150 μmol m^{-2} s^{-1} condition ($F_{2,6} = 20.7$, $P = 0.02$, Figure 5.7).

The average values of colloidal carbohydrates (over the entire sampling period) were significantly different between light conditions with greatest concentrations in the highest light intensity ($F_{3,68} = 10.47$, $P = 0.000$, one-way ANOVA, Figure 5.8a). In addition, sediment chlorophyll *a* concentrations varied significantly with greatest chlorophyll *a* concentrations in the highest light intensity ($F_{3,62} = 9.35$, $P = 0.000$, on square root transformed data, Figure 5.8b) and there was a significant positive correlation between colloidal carbohydrate and chlorophyll *a* ($P = 0.01$, $r = 0.6$, $df = 70$, Pearson Product correlation). Therefore, when colloidal carbohydrates were normalised to chlorophyll *a* (i.e. expressed as mg glucose equivalents mg^{-1} chlorophyll *a*), there was no significant trend with light intensity ($F_{3,62} = 0.73$, $P = 0.53$, on square root transformed data, Figure 5.9).

The benthic primary productivity zone was shown to be limited to approximately the upper 0.5 mm (Figure 5.10). There was variation in the distribution of oxygen in the upper 4 mm of sediment cores at different light intensities, with greatest concentrations occurring in the highest light condition (Figure 5.11). There was no indication of oxygen productivity in cores that were stored in the dark and oxygen concentrations decreased immediately below the surface (Figure 5.11d).

Estimated net average oxygen productivity rates (NAPR) pooled over the 8 day period, increased with light intensity ($F_{3,33} = 9.11$, $P = 0.000$, on log10

Figure 5.7 Chlorophyll *a*, colloidal carbohydrate (n = 5) and NAPR (n = 3) in 4 light climates on Day 8 (bars = S.D.)

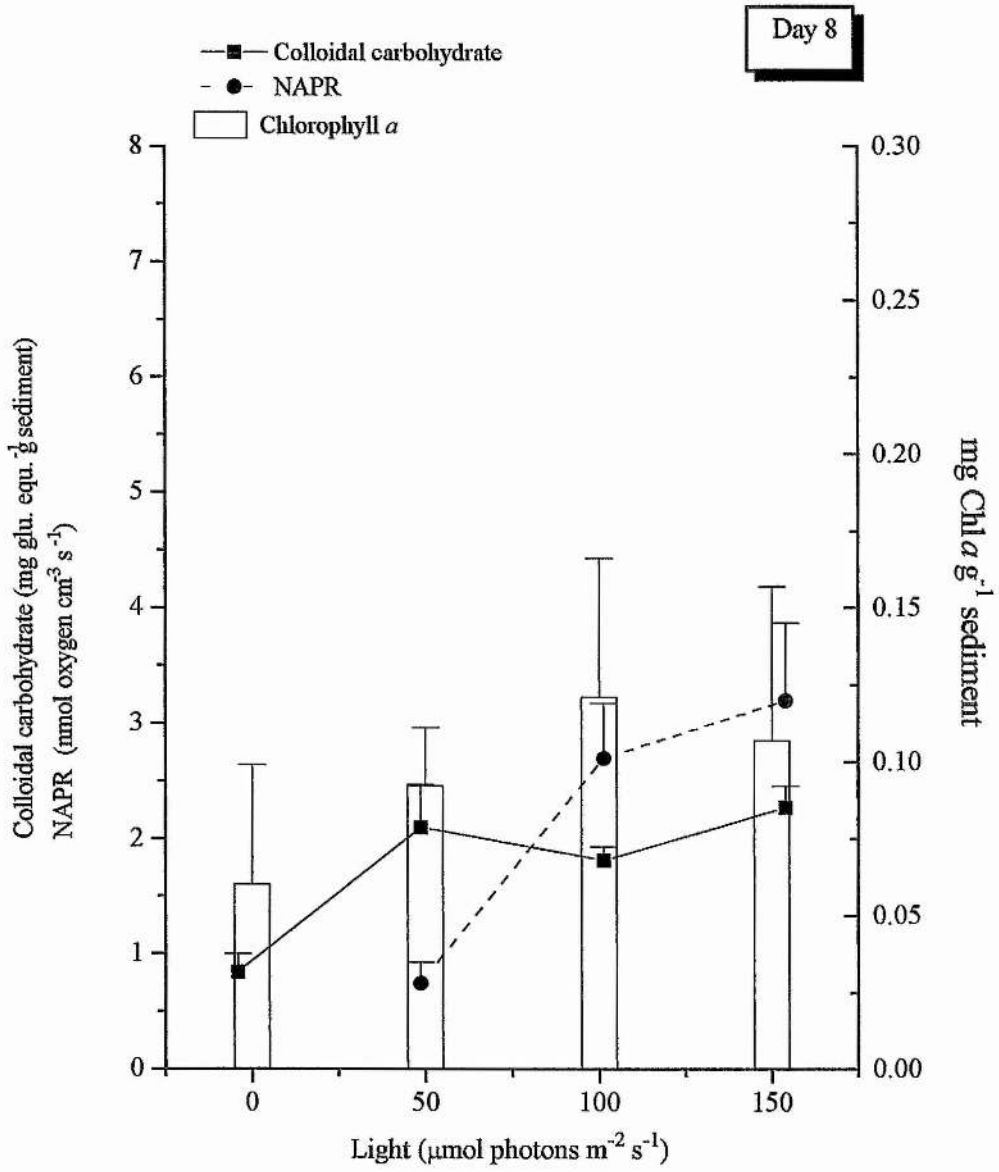


Figure 5.8 Increase in (a) colloidal carbohydrate and (b) chlorophyll *a* concentrations with light intensity (data pooled over 8 days, bars = S.E., n = 18)

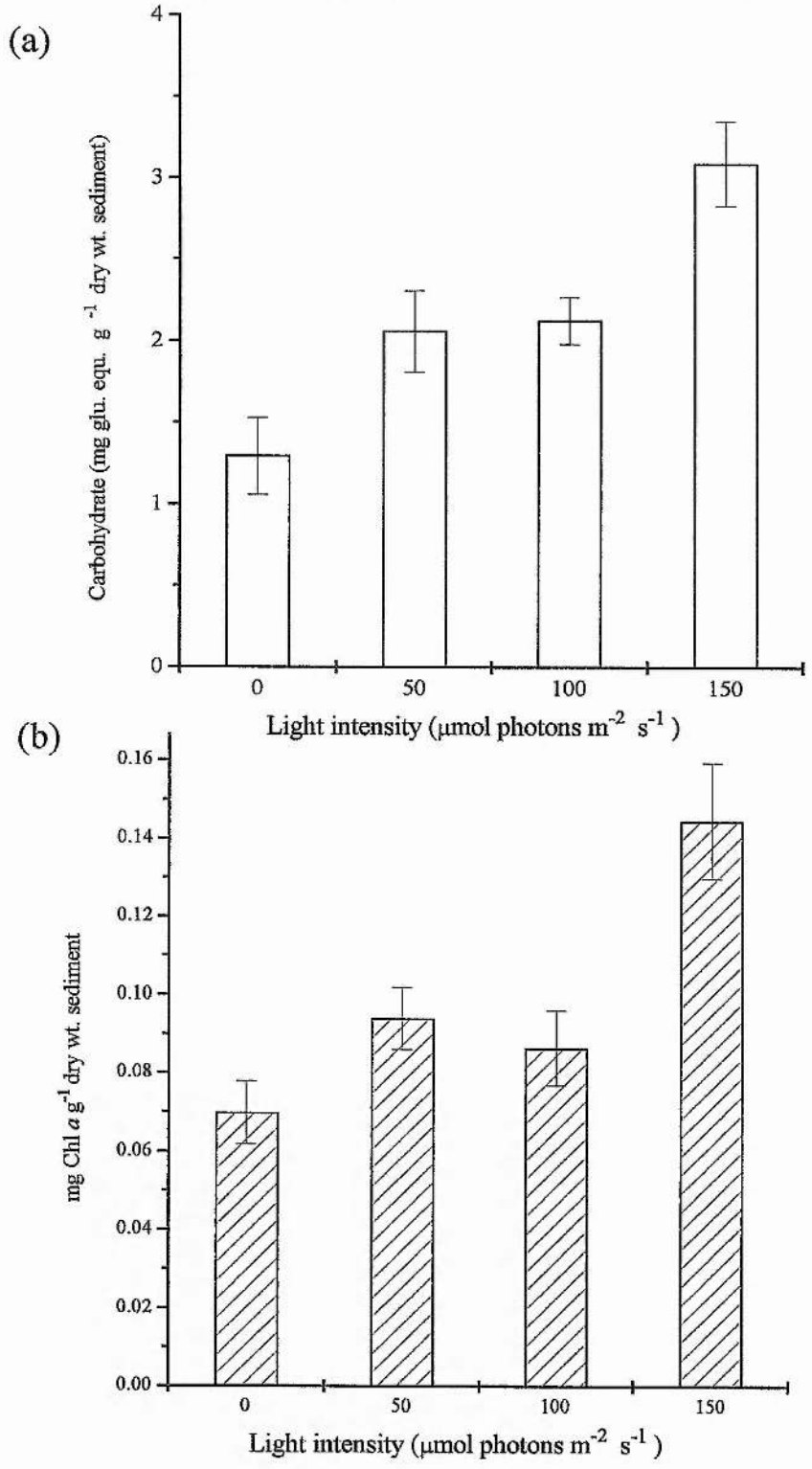


Figure 5.9 Colloidal carbohydrate per unit biomass at 4 light conditions, over 4 sampling days (bars = S.E. n = 5)

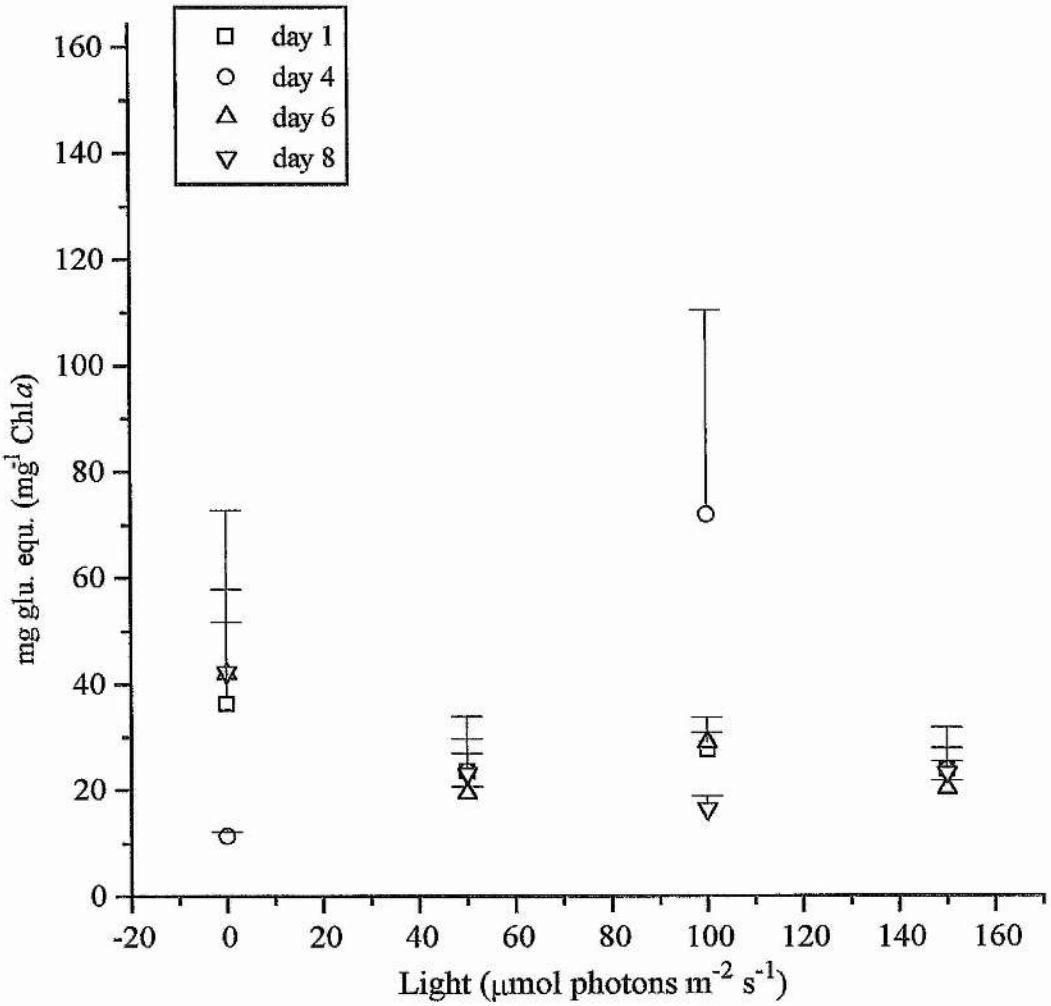


Figure 5.10 Typical depth profile of sediment core showing oxygen concentration (open squares) and gross photosynthetic rate (solid squares).

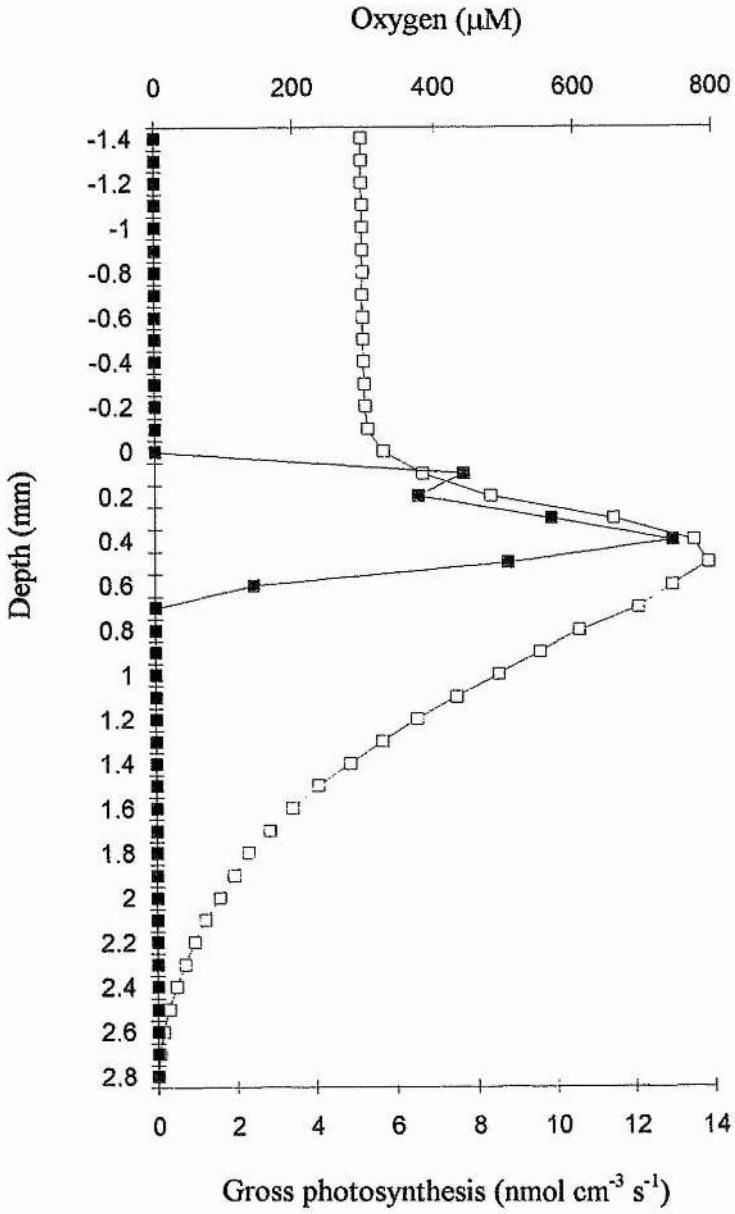
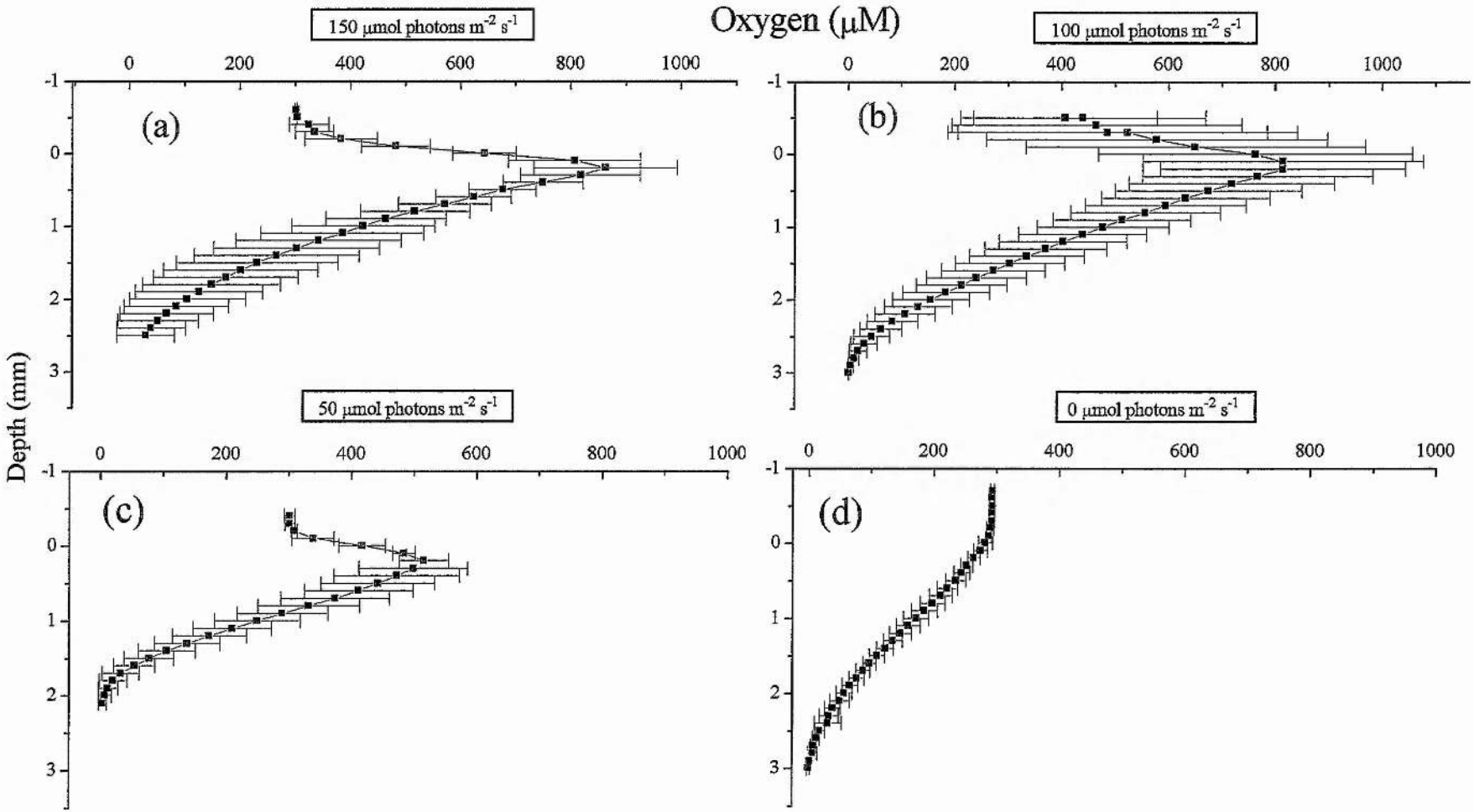


Figure 5.11 The distribution of oxygen in sediment cores at varying light intensities (bars = S.D., n= 3)



transformed data, Figure 5.12). However, when normalised to chlorophyll *a* content of the upper 2 mm (MacIntyre *et al.* 1996, Guarini *et al.* 1997), there was no significant variation in NAPR rates with light intensity ($F_{3,40}$=1.44, P = 0.25, Figure 5.13). Modelled net average oxygen productivity rates were lower than gross photosynthetic rates (Table 5.3) and the difference between these measurements represents respiration rates.

Table 5.3 Comparison of modelled net and measured gross photosynthetic rates.

Light (*μmol photons* m^{-2} s^{-1})	*Gross photosynthetic rate* (*nmol* cm^{-3} s^{-1}) *STDEV. n = 40*	*Net average photosynthetic rate* (*nmol* cm^{-3} s^{-1}) *STDEV n = 10*	*Estimated respiration rate (nmol* cm^{-3} s^{-1})
150	8.4 ± 4.8	3 ± 2.1	5.4
100	6.8 ± 4.1	2.2 ± 1.6	4.6
50	3.4 ± 2.1	2 ± 1.8	1.4
0	0	0	-

There was significant variation in sediment water content under different light climates ($F_{3,68}$ = 3.7, P = 0.016, on log transformed data, Figure 5.14). However, the only significant difference occurred between the 0 and 50 μmol photon m^{-2} s^{-1} conditions (shown by a *post hoc* Tukey test for comparison of means). Furthermore, a Pearson Product correlation found no correlation between the carbohydrate or chlorophyll *a* concentrations and variations in water content. There was no significant variation in wet sediment bulk density across the different light climates ($F_{3,68}$ = 0.91, P = 0.44).

5.2.1 Variation in measured parameters over the experimental period

No significant variation in carbohydrate concentration was found to occur over time (8 days P = 0.36, df = 71, one-way ANOVA). Similarly, there was no change in chlorophyll *a* concentration over the 8 day period (P = 0.6, df = 65, one-way ANOVA). The amount of oxygen produced in the sediment by photosynthetic organisms, (net average productivity rate, nmol O_2 cm^{-3} s^{-1}) peaked on Day 4 ($F_{3,36}$ = 9.11, P = 0.000, Figure 5.15a). Sediment wet bulk density decreased significantly on Day 4 ($F_{3,68}$ = 2.87, P = 0.04, Figure 5.15b).

Figure 5.12 Net average oxygen production (NAPR) rate at 4 light climates (bars = S.E., n = 3)

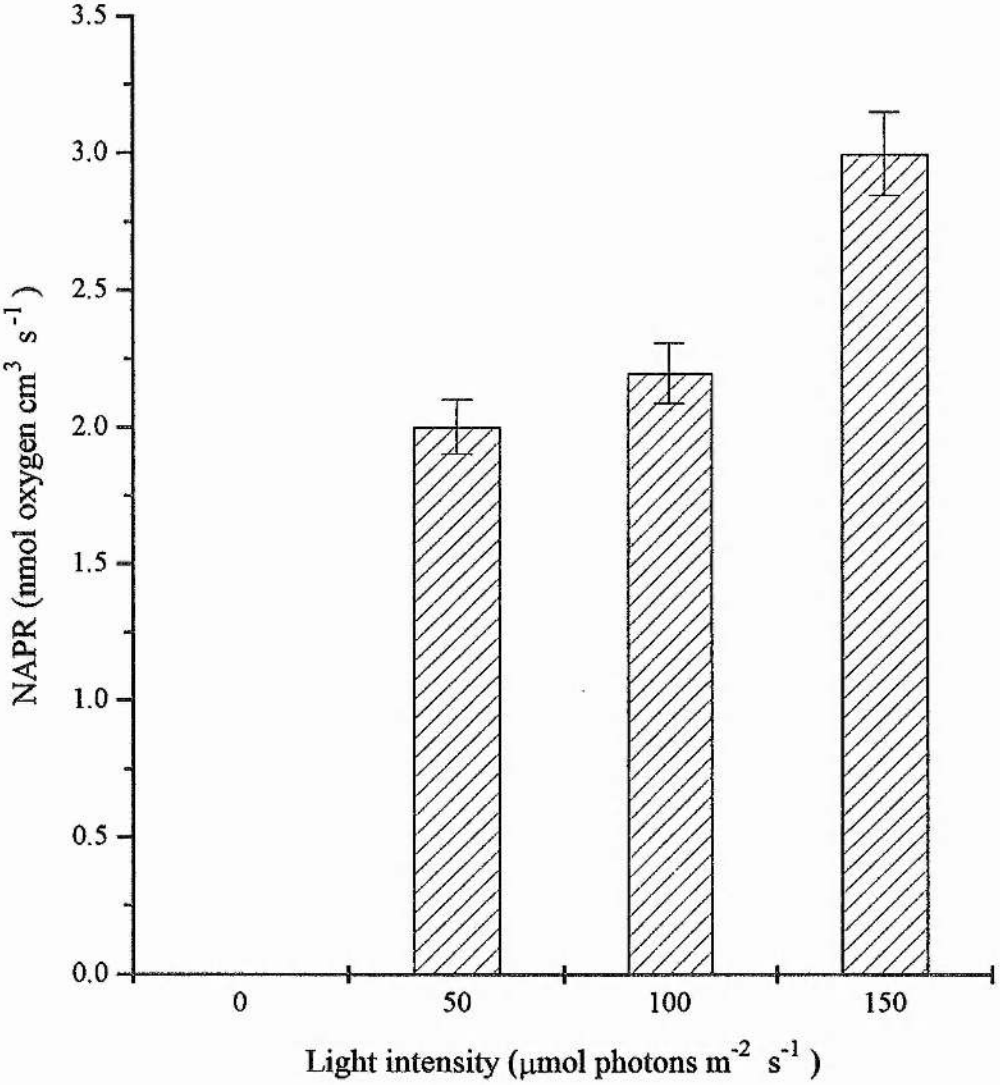


Figure 5.13 Net oxygen production per unit biomass at varying light conditions (bars = S.E., n= 3)

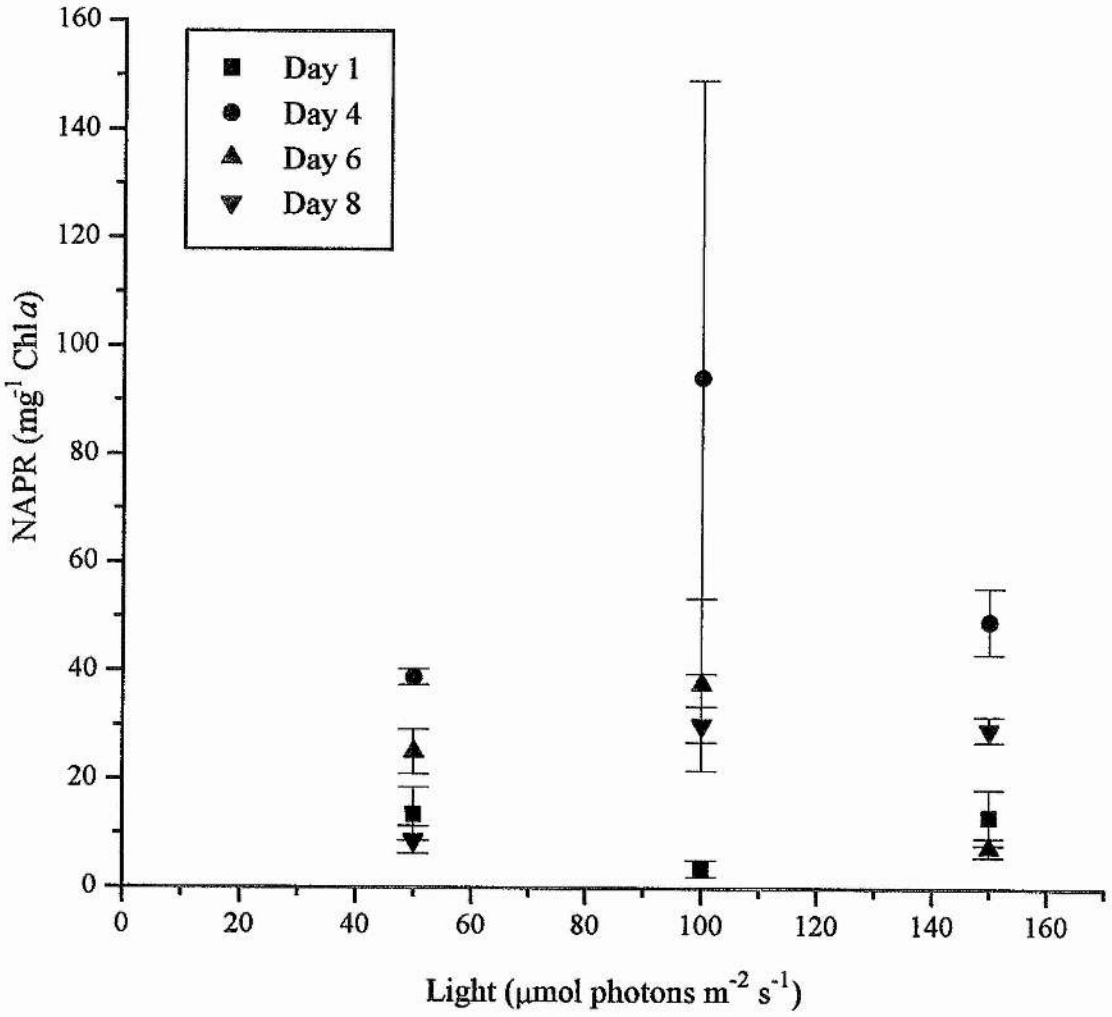


Figure 5.14 Variation in percentage water content over four light climates (bars = S.E., n = 18)

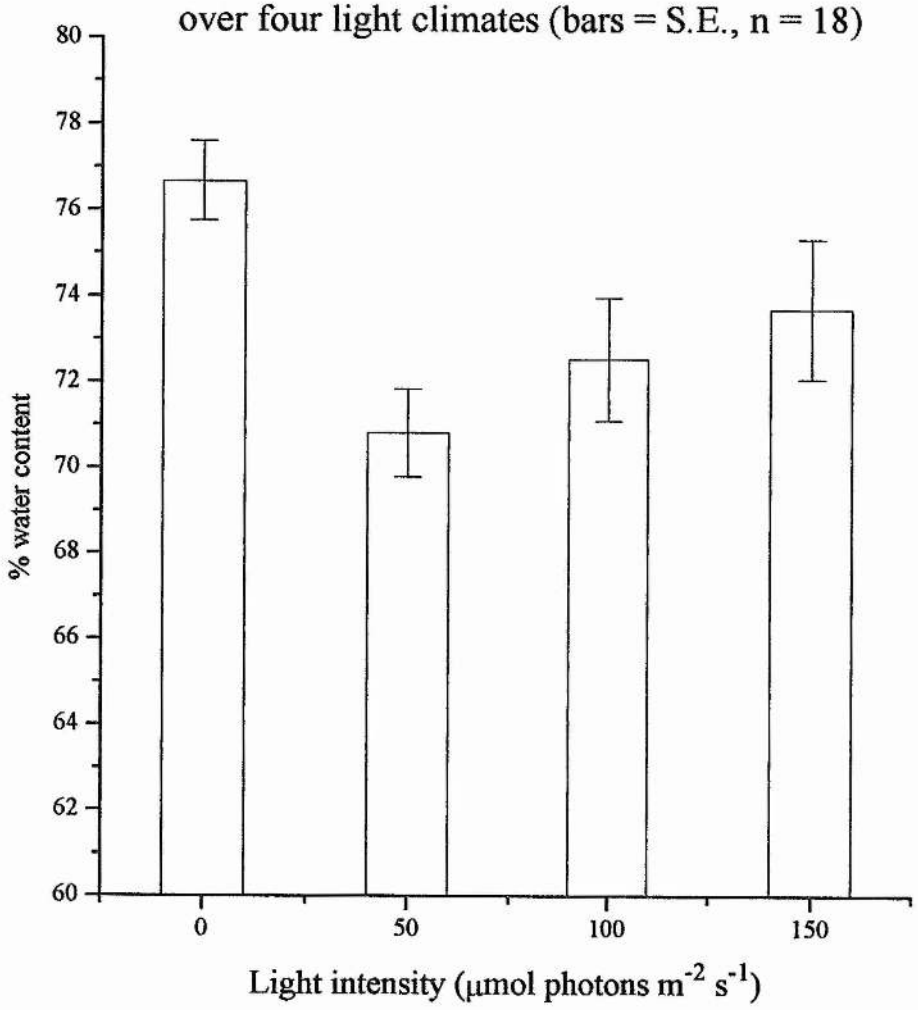
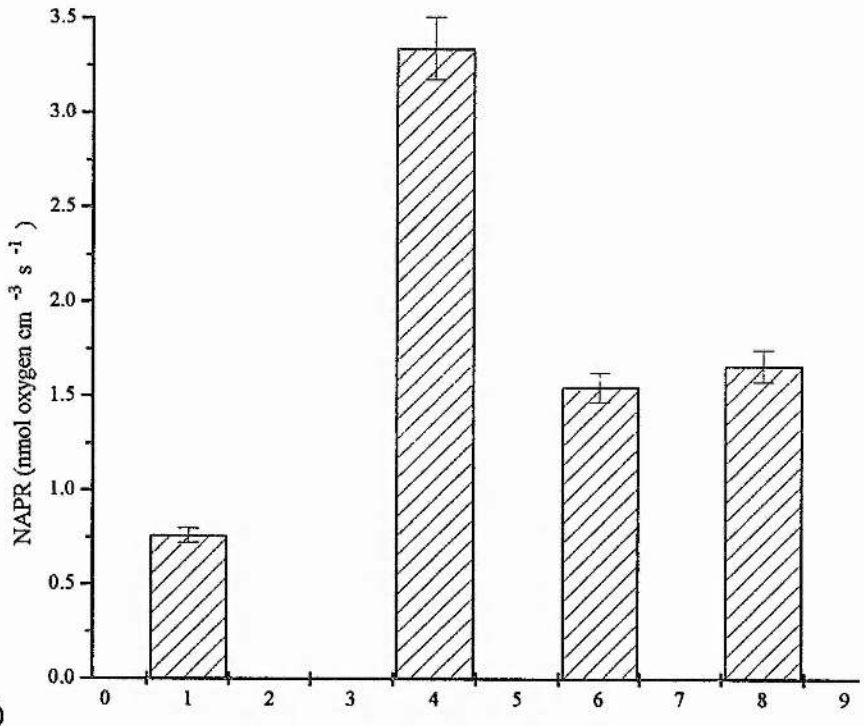
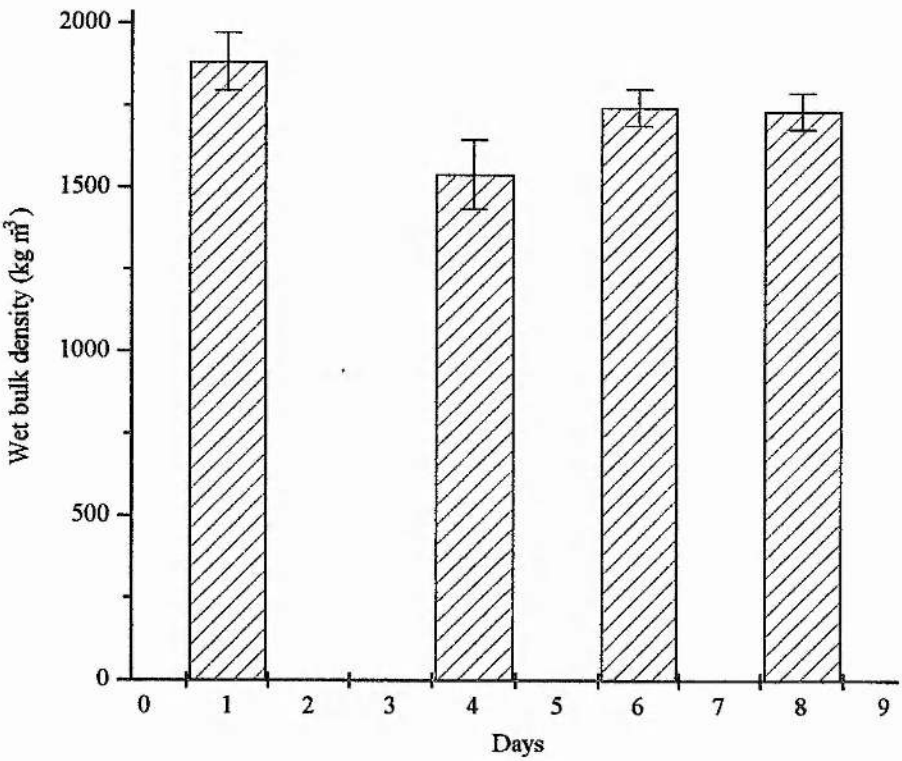


Figure 5.15 Variation in (a) net oxygen production and (b) sediment wet bulk density over time (bars = S.E., n = 12)

(a)



(b)



The sediment moisture content and salinity did not vary over the sampling period (moisture content; $F_{3,68} = 1.56$, $P = 0.2$).

5.3 Discussion - laboratory study

5.3.1 The relationship between light intensity and benthic productivity

The influence of light intensity on photosynthetic rate has been demonstrated in previous studies (Kühl *et al.* 1997). The present study went further to investigate the role of light and photosynthesis on the abundance of sediment carbohydrates. The ratio of colloidal carbohydrate: chlorophyll *a* was 28:1 and was in the range of previous studies (i.e. 2-200:1 de Winder *et al. submitted*). Average carbohydrate concentrations (mg glu. equ. g^{-1} dry sediment) were greatest in the highest light condition (150 µmol photons m^{-2} s^{-1}) however, this occurred as a function of algal biomass and not through increased EPS production per cell (e.g. no luxury EPS production). However, the light intensities used may not have been high enough to induce luxury EPS production (measured as colloidal carbohydrates).

Since sediment samples for chlorophyll *a* and colloidal carbohydrates were of the upper 2 mm it is possible that at some sampling times, the microphytobenthic assemblages were below this depth and not sampled. Migratory movements could be taken into account by conducting the experiment in a tank with simulated tidal cycles.

Algal biomass was greatest in the high light condition on three of the four sampling days, including day 1. The lower chlorophyll *a* concentrations in the dark cores on days 6 and 8 may have been due to the death and breakdown of light-starved cells.

There were significant variations in chlorophyll *a* concentrations and estimated productivity between light conditions on the first day. All the sediment sub-samples for chlorophyll *a* and colloidal carbohydrates were collected at the end of the measurement day, after the oxygen depth profiles had been taken. Therefore, variation in chlorophyll *a* concentrations between the light conditions on the first day could not be due to time variation in sampling (such as migration

effects). The sediment cores were collected randomly and arbitrarily assigned to each light condition. Therefore it is unlikely that the sediment cores placed in the highest light condition had the highest initial chlorophyll *a* concentrations. Perhaps it is more probable that chlorophyll *a* concentrations increased during the first 5 hours, from when light conditions were established until sediment cores were taken for chlorophyll *a* and carbohydrate analysis. This would imply that microalgae respond quickly to changes in light climate. Auclaire *et al.* (1982) found planktonic diatoms were capable of doubling chlorophyll *a* concentrations in 2-3 hours. Unfortunately, the analysis of sediment samples taken prior to the introduction of light conditions was unreliable due to differences in the assay equipment between Danish and St Andrews laboratories.

The colloidal carbohydrate and chlorophyll *a* concentrations were averaged over the eight day period in order to examine the effects of light without large daily variations. However, this may not be a realistic approach to investigating the relationship between photosynthesis to sediment carbohydrate concentrations. A better approach would be to analyse a greater number of sediment cores within a shorter period of time (perhaps several hours). In future studies, the same sediment cores should not be sampled at each time interval since this represents pseudo-replication and the removal of sediment material may introduce errors for subsequent measurements.

Although the greatest O_2 concentrations were measured in the highest light condition, net average oxygen productivity (NAPR), per unit biomass, did not vary significantly between light climates. This suggests that the light intensity was saturating. Although photosynthetic saturation points are often lower in winter (Admiraal 1984, MacIntyre *et al.* 1996), the laboratory light climates were lower than the ambient light intensity at the sampling site (170 μmol photons m^{-2} s^{-1}) and were unlikely to cause saturation.

The absence of a *P-I* response (i.e. increased oxygen productivity with increasing irradiance) may suggest that primary productivity was limited by other factors, such as nutrients, availability of CO_2 or the accumulation of toxic substances. Thus, cores with greatest biomass may reach the carrying capacity of

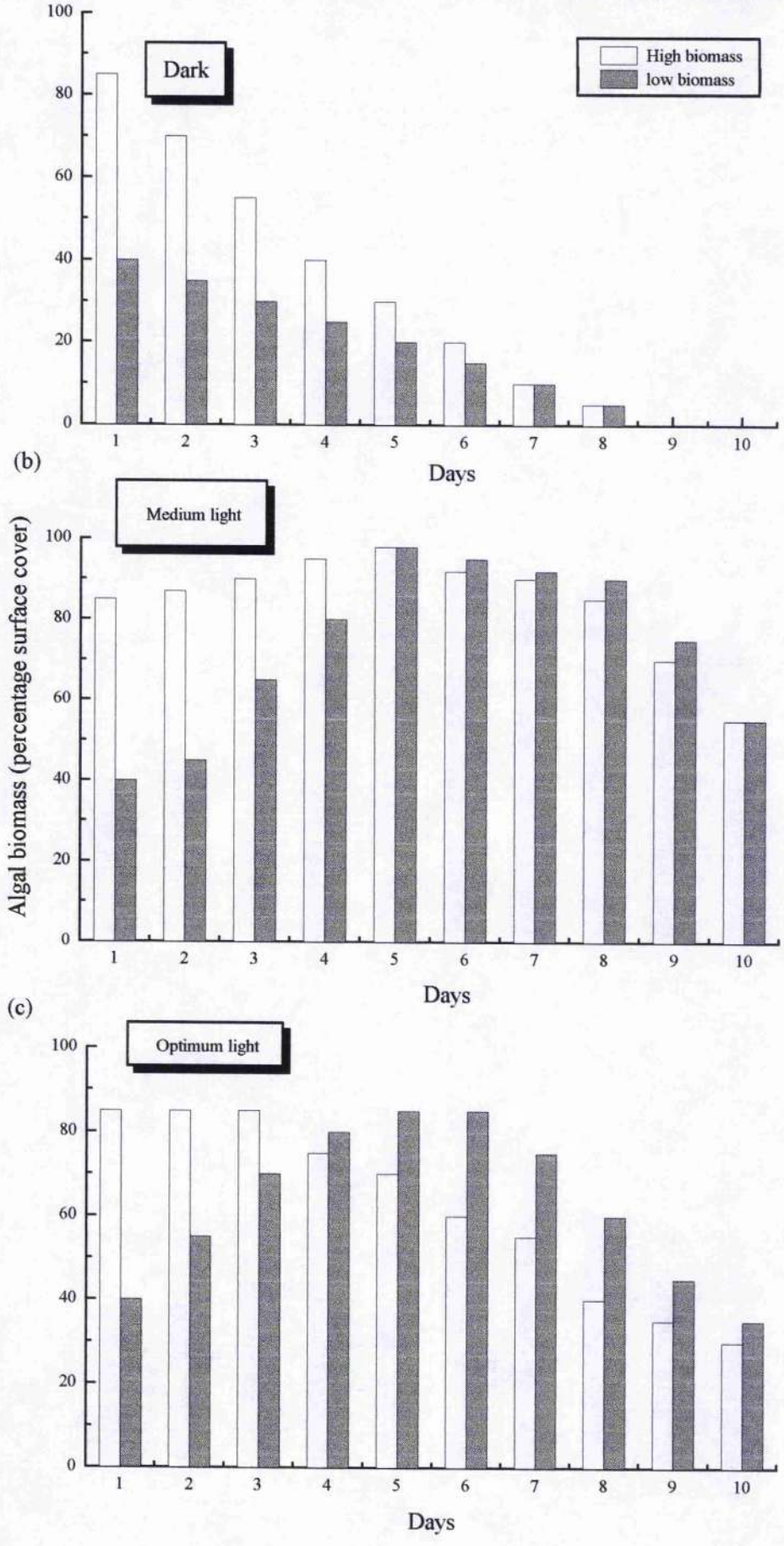
the system before cores with less biomass. In this study, cores with the highest initial biomass showed no further increases in biomass over the experimental period but biomass declined on the 8th day. Therefore, the initial biomass of a sediment core may influence future biomass development.

A conceptual model investigates the limitations on algal biomass in cores of low and high biomass levels and under dark, medium and optimum light climates. In cores where light is absent, algal biomass may decline when internal carbon reserves are depleted (Figure 5.16a, Smith and Underwood 1998b). Under medium light conditions, cores of high biomass may utilise resources rapidly and biomass would increase until growth becomes limited (Figure 5.16b). Cores with a lower initial biomass may take longer to reach the carrying capacity of the system and biomass may continue to increase for a longer period before resources are depleted. Therefore, due to other limiting factors, there would be no difference in the biomass specific productivity rates between different light conditions.

Under optimum light conditions, cores with a high initial biomass may have already reached the carrying capacity of the core (Figure 5.16c) and productivity may remain at a steady state until limiting resources cause biomass to decline, as observed in this study. Cores with low biomass levels may show increases in biomass and productivity under optimum light conditions. In these circumstances cores with high algal biomass, in the highest light condition, may have similar productivity rates to cores lower biomass levels, in lower light intensities, resulting in uniformity of productivity rates (per unit biomass) between the light conditions. However, this conceptual model assumes an equal rate of algal growth which is unlikely to exist in natural microphytobenthic assemblages.

Although the depletion of resources within the system may explain the uniformity of oxygen productivity between light climates, the secretion of EPS has been shown to increase in situations such as nutrient depletion, where photosynthetic activity is possible but cell division is inhibited (Hellebust 1965). However, increases in colloidal carbohydrates (as an index of EPS) were not shown in this study.

Figure 5.16 Proposed growth potential of low or high epipelic diatom biomass at different light intensities



The lack of a typical *P-I* response could also be due to failures in the NAPR model. While several direct measurements of photosynthetic productivity were made, the productivity model was thought to be more accurate since it was based on complete depth profiles. However, only one porosity value was used in the model although sediment density has been shown to vary greatly over the photosynthetic zone (Chapter 3), affecting the diffusion of oxygen into deeper layers of the sediment. In addition, the model assumes that oxygen productivity occurs at a constant rate throughout the photosynthetic zone, however, direct measurements of gross photosynthetic rate suggest that this may not be correct (Fig. 5.10) therefore, possibly introducing errors to the estimation of primary productivity.

The estimated respiration rates (calculated as the difference between gross and estimated net photosynthetic rate) appeared to increase with increasing light intensity and were very high (Yallop pers. comm.). High respiration rates may indicate a significant contribution from sediment bacteria. The increase in respiration rates with light intensity has been observed in a previous study (Revsbech 1989a) and it was suggested that it may represent photorespiration. It has been estimated that 1/6 of CO_2 fixed by higher plants (C3), in favourable conditions, is released again through photorespiration (Hipkins 1984 and references therein). In a diatom biofilm, photosynthetic activity can result in high oxygen concentrations (Fig 5.10, Revsbech 1989a) and photorespiration may act to restrict carbon fixation and perhaps maintain C:N ratios under favourable conditions. EPS is composed mainly of carbohydrates and has negligible concentrations of nitrogen (Hoagland *et al.* 1993 and references therein), therefore EPS secretion could regulate C:N balance, therefore a reduction in carbon fixation may have implications for the production of EPS.

5.3.2 The correlation between carbohydrates and chlorophyll *a*

There was a significant correlation between carbohydrates and chlorophyll *a* in the upper 2 mm of the sediment and this agrees with the study by Underwood and Smith (1998b) where a significant relationship was found to occur on

numerous cohesive sediments where diatoms composed more than 50 % of the microphytobenthic population.

5.3.3 Sediment water content and wet bulk density

The variation in sediment water content in the four different light climates represents a possible source of variation. Similarly, wet sediment bulk density varied significantly over the sampling period. It is preferable that these parameters are kept constant so that the effects of light alone may be examined, for example diatoms are known to produce more EPS under desiccating conditions (Peterson 1987). In addition, high sediment densities may affect the penetration of light and oxygen and limit diatom mobility. Which may, in turn, affect the depth and rates of primary productivity. However, in this study, there were no significant correlations between water content, wet bulk density, chlorophyll *a* or colloidal carbohydrate concentrations.

5.4 Baie de Marennes-Oléron

5.4.1 Results

The field study at Baie de Marennes-Oléron investigated the relationship between colloidal carbohydrates and chlorophyll *a* over the emersion period at a mid and upper-intertidal site.

5.4.1.1 Spatial variation

Colloidal carbohydrates decreased significantly within the upper 1.5 mm of sediments at Baie de Marennes-Oléron, falling from the average surface concentration of 10.7 to 2.8 mg g^{-1} dry sediment at 1.5 mm on day 1 (mid-intertidal site, $F_{4,50} = 16.20$, $P = 0.00$ one-way ANOVA on square root transformed data, Figure 5.17) and from an average of 3.9 to 1.9 (mg g^{-1} dry sediment) on day 2 (upper-intertidal site, $F_{4,145} = 4.02$, $P = 0.004$ on log transformed data, Figure 5.18). Similar to colloidal carbohydrate depth profiles at the Humber Estuary and the Eden Estuary (Figures 4.4 and 4.7), the greatest

Figure 5.17

Baie de Marennes-Oléron, colloidal carbohydrate in the upper 1.5 mm, mid-intertidal, 10/4/97 (n = 5, bars = S.E)

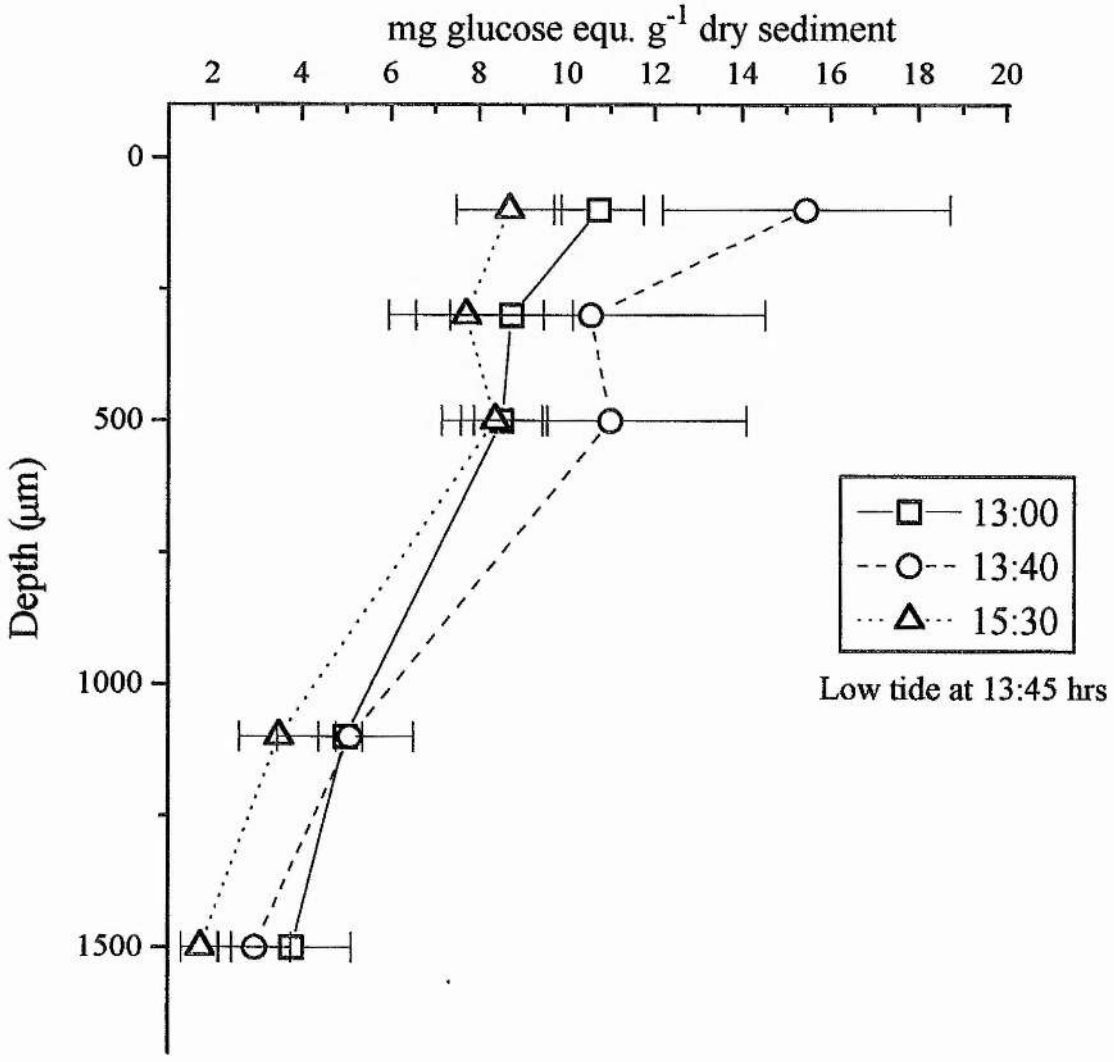
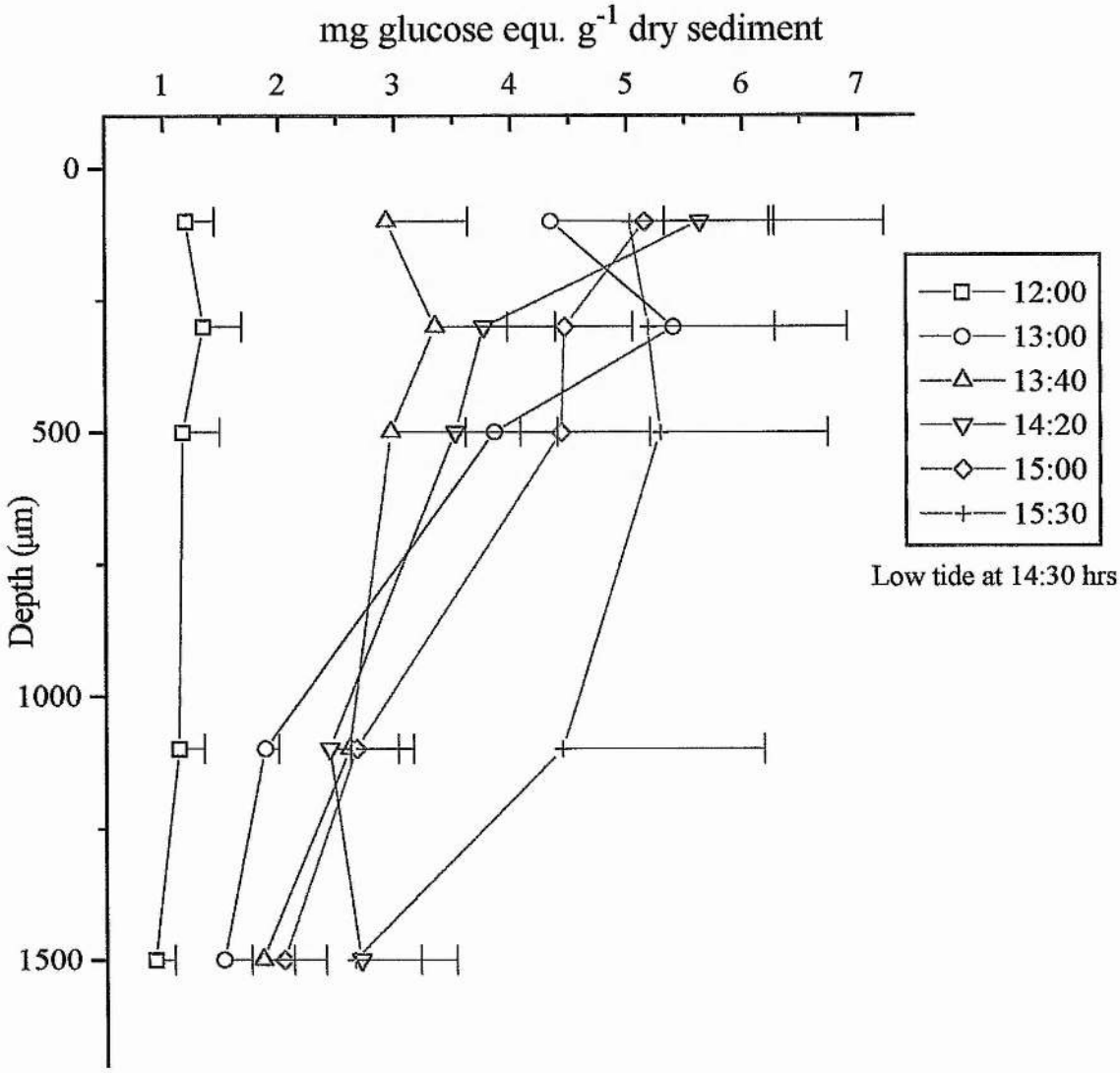


Figure 5.18 Colloidal carbohydrates in the upper 1.5 mm top shore (site 1, 11/4/97, bars = S.E., n = 5) Baie de Marennes-Oleron



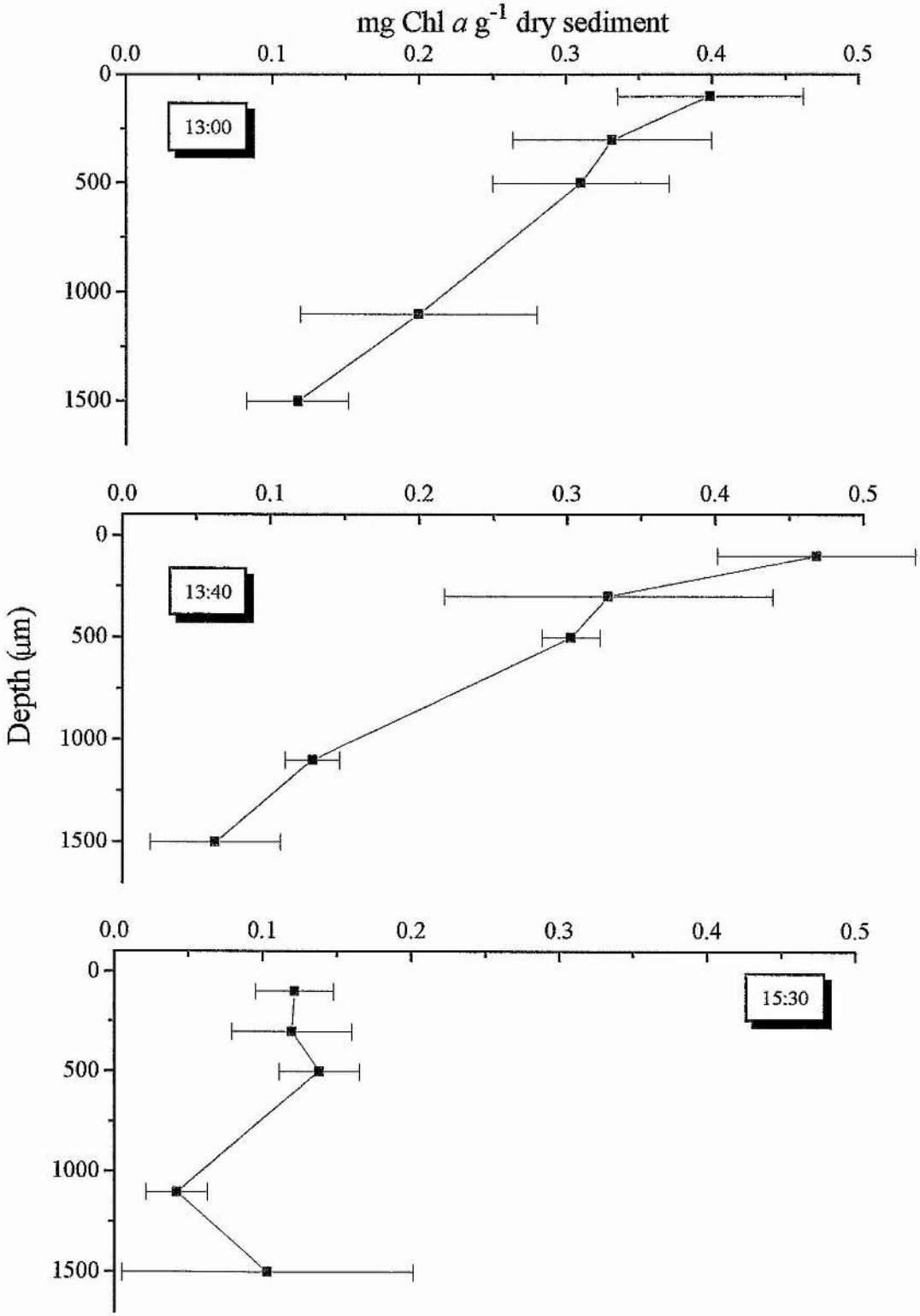
variability with time was found in the upper 200 μm section (see section 5.4.1.2. Temporal Variation, below). Also, there was a significant decrease in algal biomass (as chlorophyll *a*) with depth, falling from the mean surface concentration of 0.29 mg to 0.1 mg Chl *a* g^{-1} sediment at the mid-intertidal site (Figure 5.19) and 0.073 mg to 0.048 mg g^{-1} dry sediment at the upper-intertidal site ($F_{4,53}$ = 4.11, P = 0.006, $F_{4,149}$ = 6.35, P = 0.00 respectively, on square root transformed data, Figure 5.20). The ratio of colloidal carbohydrate to chlorophyll *a* did not vary with sediment depth, at either site (mean 54:1, $F_{4,52}$ = 0.2, P = 0.9; 52:1, $F_{4,141}$ = 0.4, P = 0.8 at mid and upper-intertidal sites respectively, log10 transformed data).

A strong correspondence in the spatial distribution of colloidal carbohydrates and chlorophyll *a* is clear from the depth profiles at both sites (Figure 5.20). However, regression analysis showed carbohydrates were only related to chlorophyll *a* at the mid-intertidal site (r^2 = 28 %, $F_{1,56}$ = 21.82, P = 0.000) with chlorophyll *a* accounting for 28 % of the variation in carbohydrate concentrations. The model describing this relationship was (sqrt. conc. coll. carbo.) = 1.92 + 2.69 (sqrt. Chl. *a* conc.). Another model predicting colloidal carbohydrate concentrations from measurements of chlorophyll *a* was described by Underwood and Smith (1998b), (log (conc. coll. carbo. + 1) = 1.40 + 1.02 (log (Chl. *a* conc. + 1). Their model accounted for 65 % of variability in colloidal carbohydrate concentrations. Both models found a significant positive relationship between chlorophyll *a* and colloidal carbohydrates. The data collected in this study was then transformed in the same way as that described by Underwood and Smith and the chlorophyll *a* data entered into the carbohydrate prediction equation. The actual colloidal carbohydrate data from this study was then compared with that predicted by the model using regression analysis

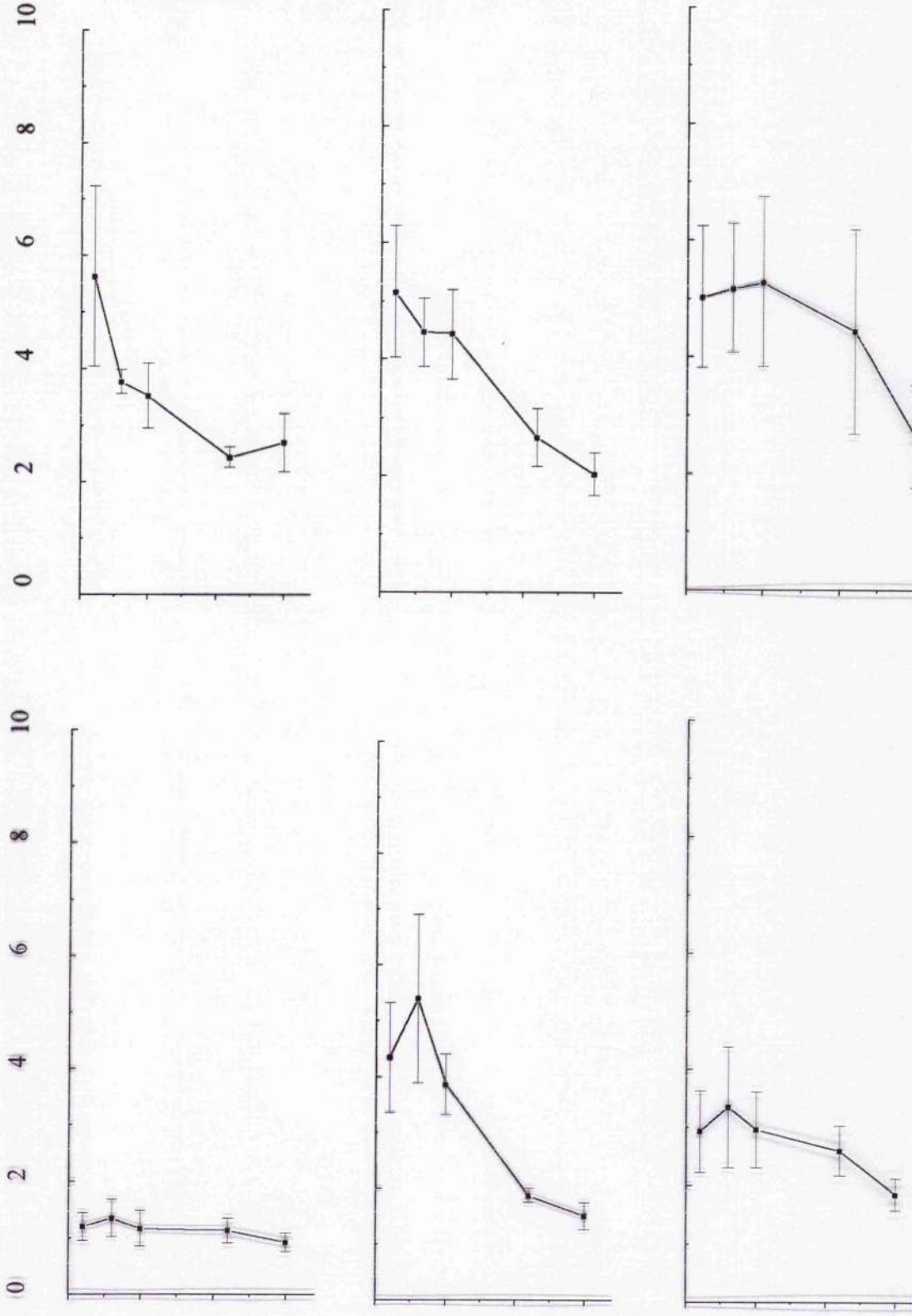
(log (carbo. conc. +1) = $\alpha + \beta$ (log (Chl a conc. +1) + β_2 (intercept) β_3 (slope)

and the slope (β) and intercept (α) of the present study was found to be significantly different to that of the model. Underwood and Smith. had used average β and α values from several data sets from English sampling sites whereas the present study describes only one data set from France.

Figure 5.19 Concentration of chlorophyll *a* in the upper 1.5 mm on 10/4/97 (n = 5, bars = S.E.)



Overlay showing similarities between colloidal carbohydrate and chlorophyll *a* profiles (mg glucose equ. g^{-1} sediment)



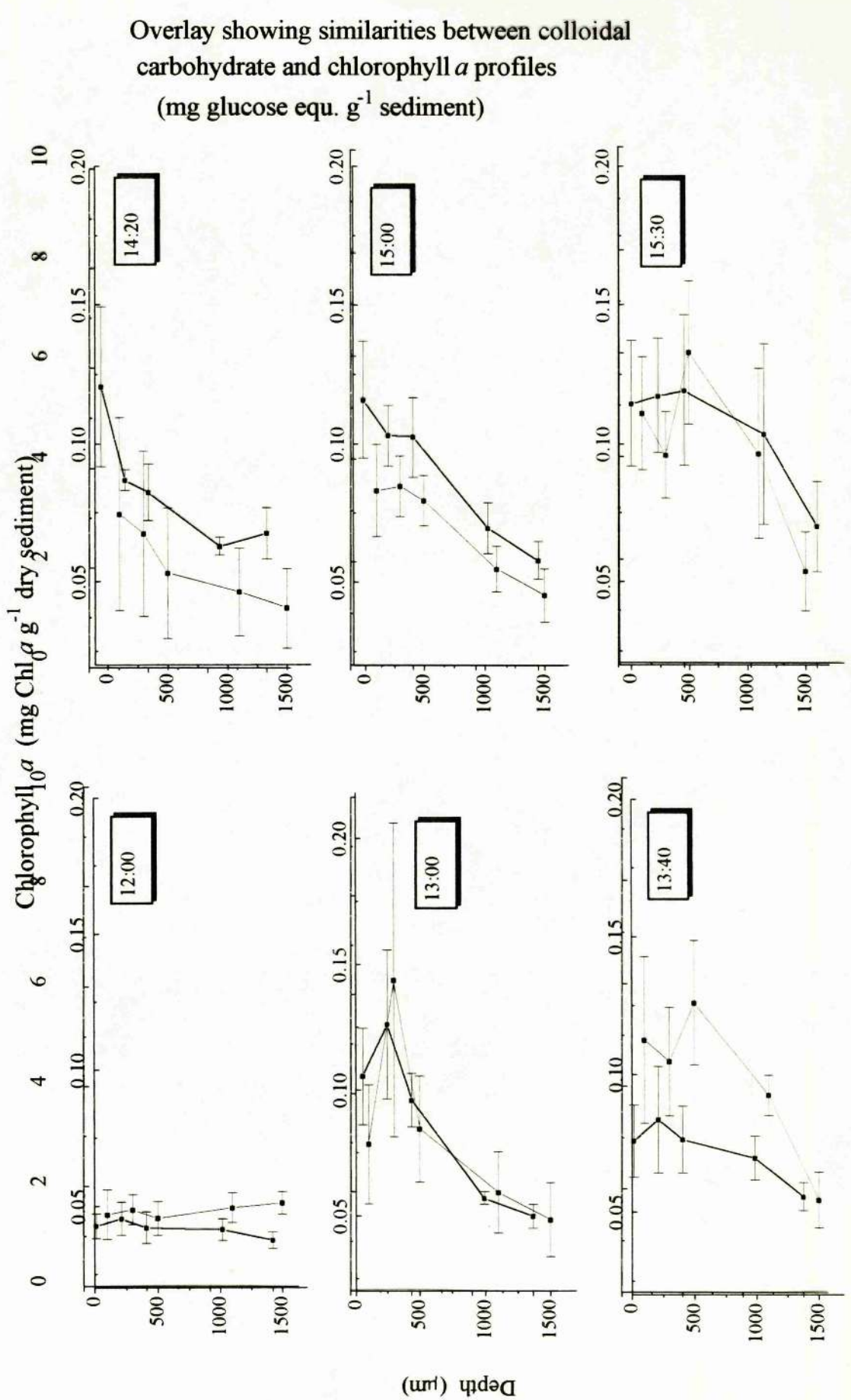
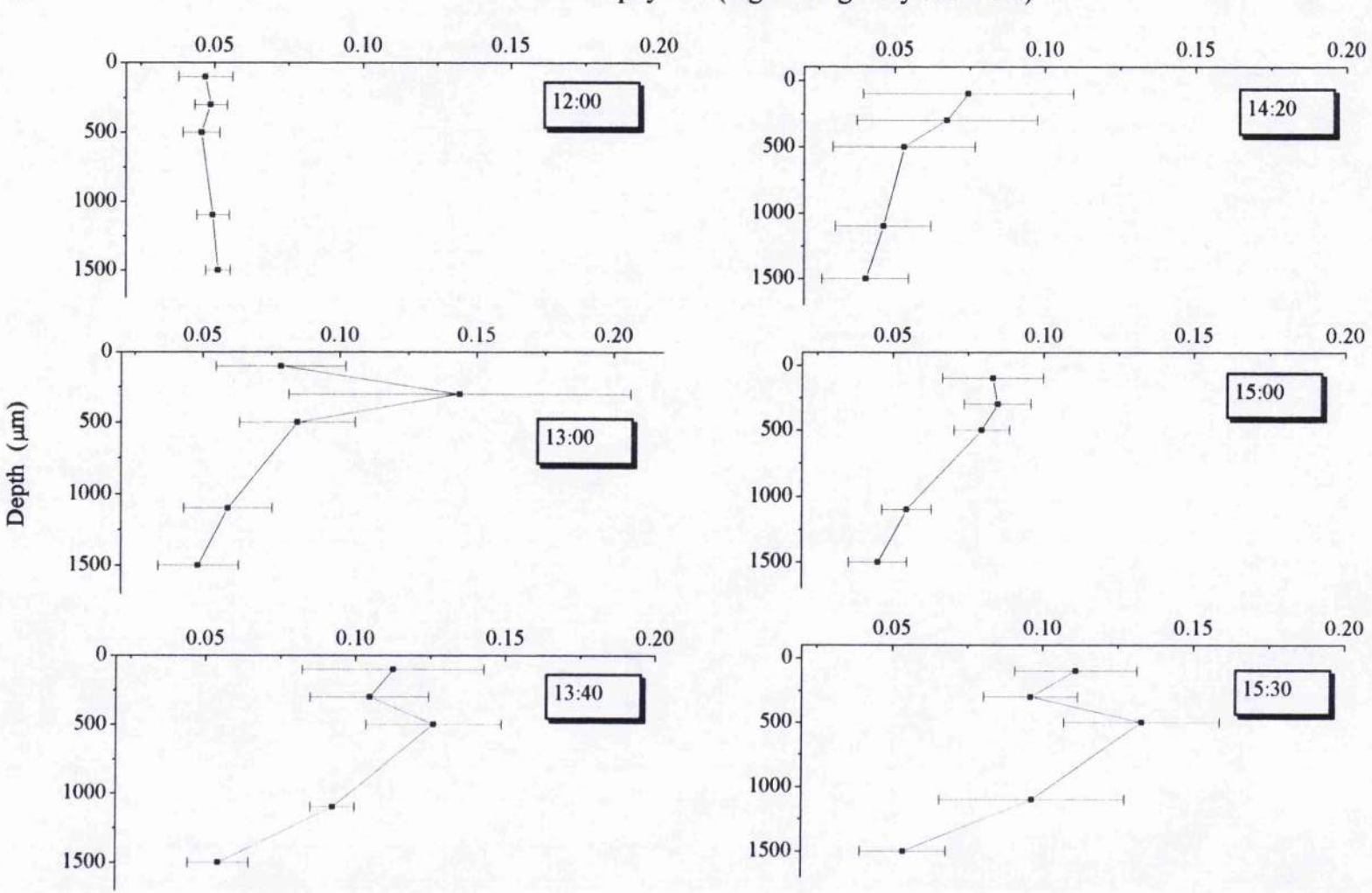


Figure 5.20 Chlorophyll *a* concentrations with depth, over tidal cycle on 11/4/97, upper-intertidal site (bars = S.E., n = 5)

Figure 5.20 Chlorophyll *a* concentrations with depth, over tidal cycle on 11/4/97, upper-intertidal site (bars = S.E., n = 5)



Therefore, although there is a positive relationship between sediment chlorophyll *a* concentrations and colloidal carbohydrates in both studies, the model described by Underwood and Smith does not encompass data in the present study.

By including sediment depth into the present model, the percentage of carbohydrate variability explained was increased to 62 % (r^2 = 62.3 %, multiple regression analysis, $F_{2,55}$ = 45.37, P = 0.00). Finally, the time that sediment samples were collected, over the emersion period, was included in the regression analysis but did not explain carbohydrate variability any further.

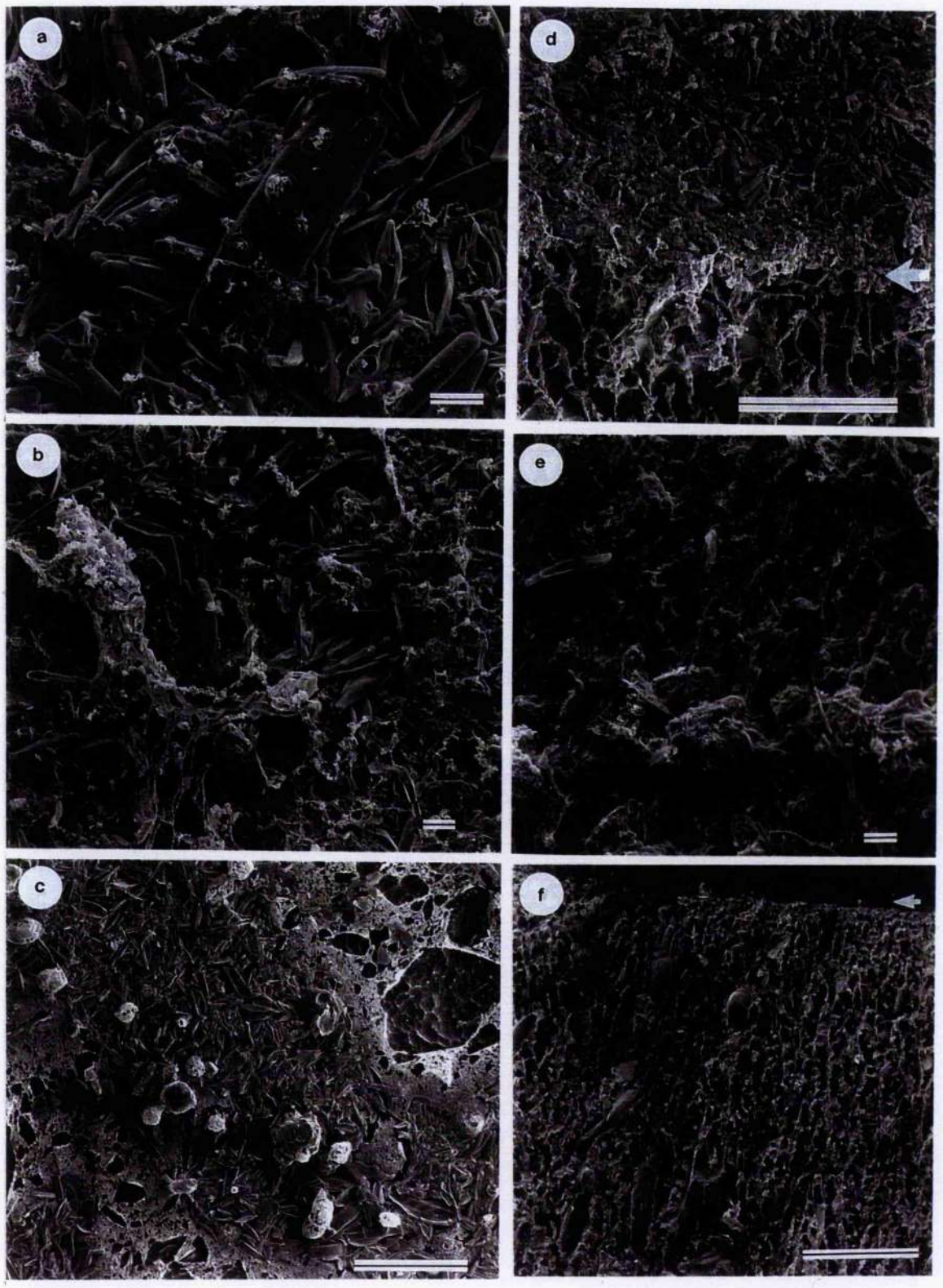
At the upper-intertidal site, chlorophyll *a* accounted for only 2 % of the variation in carbohydrate concentrations ($F_{1,147}$ = 2.97, P = 0.09). Consideration of the sampling depth in the regression accounted for 14 % of carbohydrate variability at the upper intertidal site (r^2 = 14.1 %, $F_{2,145}$ = 11.88, P = 0.000) and when sampling time over the emersion period was included, 21 % of the variability in carbohydrate concentrations was explained ($F_{3,144}$ = 13.37, P = 0.000).

The variation in biomass between the two sites was qualitatively demonstrated by low-temperature scanning electron microscopy of surface and fracture face sediment samples (Figure 5.21). Epipelic diatoms dominated the microphytobenthos at the mid-intertidal site. Fracture face views of the sediment showed diatoms to be limited to the uppermost surface layer (< 100 μm) and many of the diatoms were very small naviculoids (< 5 μm), although other larger forms, including *Nitzschia* spp., were present.

5.4.1.2 Temporal variation

During the emersion period at the mid-intertidal site, colloidal carbohydrate concentrations did not vary significantly (Figure 5.17, $F_{2,57}$ = 1.86, P = 0.165, one-way ANOVA on square root transformed data). However, there was a decrease in chlorophyll *a* ($F_{2,55}$ = 7.78, P = 0.001, on square root transformed data). Therefore, the ratio of colloidal carbohydrate: chlorophyll *a* increased from 30:1 to 76:1 ($F_{2,52}$=16.2, P = 0.000, one-way ANOVA on log transformed data).

Figure 5.21 Low-temperature scanning electron micrographs showing surface sediments from Baie de Marennes-Oléron (a) A typical surface sediment from the mid-intertidal site (13:40 hrs on 10/4/97) at Baie de Marennes-Oléron showing the surface to be covered with a variety of epipelic diatoms (scale bar = 10 μm). (b) Epipelic diatoms at the mid-intertidal site associated with large amounts of a binding material (probably EPS, scale bar = 10 μm). (c) The surface sediment of the mid-intertidal site showing many small (<30 μm) epipelic diatoms and some larger forms (scale bar = 100 μm). (d) An electron micrograph showing both the surface (above arrow) and the fracture face (below arrow) of mid-intertidal sediments. The diatoms appear to form a very thin (< 100 μm deep) skin on the surface. The striations visible in the fracture face may represent ice crystal segregation zones (Jefree and Read 1991, scale bar = 100 μm). (e) A typical surface of the upper-intertidal zone (15:00 hrs on 11/4/97). There were far fewer diatoms present than at the mid-intertidal site. In addition, the sediment particles can be clearly seen (many intertidal surface sediments are covered in an organic film i.e. Humber Estuary, Chapter 3 and Eden Estuary, Chapter 4, scale bar = 10 μm). (f) A fracture face from the upper-intertidal site, the surface is marked by an arrow. Only a few diatoms are present. There are 10 μm wide vertical tubes which may indicate the movements of epipelic diatoms (scale bar = 100 μm).


a
d
b
e
c
f

There was a significant increase in colloidal carbohydrates over the diurnal time-scale on day 2 (Figure 5.18, upper-intertidal site, $J^* = 10.9$, $P = 0.01$, $n = 32$, Jonckerre's test for ordered alternatives; this non-parametric test was chosen as the variances in the sample groups were heterogeneous, preventing the proper use of ANOVA, Siegel and Castellan 1988). However, there was no significant change in chlorophyll *a* concentrations with time at the upper-intertidal site ($J^* = -0.77$, $P = > 0.1$, $n = 31$). The ratio of colloidal : chlorophyll *a* did not vary significantly over the time series ($F_{5,149} = 2.25$, $P = 0.052$ on log transformed data), but ranged between 30:1 at 12:00 hrs. interval to a peak of 84:1 at 14:20 hrs, finishing at 50:1 at the end of the time series (15:30 hrs.). Although time and depth had a significant effect on chlorophyll *a* concentrations on day 1, there was no interaction between the two factors ($F_{8,43} = 0.72$, $P = 0.67$, General Linear Model ANOVA on square root transformed data).

5.4.2 Discussion

Epipelic diatoms were more likely to become light saturated at the light intensities present in Baie de Marennes-Oléron (approximately 1400 $\mu mol\ m^{-2}\ s^{-1}$). than those in the laboratory experiment, although light conditions in the laboratory experiment reflected those in the field at that time. This may account for the significant decrease in chlorophyll *a* over the time series on day 1.

There was a strong correlation between the spatial distribution of colloidal carbohydrates and chlorophyll *a*. However, it was apparent that the factors influencing colloidal carbohydrates differed in relative importance, between the two sites. Algal biomass was much higher at the mid-intertidal site (Figure 5.20) and colloidal carbohydrate concentrations could be predicted by chlorophyll *a* concentrations. This supports the findings of Underwood and Smith (1998b). The relationship between chlorophyll *a* and carbohydrate concentrations did not exist at the upper-intertidal site, probably because concentrations were lower. Underwood and Smith (1998b) on the subject of colloidal carbohydrate concentrations noted that; "the predictive ability [of chlorophyll *a*] is lower...where epipelic diatom biomass is lower". Therefore, physical factors,

including exposure time and sediment depth, appeared to have more influence over colloidal carbohydrate concentrations at this site. This influence may be indirect and could reflect variation in light intensity with depth and over daytime exposure which in turn, would affect the distribution of diatoms and rates of primary productivity (Kühl *et al.* 1997).

Concentrations of chlorophyll *a* and colloidal carbohydrate, may have been lower at the upper-intertidal site due to an increased density of the grazing gastropod, *Hydrobia* sp. and it is possible that some of the colloidal carbohydrates detected at this site were produced by the gastropods. Differences in mud flat morphology between the two sites may also have influenced chlorophyll *a* and colloidal carbohydrate concentrations, however there was no difference in particle size (mode 9.4, median 8.1 and mean 25 μm, Honeywill and Kelly pers. comm.) between the sites.

The average sediment particle size at Baie de Marennes-Oléron (25 μm) was lower than on other intertidal mud flats (i.e. 110 and 75 μm mean particle size at Tay and Eden Estuary sites respectively, Honeywill pers. comm.) and the diatom biofilm appeared comparatively thin (<100 μm, Figure 5.21, pers. obs.). With decreasing particle size, the penetration of light and CO_2 may be reduced (Kühl and Jørgensen 1994, Paterson *et al.* 1996) thus, restricting the depth of the photic zone and the distribution of algal biomass. Furthermore, sediment porosity may decrease with decreasing particle size and may restrict the mobility of epipelic diatom cells.

To determine if particles size could have restricted the vertical distribution of algal biomass (i.e. diatom migration), comparisons were made of depth profiles from Baie de Marennes-Oléron, the Eden and Tay Estuaries. At Baie de Marennes-Oléron, average chlorophyll *a* and colloidal carbohydrate concentrations were 60 % lower at 1 mm than on the sediment surface (0 - 0.2 mm). At the Eden Estuary, colloidal carbohydrate concentrations at 1 mm were 68 % lower than on the surface and chlorophyll *a* concentrations were 64 % lower than on the surface and in sediments from the Tay Estuary, chlorophyll *a* concentrations were 10 % lower at 1 mm than on the surface (Honeywill pers.

comm.). Therefore, chlorophyll *a* concentrations decreased more rapidly with depth at Baie de Marennes-Oléron than in the Tay Estuary. However, there was no difference in the vertical distribution of chlorophyll *a* between the Eden Estuary and Baie de Marennes-Oléron. Therefore, the particle size at Baie de Marennes-Oléron could not restrict the vertical migration of algal biomass (note that these comparisons assume that the diatom biomass has migrated to the sediment surface). Physical mixing of surface sediments during immersion may disperse chlorophyll *a* throughout surface sediments at Baie de Marennes-Oléron. This was suggested from the fluid nature of the sediment (63 % water content compared to 37 % on the Eden Estuary, Kelly pers. comm.). In addition, anoxic conditions began at a depth of approximately 7.5 cm compared with 2 - 4 cm at the Eden and infers that sediments above this depth were mixed regularly therefore the diffusion of CO_2 in the sediment would also not be restricted in these sediments. In addition, the dry mass concentration of surface sediments (0 - 200 μm) was lower at Baie de Marennes-Oléron (185 kg m^{-3}) than on the Eden (266 kg m^{-3}), therefore small particle size would not have been restricting on diatom migration.

The concentration of colloidal carbohydrates increased significantly over the emersion period at the upper-intertidal site but not the mid-intertidal site, this may reflect the more extensive time series at the upper-intertidal site or perhaps the contribution of mucopolysaccharides from *Hydrobia* (i.e. protein-carbohydrate complexes). Chlorophyll *a* concentrations decreased over the tidal period at the mid-intertidal site but not at the upper intertidal site and this may have been due to differences in light intensities over each sampling day. Alternatively, the decrease may have been more marked at the mid-intertidal site since biomass levels were an order of magnitude higher.

A comparison between the depth profiles of colloidal carbohydrates taken over the emersion periods at Baie de Marennes Oléron and those from the Eden and Humber Estuaries (Figures 4.4 and 4.7) reveals some general trends.

1. The greatest concentrations of colloidal carbohydrates were detected in the surface 400 μm of all profiles and in the upper 200 μm in many of the profiles.
2. Secondly, the variability of colloidal carbohydrate concentrations with time was greatest in the surface 200 μm section and temporal variation decreased with depth. In the profiles from Skeffling on the Humber Estuary (Figure 4.4), there was no variation in colloidal carbohydrates with time below 4 mm.
3. Where replicate depth profiles were taken (Figures 5.17 and 5.18) there was high variability between replicates taken at the same time, reflecting high spatial heterogeneity of colloidal carbohydrates.
4. Colloidal carbohydrate concentrations varied over the emersion period, there were significant increases in concentrations over the emersion period at Skeffling on the Humber Estuary (Figure 4.4) and at the upper-intertidal site at Baie de Marennes Oléron (Figure 5.18). At site 1 on the Eden Estuary, the concentrations of colloidal carbohydrates were higher at the end of the emersion period than at the beginning (Figure 4.7a), however there were large fluctuations during the intervening hours. There was no significant temporal variation in colloidal carbohydrates at site 2 on the Eden Estuary (Figure 4.7b) or at the mid-intertidal site at Baie de Marennes Oléron (Figure 5.17). Therefore, the pattern of temporal variation of colloidal carbohydrate concentrations differed within and between estuarine sites.

At Baie de Marennes Oléron, there appeared to be no interaction between time and depth factors at the mid-intertidal site, although both variables had a significant negative effect on chlorophyll *a*. The lack of interaction implies that when one variable acts negatively on chlorophyll *a* the other has little or no effect.

In order to quantify the allocation of carbohydrates to heterotrophic growth and understand the processes which degrade or change colloidal carbohydrates during emersion, extracellular enzyme activity measurements should be included in further studies (Arnosti *et al.* 1994, Paterson *et al.* 1996b).

5.5 Conclusions

A correlation between colloidal carbohydrate and chlorophyll *a* concentrations was shown in laboratory and field studies. In addition, the time of tidal emersion and depth within the sediment were found to influence the distribution of *in situ* colloidal carbohydrates and chlorophyll *a*. Microscale spatial variability was demonstrated and oxygen profiles indicated that the photosynthetic productivity zone in the laboratory study was approximately 0.5 mm thick. Rapid changes in colloidal carbohydrate and chlorophyll *a* concentrations were measured over the emersion period. Further high resolution field studies would validate the prediction of carbohydrate concentrations from measurements of chlorophyll *a*.

Chapter 6

6. Introduction

Carbohydrates comprise approximately 80% of EPS (Myklestad 1974) and are isolated from diatom cultures, water and sediments using various techniques such as hot and cold water extraction, EDTA, acid /alkali extraction and alcohol precipitation and filtration (Myklestad and Haug 1972, Handa and Mizuno 1973, Underwood *et al.* 1995, Sigleo 1996, McKnight *et al.* 1997). Those carbohydrates that are easily extracted from sediments, have been termed colloidal carbohydrates (Underwood *et al.* 1995) and are likely to be associated with porewater. A large proportion (20 - 25 %) of carbohydrates in this fraction are polymeric with the other 75 - 80 % being free sugars (Underwood *et al.* 1995). The bulk carbohydrate phase is usually found in higher concentrations than colloidal material (Chapter 3). This reflects the extraction method via direct addition of the hydrolysing reagents to sediments thus the bulk fraction will include intracellular material and carbohydrates associated with the sediment particles. However, the separation of sediment carbohydrates into colloidal and bulk fractions is operational and in natural sediments, a range of carbohydrate forms exist (Figure 6.1). The secretion of carbohydrates by epipelic diatoms results in the presence of colloidal carbohydrates in pore waters and a polysaccharide matrix associated with cells and sediment particles. The polymeric components are broken down to monomers by extracellular enzyme activity. Intracellular material is released into the environment, especially in unfavourable conditions, introducing soluble carbohydrates into the colloidal phase. Intertidal mud flats are a sink for marine and terrestrial detritus. The carbohydrates in detrital materials are initially quantified in the bulk fraction but may contribute to the pool of colloidal carbohydrates, following enzymatic degradation. Microalgae are the main source of surface colloidal carbohydrates on intertidal mud flats. Therefore, the ingestion of diatoms by grazers such as amphipods and nematodes changes the partitioning of carbohydrates from the colloidal to bulk fraction. Furthermore, sediment carbohydrates may be subject to geo-chemical transformations. For example, colloidal carbohydrates may enter the bulk fraction through aggregation into a gel, in the presence of divalent cations

Figure 6.1 A low-temperature image of a sediment biofilm used to illustrate the many forms of carbohydrate present on intertidal sediments and the processes which mediate their partitioning. **(S)** the secretion of carbohydrates by epipelic diatoms, **(m)** monosaccharides and **(p)** polysaccharide matrix present on surface sediments, **(R)** natural release of intracellular material, **(D)** marine and terrestrial detritus, **(E)** extracellular enzyme degradation, **(I)** the acid hydrolysis of intracellular sugars in the bulk carbohydrate fraction, **(G)** diatom ingestion by grazers, **(T)** geo-chemical transformations of bulk and colloidal fractions (scale bar = 10 μm).

D
R
I
S
p
m
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T
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II

(Decho 1994, Chin *et al.* 1998) or bind directly with clay particles (Martin 1971).

A lack of biochemical information on bulk and colloidal carbohydrate fractions has led to the assumption that they represent a purely operational separation. However, the operational fractions may reflect different biochemical characteristics. Decho (1994) states; *"The chemical composition is the single most important factor which affects the stability, cohesiveness and physical state of an exopolymer"*. Carbohydrate fractions were found to be distributed differently in surface sediments (Chapter 3). Colloidal carbohydrates were concentrated in the surface 200 μm (4-12 mg g^{-1} sediment) and bulk carbohydrates increased in concentration with sediment depth to approximately 14-33 mg g^{-1} at 20 mm. In addition, some carbohydrate fractions were shown to differ in their temporal variation over the emersion period with colloidal carbohydrate increasing and bulk carbohydrates remaining unchanged (Chapter 4). The spatial and temporal variability of these carbohydrate fractions prompted an investigation into their monosaccharide composition. Of the few studies into sediment carbohydrate biochemistry (see Cowie and Hedges 1984), none deals with intertidal sediments or analyses the operational separation employed in their analysis.

Several partially-relevant biochemical analyses have been performed on planktonic samples and diatom mono-cultures. In these studies, EPS was isolated from the culture medium by lyophilisation and the polymers were generally found to be anionic and contain galactose, fucose and rhamnose as major monosaccharide components while mannose, glucose, xylose and arabinose were present to a lesser degree (Myklestad and Haug 1972, Smestad *et al.* 1974). Sulphate was commonly found to comprise 7-9 % of the polymer (Allan *et al.* 1972, Bhosle *et al.* 1995). This may enhance the polymerisation of EPS and may play a role in anti-desiccation properties (Decho 1994, Bhosle *et al.* 1995). Sulphate esters and uronic acids may be responsible for the anionic nature of the polysaccharides, forming linkages through cation bridges (Ca^{2+}, Mg^{2+}), resulting in a gel structure (Decho 1994, Chin *et al.* 1998). The gel structure may be broken down with metal chelating agents,

such as EDTA (Decho 1994) and this reveals the significant role EPS plays in binding metals such as copper (Pistocchi *et al.* 1997).

An analysis of carbohydrates in the water column showed glucose to be the most common monosaccharide in suspended particles but not in the dissolved carbohydrate fraction, where carbohydrates were composed of equal percentages of glucose, galactose, fucose, rhamnose and xylose (Sigleo 1996). This suggests that different carbohydrate forms may co-exist naturally in a similar environment. Aluwihare *et al.* (1997) identified the need for research into the link between carbohydrate accumulation and algal production. This is particularly important on intertidal sediments where microphytobenthic productivity may exceed that of phytoplankton, particularly where the water is turbid (Joint 1978). However, there is no chemical information on the carbohydrates associated with epipelic diatoms. These may be fundamentally different to those released by cells in the water column since diatoms on intertidal sediments release EPS through the raphe for locomotion, whereas planktonic forms are carried by water movements. Centric planktonic diatoms often produce rigid polysaccharide fibrils to facilitate buoyancy (Hoagland *et al.* 1993), while epipelic forms resist being suspended in the water column through downward migration cycles. The differences in ecology are obvious, yet the chemical composition of the carbohydrates are assumed to be the same. The monosaccharide composition of the bulk sediment and colloidal carbohydrate fractions are compared with published studies on the types of monosaccharides secreted into the medium by planktonic diatoms.

6.1 Methods

This was a pilot study into the application of advanced biochemical techniques for the characterisation of carbohydrates from sediments and as such, it needs to be replicated in further studies.

Sediment was collected from a mid-intertidal station on the Eden Estuary, Fife, UK (56° 22' N 02° 51' E) in May 1996. Sampling took place 2 h after low tide, the sediment temperature was 22°C and the salinity 25 (‰). The surface 10 mm of an area 50 x 30 mm was transferred into a perspex tray. From this, 10

syringe cores of the upper 5 mm (1.8×10^{-3} m^3) were pooled for monosaccharide quantification and identification.

6.1.1 Carbohydrate fractionation

Sediment carbohydrates were operationally separated into colloidal and bulk carbohydrate fractions (Underwood *et al.* 1995). The colloidal carbohydrate fraction was extracted from the sediment using distilled H_2O. The water and sediment were mixed to form a slurry which was incubated at 20°C for 15 min. The liquid phase containing colloidal carbohydrates was then separated by centrifugation (2000 g for 15 min). 200 µl of the colloidal fraction was used for carbohydrate quantification. The bulk carbohydrate fraction includes all carbohydrates found in sediment after the extraction of colloidal carbohydrates. The sediment pellet (formed through centrifugation) was frozen, lyophilised and the dry weight was noted. The pellet was then homogenised and 2 mg used to determine bulk carbohydrate content.

6.1.2 Carbohydrate quantification

The carbohydrate concentrations of both fractions were quantified using the spectrophotometric assay (Dubois *et al.* 1956) described in Chapter 2. Briefly, 1 ml of concentrated sulphuric acid (H_2SO_4) was added to 200 µl of the colloidal carbohydrate fraction and 200 µl of 5 % w/v of phenol using a pump dispenser. The mixture was incubated for 35 min and sugar concentration determined against glucose standards (Liu *et al.* 1973). To quantify the bulk fraction, 2 mg of the homogenised, lyophilised sediment was added to 200 µl distilled H_2O and the assay protocol followed as before. After incubation, the sediment reagent mixture was centrifuged and the absorbance of the supernatant read.

6.1.3 Carbohydrate purification for GC-MS analysis

Carbohydrate fractions were hydrolysed with 50 ml of 0.1 kmol m^{-3} tri-fluoroacetic acid (TFA) at 100°C for 4 hours to purify the samples (Ferguson 1992). 10 g of the lyophilised and homogenised sediment (representing the bulk

carbohydrate phase) and 30 ml of colloidal carbohydrate extract were used. The solutions were cooled and filtered using sintered glass filter funnel (Quickfit SF3A33) containing cilite clay. The filtrates were placed in a rotatory evaporator (Büchi R-114) and evaporated to dryness. Ethanol was then added and evaporated off, to remove residual TFA and remaining distilled water. Samples for gas chromatography-mass spectrometry (GC-MS) were deionised using an ion exchange column (Dowex AG50, hydrogenated form). The de-ionised samples were re-dissolved in 100 µl of distilled water and carbohydrate concentrations of the bulk and colloidal samples were measured.

6.1.4 Monosaccharide analysis : Methanolysis and GC-MS

Methanolysis and GC-MS allows the identification of neutral sugars, *N*-acetyl hexoamines and sialic acid (Ferguson 1992). Trimethylsilyl (TMS) derivatization of the deionised carbohydrate samples was undertaken before the GC-MS analysis. Glass capillary tubes (SMI size J, yellow band) were prepared by heat-cleaning. One end of the tube was sealed and a carbohydrate sample, which corresponded to a concentration of 5 µg ml^{-1} of glucose equivalents, was added. Monosaccharide standards were also prepared. These contained 0.5 nmol m^{-3} each of arabinose, fucose and xylose, mannose, galactose, glucose, *N*-acetylgalactosamine and sialic acid. 1 nmol m^{-3} of *scyllo*-inositol was added to the samples. The microtubes were dried in a speed vacuum concentrator (Stratech Scientific). The contents were dissolved in 20 µl methanol and dried, then dissolved in 50 µl of 0.5 mol m^{-3} HCl in dry methanol. The capillaries were sealed and placed in a heating block at 80°C for 4 h. Methanolysis causes the breakdown of glucosidic bonds in the monosaccharides, this results in the production of methylglycosides. After cooling, the capillaries were broken open and 10 µl of 2- methyl 2- propanol was added and then dried using the speed vac concentrator, followed by the addition of 20 µl of methanol, then 10 µl of pyridine to neutralise any remaining HCl. 10 µl of acetic anhydride was added to each capillary tube and the samples incubated for 25 min at 20°C, this procedure *N*-acetylates any free amines (Ferguson 1992). The contents were again dried and dissolved in 20 µl of methanol and dried once more to

ensure thorough desiccation. Finally, 15 µl of trimethylsilyl (TMS) was added to the capillaries which were sealed with teflon tape and incubated at 20°C for 10 min.

1 µl of the standards mixture was injected into the GC-MS (Hewlett Packard). The courier gas was helium and the column was Econocap SE54 (30m x 0.25 mm). Injections were made using a split/splitless injector. The programme was as follows: injection temperature 80°C (1 min) starting temperature 140°C (1 min) then increasing at 5°C per min to 260°C and finally 15°C per min to 300°C. The run time was approximately 45 min. There were sufficient carbohydrates in the samples to use 0.5 µl of the bulk and colloidal samples. The monosaccharide composition of the sediment samples were determined through the comparison of the sediment samples with the monosaccharide standards. The concentration of each monosaccharide in the samples (nmol l^{-1}) may be calculated after Ferguson (1992). The configuration of glucose in the colloidal sample was further examined using nuclear magnetic resonance (NMR, Brukur 500 MHz with deuterium oxide as a solvent).

6.2 Results

6.2.1 Monosaccharide GC-MS analysis

Total Ion Chromatograms (TICS) show that both samples were dominated by glucose and contained the same variety of pentoses and hexoses (Fig. 6.2). However, the ratio of glucose to other monosaccharides in the carbohydrate fractions were markedly different (Fig. 6.3, Table 6.1). The colloidal fraction had a greater proportion of glucose to other monosaccharides (82 %) than the bulk fraction (37 %). The importance of primary chemical purification by TFA hydrolysis was shown by a comparison of two colloidal carbohydrate samples (Fig. 6.4). The untreated colloidal sample appeared brown in colour and the baseline of the TIC was unstable (Fig. 6.4a), suggesting contamination of the sample with other compounds, possibly lipids. Furthermore, monosaccharide abundances in the untreated colloidal sample were much lower than in the TFA treated sample (Fig. 6.4b), which may indicate that non-carbohydrate substances in the sample were masking the signal of the sugars.

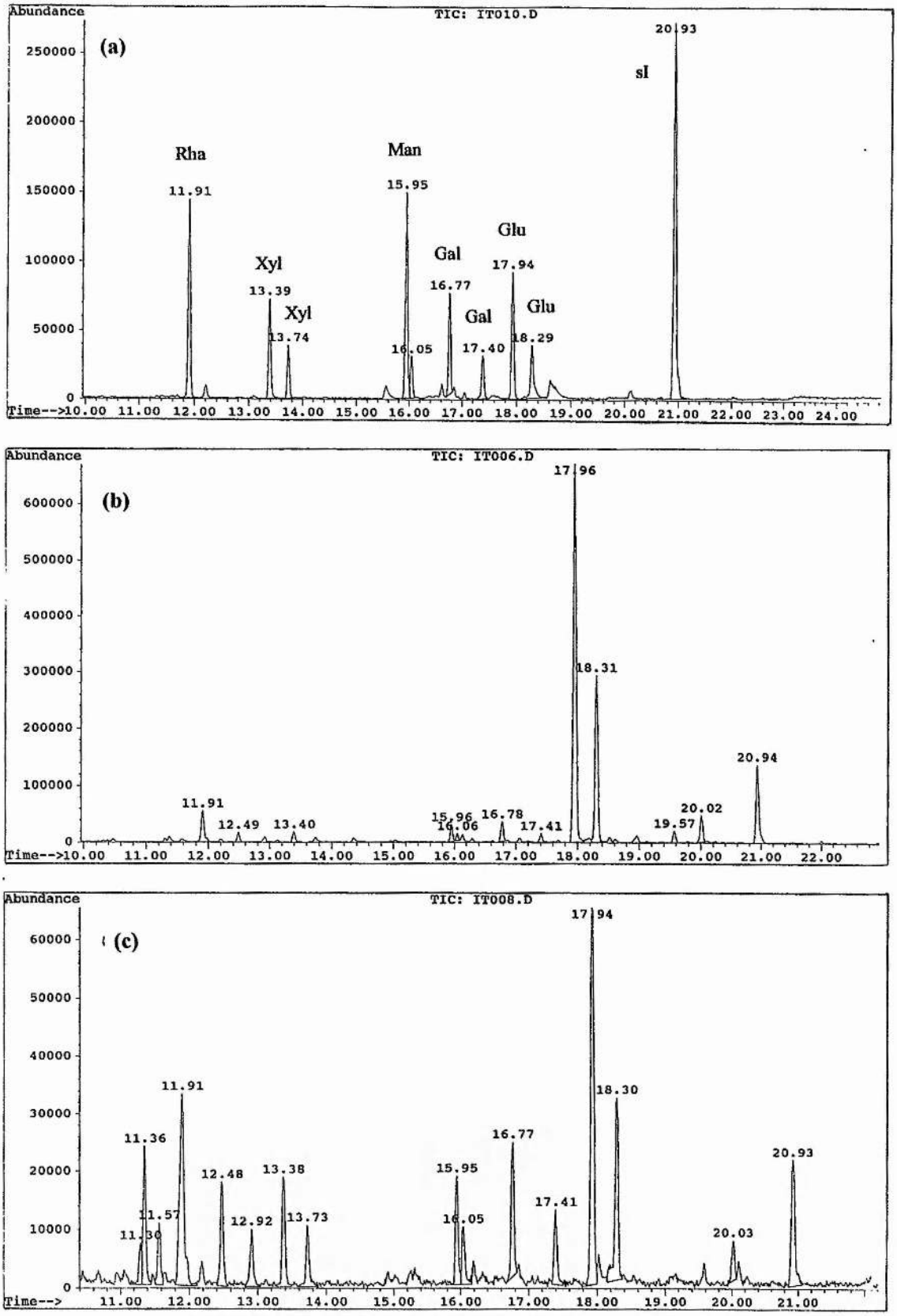
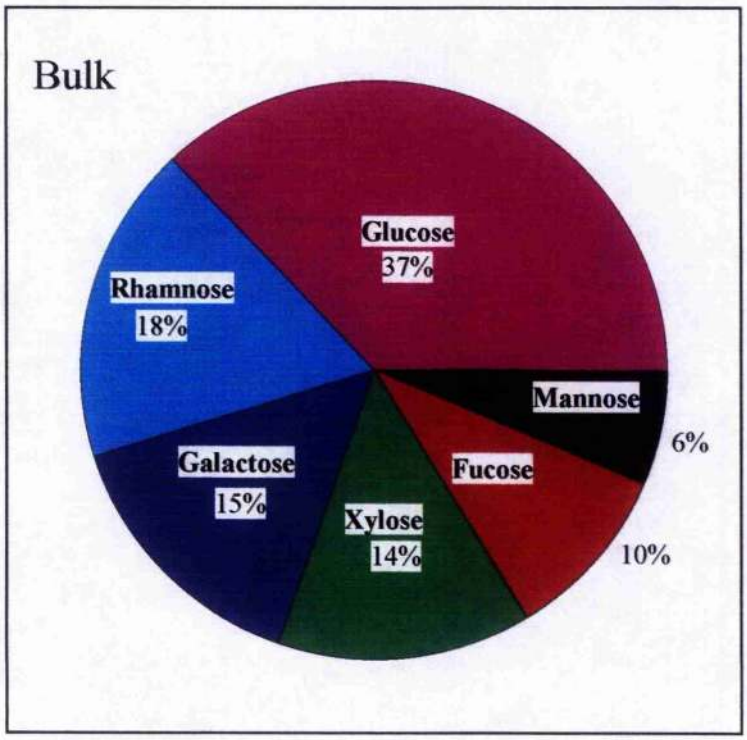
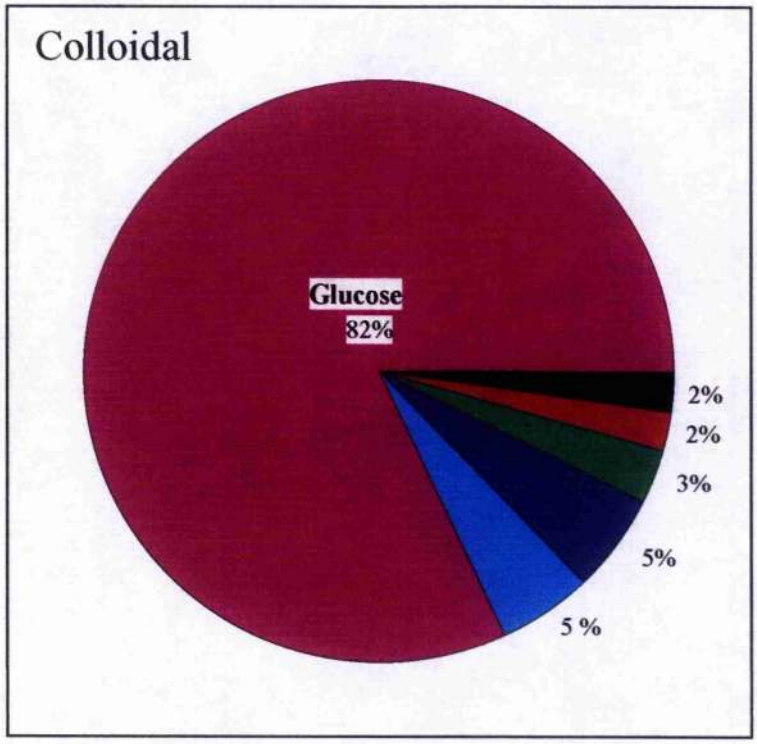


Figure 6.2 Total Ion Chromatograms (TIC) of the GC-MS carbohydrate analysis, (a) sugar standards (b) colloidal carbohydrate sample (c) bulk carbohydrate sample (Gal: galactose, Glu: glucose, Man: mannose, Xyl: xylose, Rha: rhamnose, sI: *scyllo*-inositol).

Figure 6.3 Percent variation in monosaccharide composition of colloidal and bulk carbohydrate samples





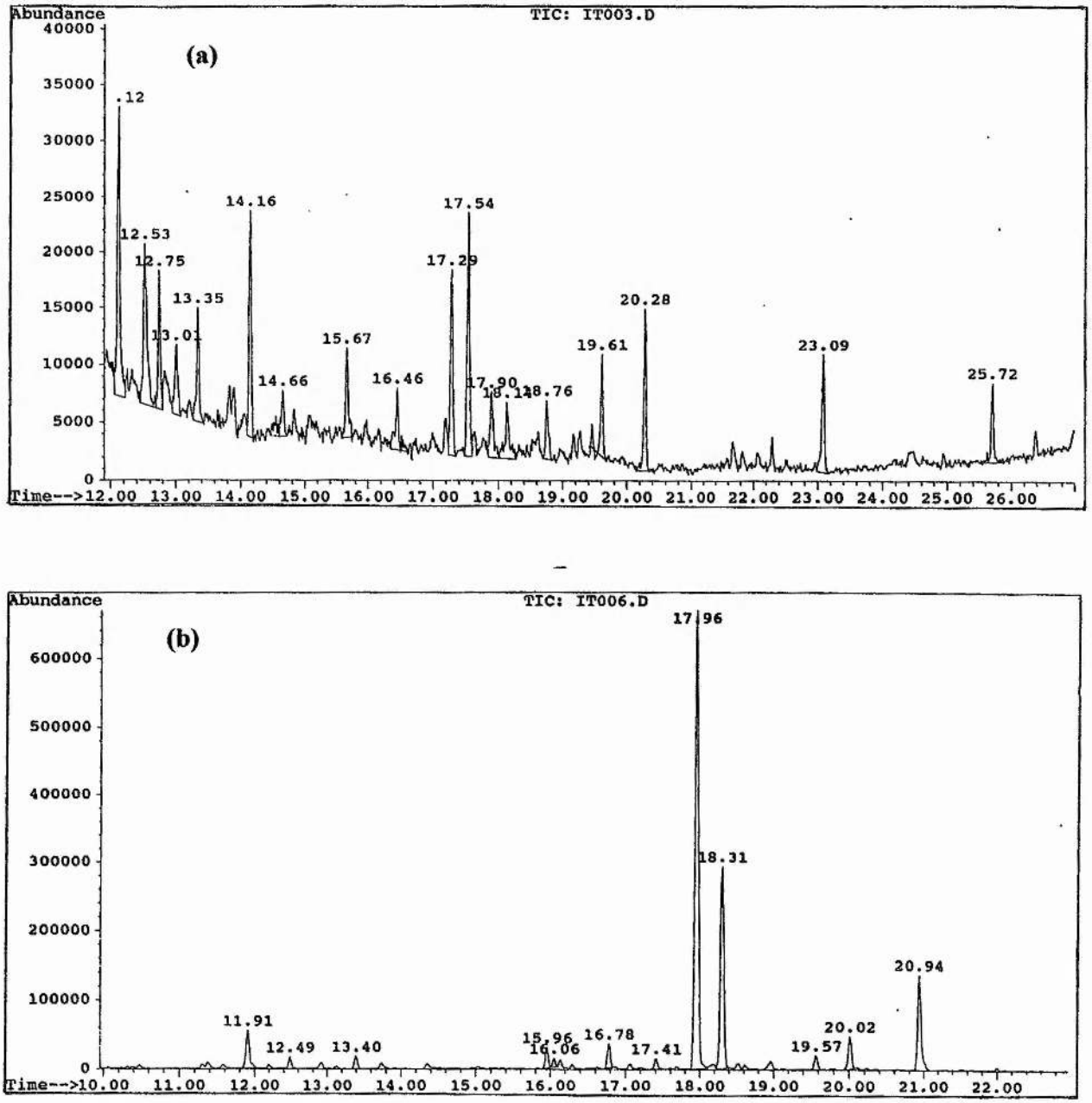


Figure 6.4 Total ion chromatographs (TIC) illustrating the effect of TFA purification on sample integrity during GC-MS analysis.

A compound was isolated from both bulk and colloidal fractions which appeared after the hexose sugars and before the *scyllo*-inositol standard. This was initially thought to be a heptose sugar (7 carbon monosaccharide) however, a comparison of the unknown compound with a heptose standard, using a mass spectrometer, found differences in the ring structure. The structure of the unknown compound was later matched with that of galacturonic acid.

Table 6.1 *The concentrations of individual monosaccharides identified in each carbohydrate fraction by GC-MS analysis.*

Monosaccharide	Bulk (nmol)	Colloidal (nmol)	Ratio (b : c)
arabinose	0.19	0.02	1 : 0.1
rhamnose	3.84	0.91	1 : 0.2
fucose	2.14	0.37	1 : 0.2
xylose	3.06	0.52	1 : 0.2
mannose	1.38	0.38	1 : 0.3
galactose	3.21	0.96	1 : 0.3
glucose	8.03	14.2	1 : 1.8

The NMR analysis of the colloidal carbohydrate sample found the glucose present to be predominately in the alpha configuration, where the hydroxyl group at carbon number one is above the plane of the ring.

6.3 Discussion

The results of this pilot study reveal new distinctions between carbohydrates traditionally separated into operational fractions (Underwood *et al.* 1995, Taylor and Paterson 1998), however these procedures need to be repeated with a number of samples perhaps from different sediments.

The monosaccharide composition of both samples were dominated by glucose but relatively more was found in the colloidal sample (82 %). Monosaccharides have been estimated to comprise 75 - 80 % of the colloidal carbohydrate fraction (Underwood *et al.* 1995). This suggests that colloidal carbohydrates may be easily broken down by bacterial extracellular enzymes and those detected in the pore water would be of recent origin. This is supported by the field carbohydrate measurements which showed colloidal carbohydrate concentrations to increase significantly over a short period of time corresponding to the migration and activity of diatoms at the sediment surface (Chapter 4).

However, it is possible that some of the monosaccharides detected in the sediment may have originated from macroalgal or terrestrial detritus.

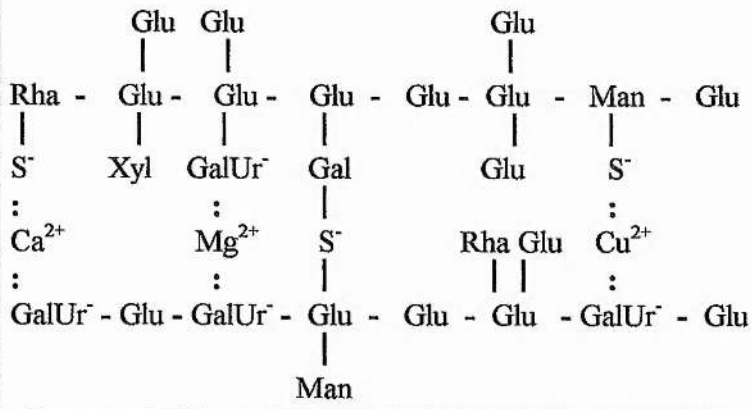
The finding of galacturonic acid in both bulk and colloidal fractions is similar to the analysis of diatomaceous EPS (Decho 1994, Bhosle *et al.* 1995). Although uronic acids have been found to compose only 7-9 % of diatom EPS, they can greatly affect the tertiary structure of the polymer. They introduce carboxyl groups of a negative charge which may be important in polymer bridging and metal adsorption (Decho 1994, Bhosle *et al.* 1995). NMR analysis on the colloidal sample found the alpha form of glucose to be present. This is commonly found in structural polysaccharides such as starch. However, since the colloidal sample was purified for GC-MS analysis, linkage analysis derived from such a sample was unreliable. In future NMR analysis, an extract of EPS (rather than colloidal or bulk carbohydrates) which had been dialysed to remove low molecular weight salts and sugars would be ideal for linkage analysis.

Due to the large number of hydroxyl atoms on any monosaccharide, linkages of *in situ* polysaccharides are likely to be highly complex . Alberts (1989) stated *"With current methods it takes longer to determine the structure of half a dozen linked sugars than to determine the nucleotide sequence of a DNA molecule containing many thousands of nucleotides,"* and this is still the case. Also, the sample of carbohydrates from the Eden Estuary contained a complex mixture of other organic compounds. Even after purification, traces of these substances could be seen as a hydrophobic sheen on the glassware, making linkage analysis difficult. The importance of sample purification was shown by the comparison of TFA hydrolysed material with an untreated colloidal extract which proved to be impossible to analyse. Although the present GC-MS analysis does not provide linkage information, the difference in the monosaccharide ratios suggests that the polymeric structures of the two fractions are different (Bertocchi *et al.* 1990). The production and consumption of the carbohydrate fractions may occur at different rates and be influenced by different environmental conditions (Sigleo 1996). Using compositional studies of diatom culture exudates and metal binding properties (Allan *et al.* 1972, Decho 1994, Bhosle *et al.* 1995), it is possible to hypothesise

some structural properties (Fig. 6.5). GC-MS analysis shows that colloidal carbohydrates contain mainly glucose, this agrees with the analysis of EPS from *Navicula subinflata* (Bhosle *et al.* 1995), therefore, repeated glucose - glucose units (covalent bonds) may dominate the structure forming an important backbone, with less common monosaccharides occurring occasionally. This would be similar to the polysaccharide structure described for the cell envelope of cyanobacteria (Bertocchi *et al.* 1990), however sediments would have to be extracted with EDTA in order to release capsular or cell wall sugars.

Figure 6.5 *Hypothesised structural features of in situ colloidal carbohydrates*

: = ionic bond
- and | = covalent bond



The presence of glucose-glucose bonds is supported by detection of glucosidase extracellular enzyme activity in diatom biofilms (Karner and Rassoulzadegan 1995, Ruddy *et al.* 1996). The sugar backbone may contain a certain degree of branching and cross linking with other sugars, uronic acids and sulphate (Allan *et al.* 1972, Bhosle *et al.* 1995). The presence of these negative groups would attract positively charged ions, forming ionic bonds. This would account for the ability of diatom EPS (of which colloidal carbohydrate is an index) to absorb metals such as copper (Stehr 1995, Pistocchi *et al.* 1997). These cation

bridges (Decho 1994) may give the polymer a gel structure but ionic bonds are not stable and may break readily in the presence of a chelating agent, such as EDTA.

Glucose was shown to be less important in colloidal carbohydrates from marine and estuarine water samples, than in the intertidal sediments of the present study (Mopper *et al.* 1995, Sigleo 1996, Aluwihare *et al.* 1997, Table 6.2). The compositional differences in these colloidal carbohydrates are due to differences in the origins of the carbohydrates. In sea water, colloidal carbohydrates are by-products of phytoplankton primary production and cell lysis (Sigleo 1996), whereas, the main contributor to colloidal carbohydrates (as an index of EPS) on intertidal sediments are benthic diatoms (Underwood *et al.* 1995), which produce EPS to serve a mechanical role in cell locomotion. In addition, the rate of carbohydrate breakdown is probably faster on intertidal estuarine sediments than in the water column, through higher bacterial cell numbers and a greater supply of nutrients. Also, epipelic diatoms utilise EPS in dark and light limiting conditions (Paterson *et al.* 1996b). Therefore, the occurrence and composition of carbohydrates in sediments is quite distinct to those in surface waters and merits separate consideration.

Table 6.2 Comparison of monosaccharide abundances in water column and sediment samples

Percentage monosaccharide abundance	*Sediments containing epipelic diatoms (present study)*	*Potomac estuary surface water (Sigleo 1996)*	*Mid Atlantic surface waters (Aluwihare et al. 1997)*	*Pacific coastal waters (Mopper et al. 1995)*
Gal	5	21	20	30
Glu	82	15	16	40
Rha	5	14	16	5
Fuc	2	16	15	20
Man	2	9	13	-
Xyl	3	16	12	-
Ara	1	7	7	5
Rib	-	4	-	-

Differences in monosaccharide composition between colloidal and bulk sediment carbohydrate fractions may provide a greater understanding of the mechanism of sediment stabilisation by EPS and colloidal carbohydrates (as an

index of EPS). Approximately 20 - 25% of the colloidal carbohydrates are polymeric (Underwood *et al.* 1995), these may increase the resistance of a given sediment to erosion stress by inter-grain binding and floc formation and also through reduction of surface roughness (Delgado *et al.* 1991, Black and Paterson 1997, Sutherland 1996). The concentration of colloidal carbohydrates increases over the emersion period and the stabilisation effects become greater (Black and Paterson 1997). The free sugars which comprise 75-80 % of colloidal carbohydrates, are mainly glucose. Since this is the most easily used form of carbon (Dawes and Sutherland 1992), the production of colloidal carbohydrates by diatoms may have a major role in the success of the heterotrophic microbial community. These may, in turn, produce other forms of polymeric materials. The bulk carbohydrate fraction is an important source of carbon to the heterotrophic community. However, bulk carbohydrates are not dynamic and have not been associated with short-term changes in carbohydrate concentrations or variations in sediment stability. They represent the more refractory carbohydrate material remaining after the breakdown of labile constituents.

6.4 Conclusion

Sediment carbohydrates have been operationally separated into colloidal and bulk fractions which are assumed to be similar in other properties. However, a biochemical distinction between the commonly extracted colloidal and bulk carbohydrates was shown. Both carbohydrate fractions were found to contain the same sugars although in markedly different proportions. This suggests that the two carbohydrate fractions are derived from a range of organic material (from glucose-rich exudates to recalcitrant detritus) and are affected by different environmental processes. An awareness of the extraction procedure used and the carbohydrate fraction measured is advised when relating *in situ* carbohydrate concentrations with biological metabolism or sediment characteristics such as erosion resistance.

Chapter 7

7. General discussion

Sediment carbohydrates are an important carbon source for bacterial metabolism and are of major importance to studies of biostabilisation (Grant and Hargrave 1987, Yallop *et al.* 1994, Smith *et al.* 1995, Paterson *et al.* 1996b). Carbohydrates on intertidal sediments are also a major component of the dissolved organic matter (DOM, Cowie and Hedges 1984) and as such, are an important source of reduced carbon to the oceans (Hedges *et al.* 1994, Bauer and Druffel 1998). The average concentrations of colloidal carbohydrates in the upper 1 mm at Skeffling were 1.2 ± 0.6 g glucose equ. m^{-2} ($n = 4$). If we assume that epipelic diatoms cover 40 % of the mud and sand flats at Skeffling (total area of 56 km^2, Figure 7.1), then a total of 272 kg of colloidal carbohydrates will be contained in the upper millimetre of this area. The typical ratio of colloidal to bulk carbohydrates on intertidal mudflats is 1:10 - 1:40 (Chapter 3) therefore, the same area of intertidal mud flat would contain 3 - 11 tons of bulk carbohydrates. Organic matter from river basins also settles out in the depositional environment of estuarine mud flats. Some of this organic matter will be re-mineralised by sediment bacteria, some may be buried within the sediments or transported into coastal waters (Ruddy *et al.* 1996, Tipping *et al.* 1997). The burial rate for carbon in the shallow North Sea has been estimated at 1×10^5 ton $year^{-1}$ (de Haas *et al.* 1997) and 90 % of this carbon is associated with mineral surfaces (Keil *et al.* 1994). The sorption of organic matter to mineral surfaces slows the re-mineralisation rate by up to 5 orders of magnitude and accounts for the preservation of labile materials such as carbohydrates in marine sediments (Keil *et al.* 1994). Intertidal mudflats are a sink and source of carbon, therefore, an understanding of the factors which govern carbohydrates and their distribution in sediments is important to the study of global carbon cycling. Colloidal carbohydrates on intertidal mud flats are associated with diatom assemblages (Underwood *et al.* 1995), therefore measurements of carbohydrates were conducted, for the first time, on a relevant scale to the distribution, size (10-300 μm, Figure 7.2b) and migratory rhythm of diatoms (Perkins 1960, Palmer and Round 1967).

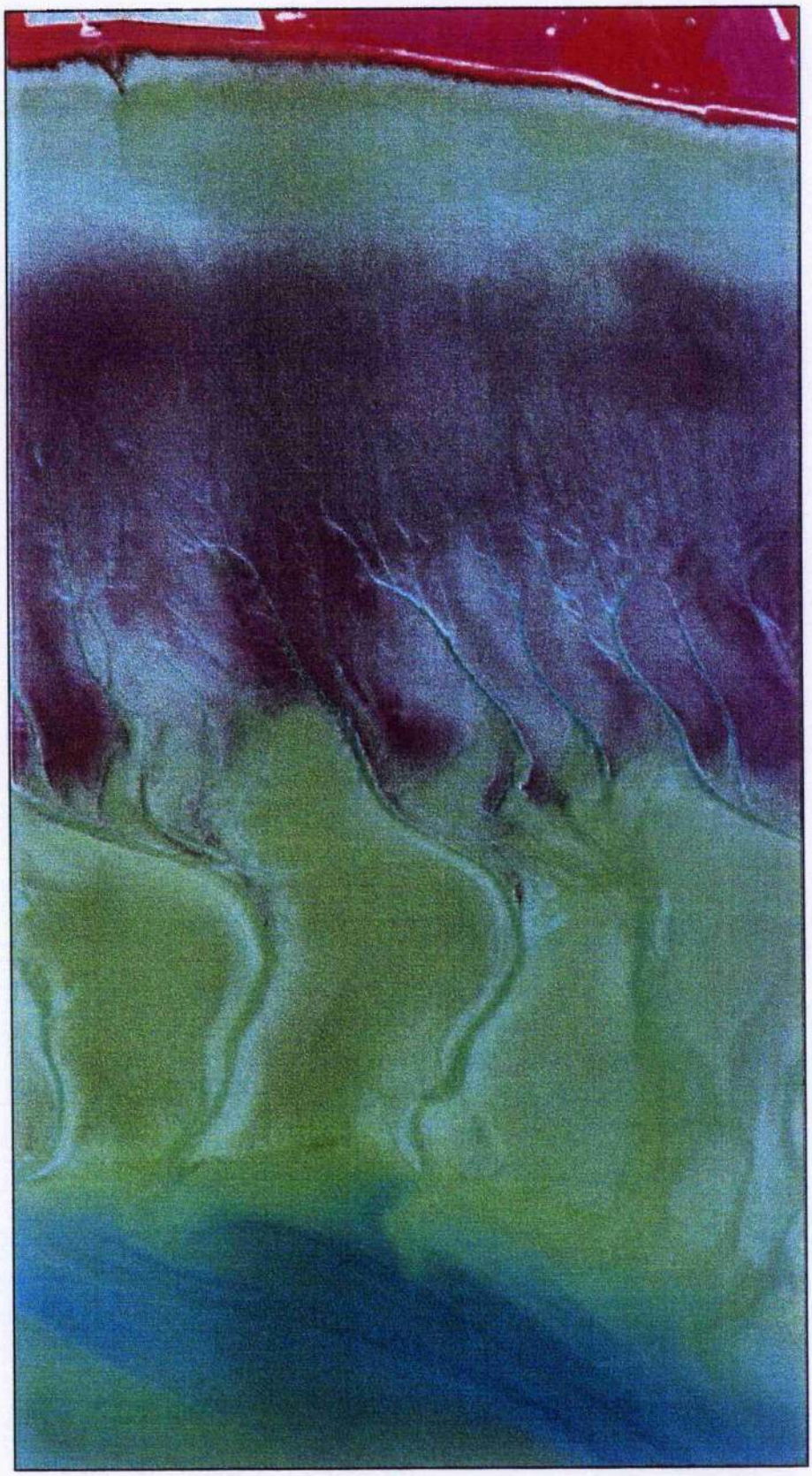
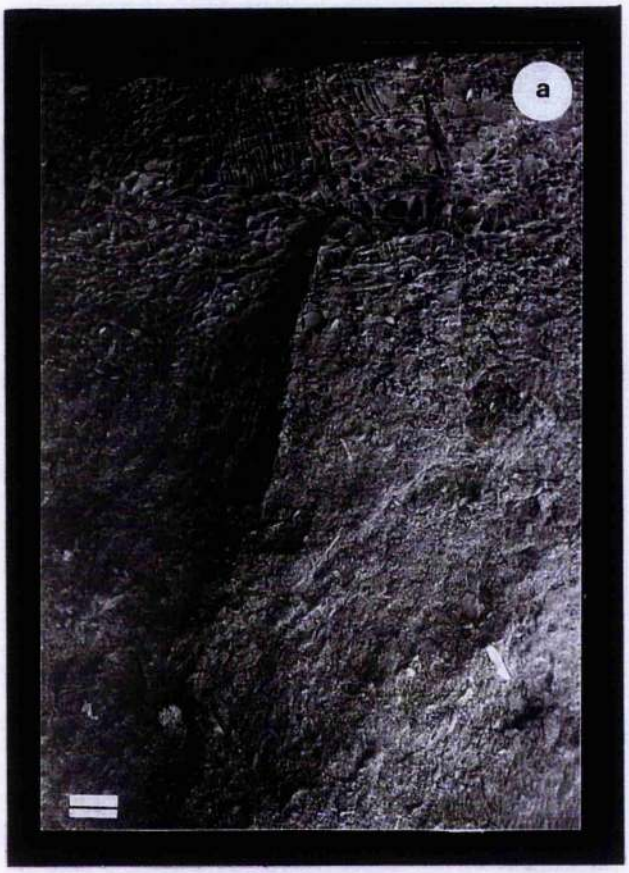
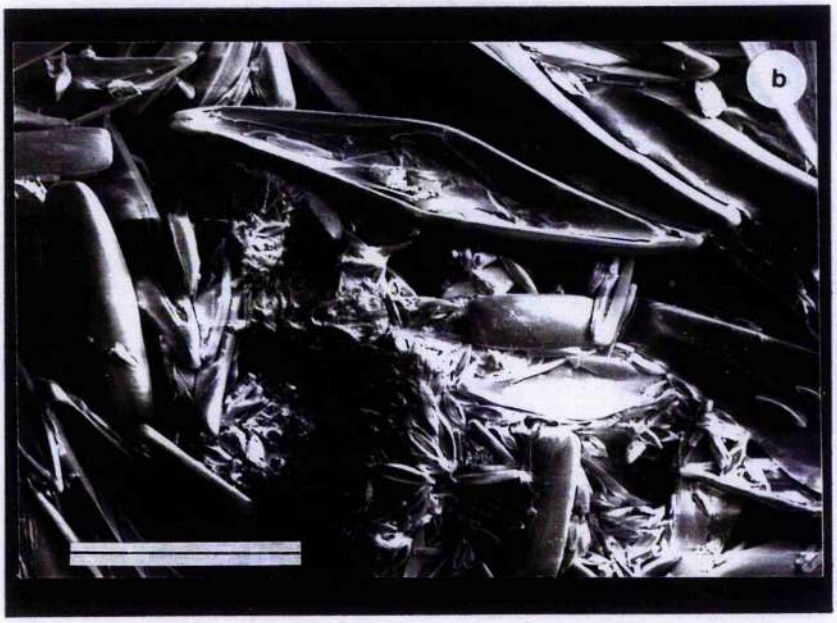


Figure 7.1 Aerial photograph showing the spectral reflectance signal of microphytobenthic chlorophyll *a* at Skeffling mudflats (courtesy of Kevin Morris, PML). Scale: 1cm≈220 m.

Figure 7.2 Low-temperature scanning electron micrographs showing (a) the increase in sediment density with sediment depth and (b) the large size range of epipelic diatom cells (scale bars = 100 μm).


a


b

7.1 The scale of ecological measurement

At migration speeds of approximately 0.19 μm s^{-1}, epipelic diatoms are often limited to a displacement distance of < 1 mm (Hopkins 1963, Hay *et al.* 1993). However, many studies of sediment carbohydrate and chlorophyll *a* concentrations have been conducted at a resolution of 1 cm (Brotas and Serôdio 1997) or 1 mm (Pinckney *et al.* 1994). The vertical resolution of this study was 0.2 mm. Observations using low-temperature scanning electron microscopy and oxygen microelectrode studies, show that the photosynthetic zone is often limited to the uppermost layer (0.2 - 0.5 mm, Revsbech 1989a, Paterson 1995). The majority of chlorophyll *a* and carbohydrates are found in the top 0.2 mm of intertidal sediments and this volume accounts for only 4 % of a 5 mm sediment core. Therefore, if chlorophyll *a* and carbohydrates are quantified over a 5 mm deep core, the surface concentrations will be greatly under-estimated (MacIntyre *et al.* 1996, Kelly and Honeywill pers. comm.). A knowledge of chlorophyll *a* and carbohydrate concentrations on the surface is important for studies of microalgal primary production and biostabilisation since this sediment/ air / water interface is where gas exchange occurs and where sediment erosion is initiated.

Although high resolution measurements provide information over a small area, they are crucial to the understanding of large-scale variability in sediment transport, due to the occurrence of diatoms on the sediment interface. Fine-scale processes such as diatom migration can have a significant effect on the estimation of surface microalgal biomass using remote sensing techniques (Paterson *et al.* in review). Similarly, large-scale mapping of estuary sediment particle sizes can help to predict diatom growth only if there is sufficient understanding of the ecology on a smaller scale (Yates *et al.* 1993). The processes which govern EPS production and turnover (and colloidal carbohydrates, as an index of EPS) , such as photosynthesis and enzymatic degradation are variable over very short distances. For example, light penetration is limited in cohesive sediments. The depth of scalar irradiance was restricted to 0.5 mm in sediments < 63 μm and the attenuation coefficient of light was 0.005 K m^{-1} (Paterson *et al.* 1996a).

As well as microscale studies, the spatial variability of sediment carbohydrates was investigated over a centimetre scale on the mud flats of the

Humber and Eden Estuaries and on a 100 m scale on the Humber and Baie de Marennes Oléron. There was significant variation between colloidal carbohydrate concentrations at each scale. Variation in sediment colloidal carbohydrates was lowest between sediment samples collected 10 m apart (coefficient of variation, cv = 52 %, short transect at Skeffling) and greatest between samples collected > 1000 m apart (cv = 72 %, Baie de Marennes Oléron), perhaps due to different chemical conditions between 1000 m stations. However, there was similar variation between samples collected 10 m apart (cv = 64 %, Eden Estuary) and 100 m apart (cv = 65 %, Humber Estuary). Therefore, variation in colloidal carbohydrate concentrations does not simply increase with increasing sampling scale. Other factors, including the size of an estuary, will affect the amount of variation that occurs over a certain distance. For example, the mid-intertidal site was 4010 m from shore in the Humber Estuary and 2000 m from shore at Baie de Marennes Oléron. Although the intertidal areas are different in size, the mid-intertidal sites may experience similar tidal exposure periods, therefore, any variability associated with tidal levels will occur over a shorter distance in the smaller estuary.

Concentrations of colloidal carbohydrates increased with tidal level on the Humber Estuary and decreased down shore at Baie de Marennes Oléron. Exposure time is a major factor which influences the abundance of diatoms and the species present (Colijn and Dijkema 1981, Saburova *et al.* 1995, MacIntyre *et al.* 1996) and in turn, affects carbohydrate concentrations over the tidal flat. In addition, the concentrations of colloidal carbohydrates may vary over the tidal flat with changes in grazer density and sediment composition (Gerdol and Hughes 1994, Brotas *et al.* 1995). The occurrence of diatom biofilms on intertidal mud flats was patchy with some patches approximately 5 cm in diameter. This is similar to the order of aggregation for all forms of microphytobenthos reported on an intertidal sand flat (Saburova *et al.* 1995). They also identified clumping of microphytobenthos aggregations at different scales i.e. 10 cm^2 patches and 100 m^2). As a result of spatial heterogeneity, some samples collected randomly at Baie de Marennes Oléron were very variable. The heterogeneity of this data did

not fulfil the criteria required for parametric statistics and conservative non-parametric tests were used.

The aggregation of microphytobenthos growing on relatively homogenous surfaces may reflect high reproduction rates, which often exceeds predation affects. The uniformity of patch size across taxonomic groups suggests that they respond to environmental limitations in a similar way and that there may be an optimal patch size (Pinckney and Sandulli 1990, Saburova *et al.* 1995). Nitrogen compounds are not likely to be limiting on intertidal mud flats (Underwood *et al.* 1998c) however, the compact nature of the biofilm will create competition for light and restrict diffusion of inorganic carbon and oxygen (Admiraal 1984, Saburova *et al.* 1995).

The majority of field work in this study was conducted as part of multi-disciplinary projects, measuring a diverse range of mud flat parameters. This approach was useful for the identification of key factors in the complex mud flat ecosystem. A multi-disciplinary programme (INTRMUD) established to classify North West European estuaries, includes colloidal carbohydrates as a key property in the description of each estuary. Following this study, measurements of colloidal carbohydrates and pigment composition in the INTRMUD programme have been conducted on a microscale relevant to microphytobenthos.

7.2 Microscale distribution of sediment carbohydrates

Despite the importance of carbohydrates to sediment processes, there was very little information available on spatial variability (Vallentyne and Bidwell 1956, Rogers 1965, Paterson *et al.* 1996b). Parahistological thin sediment sections served to visualise cyanobacterial biofilms and EPS associated with sediments (Wachendörfer *et al.* 1994). In this study, new high resolution sectioning techniques revealed a significant variation of carbohydrates within the upper 2000 μm and an inverse correlation between the distribution of two artificially separated carbohydrate fractions.

The operational separation of carbohydrate fractions distinguishes extractable carbohydrates from those associated with sediment particles. When there is a gradient in the concentration of sediment particles, i.e. significant changes in sediment density, the concentration of sediment associated

carbohydrates may be significantly affected. Therefore, it is important to measure changes in the bulk properties of the sediment, i.e. sediment density increases significantly with depth in the upper millimetres of intertidal mud flats (Chapter 3, Table 7.1). The concentration of bulk carbohydrates in 1 mg of sediment does not increase with depth but since the weight of sediment in each section increases through compaction, the amount of bulk sediment carbohydrates increases with depth. In this study, bulk sediment carbohydrates were expressed as kg m^{-3} and were positively correlated with depth (Table 7.1). Colloidal carbohydrates may exist mainly in pore waters and show a negative correlation with depth when expressed as a weight or by volume (Table 7.1). Expressing colloidal carbohydrates per volume rather than per weight was found to improve the relationship with erosion threshold τ_c (0), in erosion studies on the Humber mud flats (Amos *et al.* 1998).

Colloidal carbohydrates were correlated to diatom biomass (measured as chlorophyll *a*) in sediment cores maintained under laboratory conditions and *in situ* at a mid-intertidal site at Baie de Marennes Oléron. However, this relationship did not exist at low biomass levels (Chapter 5, Underwood and Smith 1998b). The ratio of colloidal carbohydrates to chlorophyll *a* was 28:1 in sediment cores from Norsminde Fjord, Denmark and 54:1 on the mud flats at Baie de Marennes Oléron and were within the range of previous studies (Underwood and Smith 1998a, de Winder *et al. submitted*). There was no variation in the ratio of colloidal carbohydrates to chlorophyll *a* with sediment depth. Therefore, the vertical distribution of colloidal carbohydrates co-varies with the occurrence of diatoms in the photic zone.

The depths to which cells may descend on the approach of darkness or tidal cover is limited by:

1) the velocity of diatom locomotion

2) the need to re-surface and time of migration

3) and the density and composition of the medium

The velocity of diatom locomotion may slow with depth as sediment density increases or the migratory movements of diatoms may maintain the porosity of

surface sediments. However, to date, there is little information on the influence that diatoms may have on sediment structure.

Table 7.1 *Correlations between carbohydrate fractions and sediment parameters on a weight or volumetric basis (data from Skeffling, Humber Estuary, Pearsons product moment correlation, n = 24)*

	colloidal carbo. (g^{-1})	**colloidal carbo. (m^{-3})**	**bulk carbo. (g^{-1})**	**bulk carbo. (m^{-3})**	**depth (0-1.5 mm)**	**dry mass conc.**
colloidal carbo. (m^{-3})	++					
bulk carbo. (g^{-1})	ns	ns				
bulk carbo. (m^{-3})	- -	- -	ns			
depth	- -	- -	ns	++		
dry mass conc.	- -	- -	ns	++	++	
wet sediment density	- -	- -	ns	++	++	++

++ highly significant positive correlation (P = < 0.01), -- highly significant negative correlation (P = < 0.01), ns = no correlation, carbo. = carbohydrate.

Bulk carbohydrates lacked temporal variability and this suggests that they remain conserved in the sediment and accumulate in deeper layers, existing in a form which resists bacterial degradation. If bulk carbohydrates from a certain area could be identified by their chemical composition, they may be useful for tracing the transport and fate of organic matter in sediments over time (Poynter and Eglington 1991). This would provide an estimation of the flux of carbohydrates from rivers and estuaries into coastal and open waters.

The decrease in colloidal carbohydrates in a number of depth profiles, was compared to establish if there is a common gradient of carbohydrate decline in profiles taken in the same Estuary or at the same season. Such a relationship would allow predictions of the distribution of colloidal carbohydrate to be made from a single measurement. Comparisons were made between depth profiles of colloidal carbohydrates taken at different tidal heights and season on 3 intertidal mud flats (Table 7.2). There was no significant difference in the decline in colloidal carbohydrates in depth profiles taken at the one time. However, only approx. 50 % of comparisons between different dates were related (two-tailed *t*-test, Fowler and Cohen 1990). Furthermore, there was no inherent similarity between slopes of the same tidal height, estuary or season. Therefore, similarity between the slopes of colloidal carbohydrate decline occurred by chance. The variability may be due to many other factors including changes in sediment density and particle size, which may in turn, influence the penetration depth of light (Kühl and Jørgensen 1994). Indeed, steep declines in colloidal carbohydrate concentration with depth, were matched with steep increases in sediment density (Chapter 3). Since there was no predictive relationship between depth and colloidal concentration, it was not possible to estimate the distribution of carbohydrates within the upper millimetres. Therefore, high resolution depth profiles are necessary to explore the relationship between density and depth and to provide information on biofilm properties at individual sites.

Table 7.2 Comparisons of the gradient of colloidal carbohydrate decline with depth. (b1 versus b2, two tailed *t*-test, $P = < 0.05$)

	Ed/April/ Upp	**Ed/July/ Low**	**Ed/Nov./ Upp**	**Tay/Feb./ Upp**	**Tay/Nov. /Upp**	**Hu/April/ Mid**
Ed/July/Low	-					
Ed/Nov./Upp	-	+				
Tay/Feb./Upp	-	-	+			
Tay/Nov./Upp	+	+	+	+		
Hu/April/Mid	-	-	+	-	+	
Hu/Nov./Mid	+	-	-	-	+	+

(+ = slopes similar, - = slopes not similar, Ed = Eden Estuary, Hu = Humber Estuary, Tay = Tay Estuary, Upp = upper-intertidal, low and mid-intertidal, Nov. = November, Feb. = February). (4 of the data sets were compiled by Kelly and Honeywill).

Depth profiles of colloidal carbohydrates showed concentrations increased nearer the surface over the emersion period but not in deeper layers (Chapter 4, Figure 4.4, 4.7). This suggests that in areas without substantial bioturbation, colloidal carbohydrates measured more than 1 cm below the surface may represent background levels which are unlikely to be an index of EPS secretion by epipelic diatoms.

7.3 Microscale studies of sediment fabric

In addition to carbohydrate measurements, the high resolution sectioning technique allowed the physical nature of surface sediment to be examined. As previously mentioned, the mobility of diatoms may affect, and be affected by, the density of the medium. However, previous studies have not considered the possibility of significant changes in density within the upper millimetres of the sediment. This study provided quantitative evidence of significant sediment compaction in the upper 2 mm which had been previously observed using scanning electron microscopy (Figure 7.2a, Taylor and Paterson 1998). The relationship between density and diatom mobility could be explored further in laboratory studies which measure the velocity of migratory movements in sediments of different densities (i.e. those found in the upper 1 cm, 200 - 600 kg m^{-3}, Chapter 3).

Microscale changes in sediment density have important implications for the modelling of sediment erosion, in which the properties of surface sediments can dictate the erodibility of deeper layers. Although a positive correlation between colloidal carbohydrate and sediment stability was not shown in this study, many other studies (Yallop *et al.* 1994, Sutherland 1996) have shown that microphytobenthic biofilms increase sediment stability by inter-grain binding, the formation of a network of filaments and the reduction of surface roughness. The high resolution sectioning technique allows both physical and biological studies of the sediment surface to be conducted at the same scale and provides a better means for understanding of biologically mediated sediment stability.

In some erosion studies, sediment stability increased over the tidal emersion period when there was no significant change in sediment moisture content or density, suggesting that non-physical processes such as biostabilisation

were involved (Chapter 4, George 1995, Sutherland 1996, Amos *et al.* 1998). In other studies, increasing sediment stability corresponded with a reduction in moisture content and limited biological activity (Paterson and Black *in press*). These results demonstrate the complexity of sediment systems, where the factors which influence erodibility can vary rapidly at a single site, making accurate predictions of stability difficult.

This study has shown *in situ* carbohydrate concentrations to vary with sediment topography. There were greater concentrations of sediment carbohydrates in runnel depressions than on the sediment ridge. In this case, topographical variation had created significant differences in sediment moisture content less than 1 m apart, which may have influenced diatoms biomass levels. Similarly, a study of an intertidal sand flat showed densities of epipelic diatoms to be greater on the ridge crest than in the depression due to differences in the sediment particle size distribution (Saburova *et al.* 1995). Therefore, the response of diatoms to topographic features and the conditions created by them, will have important implications for the prediction of sediment transport in estuarine systems with varied bed morphologies. As yet there are no sediment transport models available at this scale (Wood *et al.* 1998).

The measurement of colloidal carbohydrates over an erosion event (induced using the microcosm device) found a peak concentration in one water sample directly before bed failure (Chapter 2). It has been suggested that the concentration of colloidal carbohydrates in the water column may reflect the strength of the sediment bed (Sutherland 1996) however, this value was isolated and the experiment should be repeated on sediments with a higher diatom biomass. In addition, the concentration of carbohydrates in the water were often below the detection limits of the Dubois assay and concentrating carbohydrates through lyophilisation was found to under estimate carbohydrate concentrations. However, the polymeric components of the carbohydrates may be concentrated through precipitation. The analysis of carbohydrates from the water column, using this method, were unsuccessful due to low diatom biomass at the time of microcosm deployment. However, this methodology is being used successfully in current microcosm studies which may provide a greater understanding on the fate

of surface carbohydrates upon the return of the tide (Paterson 1989, Smith and Underwood 1998b). Some colloidal carbohydrates may be carried towards the shore with the flood tide but a greater amount may remain associated with surface sediments since repeated extractions of colloidal carbohydrates show that not all are released (Underwood *et al.* 1995). Furthermore, microcosm studies suggest that components of the mud flat (i.e. colloidal carbohydrates, diatoms and sediment particles) may have different critical erosion thresholds (Chapter 2).

7.3.1 The effect of moisture content on the dynamics of carbohydrate fractions

With increasing desiccation, a point may be reached where cells lyse and release intracellular sugars which increase the pool of extracellular sugars in the colloidal fraction. The partitioning of bulk and colloidal carbohydrates may be altered by physical and chemical transformations or changes in the rates of enzymatic breakdown (Chin *et al.* 1998). Neap / spring tidal phases and atmospheric evaporation influence sediment moisture content most significantly on the upper-intertidal zones (Amos *et al.* 1998), and could therefore, produce oscillations of carbohydrate concentrations and subsequent bacterial metabolic rates. For example, when the tidal phase approaches spring tide, the increase in water content at the upper-intertidal zone may boost bacterial extracellular activity and growth. When the tidal phase begins to return to neaps and sediment water content falls, epipelic diatoms may produce more EPS to protect against desiccation stress (Peterson 1987) leading to an increase in the concentration of colloidal carbohydrates. However there is no data to support this theory and this scenario assumes no variation in precipitation and does not take into account any variation in primary production due to changes in exposure time.

7.4 Temporal variability of carbohydrate concentrations during emersion on intertidal mud flats

Colloidal carbohydrates increased over the emersion period at upper and mid-intertidal sites on the Humber Estuary, at site 1 on the Eden Estuary and at the upper-intertidal site at Baie de Marennes Oléron. However, on other occasions, colloidal carbohydrate concentrations fluctuated or did not vary significantly over the emersion period (Chapter 5).

At Baie de Marennes Oléron, the ratio of colloidal carbohydrate to chlorophyll *a* was shown to increase over the emersion period, implying that the increases were not merely due to increasing diatom biomass, supporting the findings of Underwood and Smith (1998b). Short-term variability of colloidal carbohydrates indicates they are actively secreted and are of recent origin, whereas bulk sediment carbohydrates did not vary over a number of hours (Humber Estuary) and may be more recalcitrant phase which is less representative of microphytobenthic production.

Temporal variation in colloidal carbohydrate concentrations are likely to be due to several factors. In studies of intertidal sediments on the Humber and Eden Estuaries, primary production was found to increase towards midday coinciding with increasing light intensities and temperature (Miles and Paterson 1996, Underwood and Smith 1998a), resulting in more photo-assimilated carbon for possible EPS secretion. Therefore, daily and seasonal changes in light intensity may play a significant role in determining the amount of colloidal carbohydrates on intertidal estuaries. However, *in situ* photosynthetic rates often decline towards the end of an afternoon emersion (Sevecke and Krumbien 1996, Smith and Underwood 1998b), whereas carbohydrates have been shown in some cases to increase until the return of the flood tide (Chapter 4, Smith and Underwood 1998b). Although cell numbers increase on the sediment surface, the migration often occurs within the first hour of tidal retreat, after which, cell numbers remain steady (Palmer and Round 1967, Paterson *et al.* in review). Therefore, migration alone may not account for increases in carbohydrates which were measured over the emersion period at the Humber, site 1 on the Eden Estuary and on the upper-intertidal at Baie de Marennes Oléron. Furthermore, chlorophyll *a* concentrations (as an indicator of biomass) did not increase over the emersion period at the Eden or Humber Estuaries or at Baie de Marennes Oléron, France.

The accumulation of colloidal carbohydrates over the emersion may be due to the horizontal movement of epipelic diatoms once they have surfaced. Although this probably occurs, it does not explain elevated colloidal carbohydrates at the end of the emersion, since diatom cells descend into the

sediment before tidal inundation (Palmer and Round 1967). A more probable explanation is a decrease in the rate of temperature-dependant extracellular enzyme breakdown processes, when sediment temperatures decrease towards the end of the emersion.

A second process which results in a loss of EPS (and therefore, colloidal carbohydrates) on surface sediments is the utilisation of EPS by diatoms themselves. Algal heterotrophy and photo-heterotrophy were observed in a laboratory study in which they accounted for 37 and 48 % breakdown of labelled EPS after 24 hours in dark and low light conditions (Paterson *et al.* 1996b). Heterotrophic growth may allow diatoms to survive during periods of burial below the photic zone (Smith and Underwood 1998b). The turnover of colloidal carbohydrates is also affected by changing conditions within the biofilm. Highly active biofilms are subject to extreme biochemical variations such as oxygen concentrations, salinity and pH, which can reduce the availability of carbon dioxide (Rasmussen *et al.* 1983). In such conditions, it may be beneficial for the diatom cell to secrete more EPS for internal pH regulation or as a buffer against external fluctuations (Hoagland *et al.* 1993, Freeman and Lock 1995). In addition, evaporation of surface water from intertidal sediments during emersion may draw EPS from lower sediment depths to the surface (Paterson pers. comm.), however, depth profiles have shown colloidal carbohydrates (as an index of EPS) to decline significantly below the surface 200 μm. Therefore, colloidal carbohydrate can be thought of as a transient pool of carbohydrates, the concentrations of which depend on the production of EPS from epipelic diatoms, the rate of enzymatic degradation, diatom heterotrophy and physico-chemical transformations (Smith *et al.* 1995, Paterson *et al.* 1996b, Chin *et al.* 1998, Underwood and Smith 1998b).

7.5 Biochemical characterisation of *in situ* sediment carbohydrate fractions

Previous studies of diatom carbohydrates and EPS have focused on planktonic diatom cultures (*Chaetoceros affinis, Thalassiosira spp., Skeletonema costatum,* Allan *et al.* 1972, Myklestad 1977, Mopper *et al.* 1995). Planktonic cells are morphologically and ecologically different from epipelic forms which exist on intertidal mud flats. They encounter different environmental challenges

for example, epipelic diatoms produce EPS in order to provide mobility and to protect against desiccating conditions (Edgar and Picket-Heaps 1984, Peterson 1987), such processes do not affect planktonic cells. Therefore, epipelic diatoms may produce structurally different EPS in response to these ecological differences (Hoagland *et al.* 1993) which could be detected in the colloidal carbohydrate fraction or in precipitates of EPS. This study addressed the need for biochemical analysis of sediment carbohydrates fractions.

The operationally separated sample of bulk and colloidal carbohydrate fractions was purified and examined. Monosaccharide analysis supported the conclusions of spatial and temporal studies which suggested that colloidal carbohydrates were different from bulk sediment carbohydrates. Although both carbohydrate fractions contained the same monosaccharides, the ratios in which monosaccharides were present differed markedly between fractions and from published characterisations of phytoplankton EPS (Chapter 6, Allan *et al.* 1972, Aluwihare *et al.* 1997). In future studies, an examination of EPS precipitates (rather than bulk and colloidal fractions) would provide a more relevant comparison to planktonic EPS. Unlike the EPS of planktonic diatoms, glucose was the dominant monosaccharide in the carbohydrate fractions isolated from natural sediments. The colloidal fraction was proportionally richer in glucose, the monosaccharide most readily utilised in bacterial metabolism (Cowie and Hedges 1984, Dawes and Sutherland 1992, Hernes *et al.* 1996). Temporal variability and spatial relationship between colloidal carbohydrates and chlorophyll *a* suggests that colloidal carbohydrates are more representative of recently secreted EPS and diatom activity than bulk carbohydrates, which are absorbed to sediment particles and are more likely to accumulate in sediments.

The difference in monosaccharide composition between the two fractions suggests structural differences in their polysaccharide components (Bertocchi *et al.* 1990). Using monosaccharide information, previous analyses of planktonic EPS and *in situ* enzyme activity, a hypothesised structure for epipelic EPS was presented, encompassing chemical features such as a monosaccharide backbone dominated by glucose, the presence of negatively charged uronic acids and sulphates (de Winder *et al. submitted*). These can bind directly to positively

charged sites on the edges of clay particles (Martin 1971) or form ionic bonds with divalent cations such as Mg^{2+} Ca^{2+} and Cu^{2+} resulting in a polysaccharide gel (Decho 1994, Chin *et al.* 1998).

Carbohydrates in natural sediments arise from many sources and occur in a wide range forms including the intracellular sugars (which are less likely to influence sediment stability directly). Previous studies on biostabilisation and microbial processes have found the operational separation of carbohydrates fractions to be useful and the biochemical characterisation in this study contributes to the understanding of such investigations.

7.5.1 Environmental factors which may affect colloidal carbohydrate turnover

At typical hydration levels, the concentration of colloidal carbohydrates may be dependant mainly on the production of EPS and the enzymatic breakdown rate (Ruddy pers. comm.). A general ratio of 25 % EPS and 75 % monosaccharides exists within the colloidal carbohydrate pool of many intertidal sediments (Underwood *et al.* 1995). The monosaccharides in this fraction may represent EPS which has been degraded by extracellular enzymes but has not yet been utilised. A laboratory study, (Arnosti *et al.* 1994), on the degradation of algal polysaccharides by anaerobic sediment bacteria showed monosaccharide hydrolysis products were not utilised until the majority of polysaccharides were broken down (Arnosti *et al.* 1994). These workers suggested that the rate of enzymatic activity, which converts polysaccharides to monosaccharides, exceeds the rate at which monosaccharides are used therefore, monosaccharides accumulate during a lag period between enzymatic breakdown and increased bacterial numbers. However, Smith and Underwood (1998b) showed the proportion of monosaccarides in the colloidal carbohydrate fraction of axenic cultures to decrease in darkness, suggesting that photosynthetic activity, rather than enzymatic activity, may determine the monosaccharide : polysaccharide ratios.

Bacterial numbers may not reach densities capable of utilising all the breakdown products within the time that migratory diatom cells are at a particular depth (Arnosti *et al.* 1994). There is a need for a greater understanding of the

relationships between carbohydrate turnover and bacterial and diatom activity (Smith *et al.* 1995, Ruddy *et al.* 1996).

7.6 A conceptual model of carbohydrate dynamics on surface sediments

The production of a conceptual model clarifies the interactions influencing sediment carbohydrate concentrations. Biochemical information coupled with acknowledge of carbohydrate fraction extractability, may help to explain the distribution patterns and longevity of carbohydrates fractions in natural sediments.

7.6.1 An established biofilm

Colloidal carbohydrate material is energetically rich and requires little metabolic breakdown. Therefore, colloidal carbohydrates have a short 'half-life' and those detected are of recent origin, existing only in areas corresponding in time and space with diatom populations. However, under highly desiccated or freezing conditions (Chapter 3, Shayler pers. comm.) high colloidal carbohydrates concentrations may be detected. These do not reflect active secretion of EPS but rather the death of sediment organisms and the subsequent release of intracellular carbohydrates. Furthermore, such extreme conditions (including rapid pH changes) restrict the activity of extracellular enzymes thereby allowing colloidal carbohydrates to accumulate. Under more typical, hydrated conditions epipelic diatoms follow a migration pattern in response to lunar and solar cycles. This results in one upward migration to the sediment surface during daytime exposure and a downward migration before the flood tide (Fig. 7.3a, Palmer & Round 1967, Perkins 1960, Paterson 1986, Pinckney *et al.* 1994). Such a migration will maximise photosynthesis whilst protecting cells from tidal flow and wave action. Since colloidal carbohydrates are an index of EPS which is released through cell locomotion, migration may influence surface colloidal concentrations over the exposure period. Several field measurements have shown surface carbohydrate values to be low at the beginning of the emersion period (site A on the Humber Estuary, Figure 4.3a, sites 1 and 2 on the Eden Estuary, Figure 4.6 and upper-intertidal at Baie de Marennes Oléron Figure 5.18), when diatoms cells have yet to surface. One possible scenario of this is that colloidal carbohydrate

Figure 7.3a Conceptual diagram showing how migration may influence the surface concentration of colloidal carbohydrates

An established biofilm

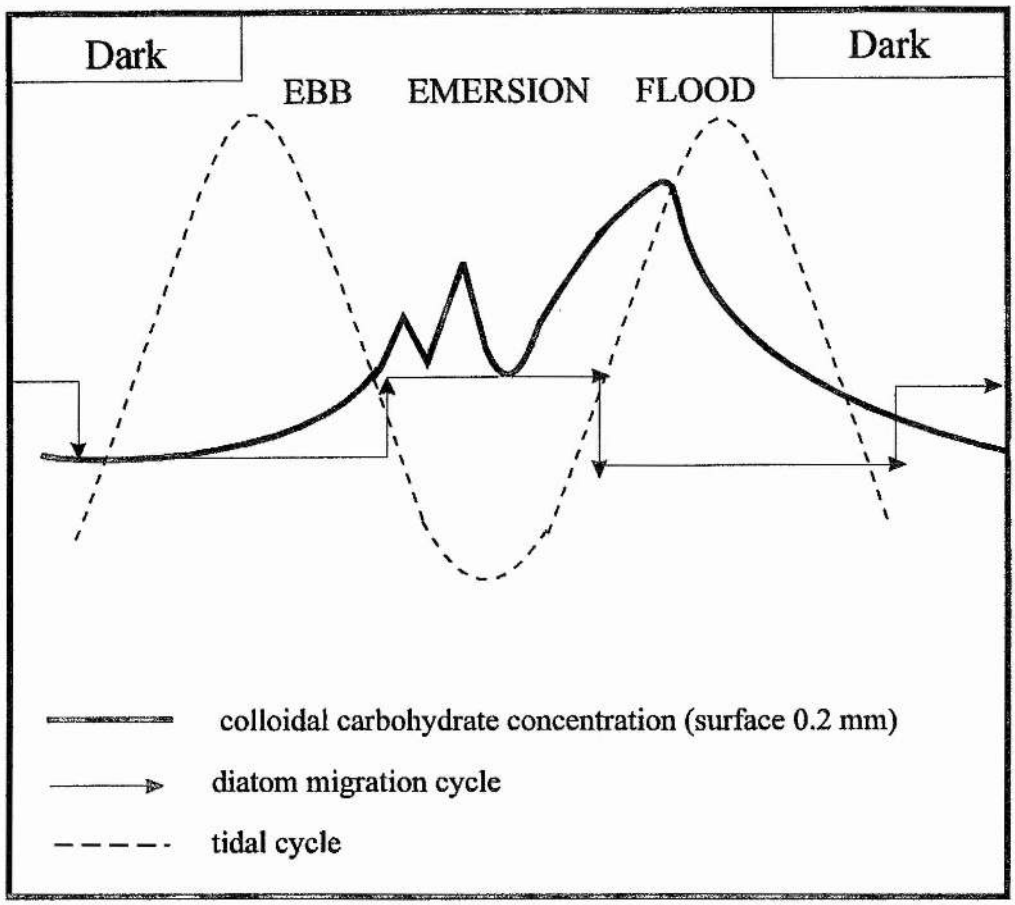
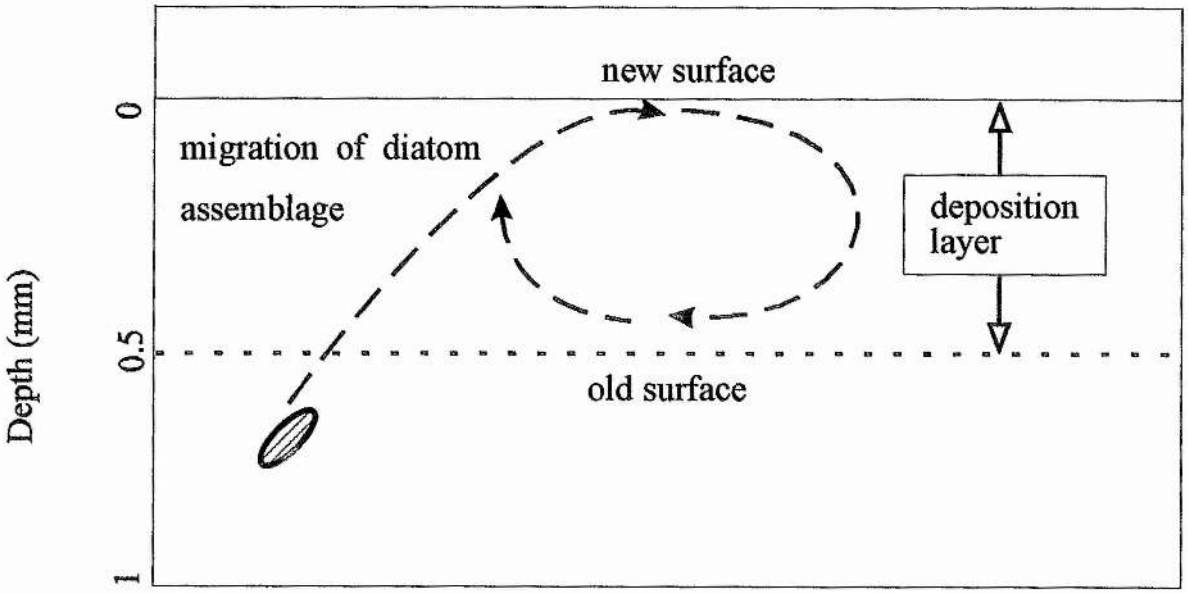
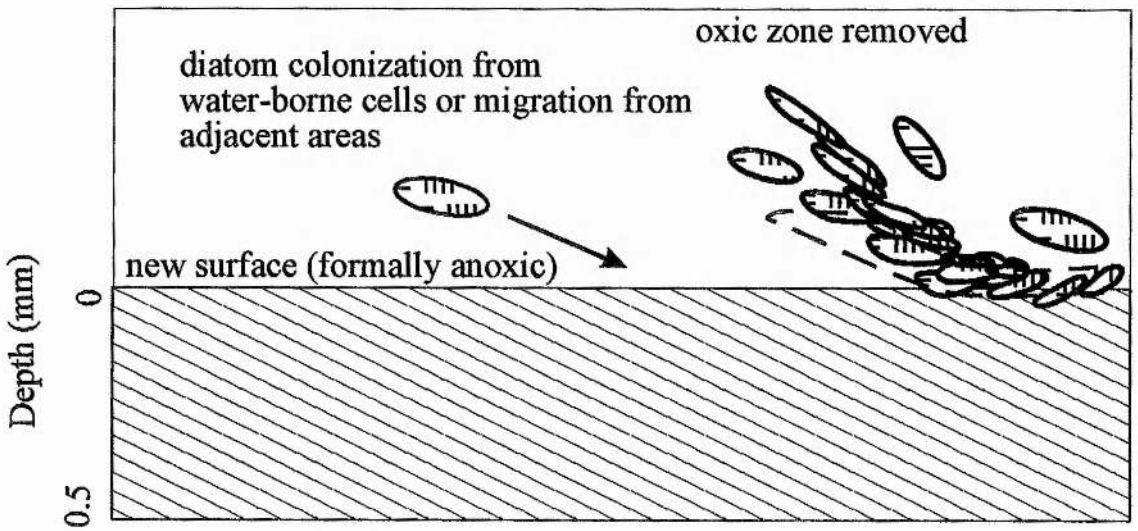


Figure 7.3 (cont.)

(b) *Following deposition*



(c) *Erosion*



concentrations will peak below the sediment surface (Fig. 7.3a). Once cells migrate to the surface, colloidal carbohydrate concentrations may fluctuate or increase towards the end of the emersion period (as measured on the Humber Estuary and upper-intertidal at Baie de Marennes Oléron). A proportion of the surface carbohydrates will be lifted into the water column with the onset of the flood tide (this is often visible as a froth on the tidal front).

Algal polymers change the rheological properties of the water column, increasing viscosity and damping turbulence (Jenkinson 1990). These carbohydrates will be broken down by bacteria in the water column or may adsorb suspended particulate matter, forming flocs which subsequently settle out (Dugan 1987, Karner and Rassoulzadegan 1995, Smith *et al.* 1995, Chin *et al.* 1998). A background level of colloidal carbohydrates may then be measured at the beginning of the next emersion period.

7.6.2 Following deposition

Where hydrodynamic energy is low, sediment flocs will settle onto the bed, creating a new surface layer. The concentration of surface carbohydrates may be low, since the newly deposited particles were subjected to bacterial activity in the water column. Therefore, a depth profile of the surface layers may show low colloidal concentrations in the new deposits and a underlying zone of high concentrations corresponding with the diatom biofilm (Hay *et al.* 1993). The new deposit with low colloidal carbohydrate content may be easily resuspended. Depending on the thickness of the deposition layer, diatom cells will migrate through the fresh deposits to the surface in one or more tidal cycles (Fig. 7.3b). The distribution of colloidal carbohydrates in the sediment will be linked to this upward movement of cells. Such a dynamic system helps to explain the observed decrease of colloidal carbohydrates with depth in the upper sediment layers (Taylor and Paterson 1998).

7.6.3 Following erosion

Erosion has a dramatic effect on surficial carbohydrate production on intertidal mud flats, since it removes the upper sediment layers containing diatom

cells and carbohydrates. A new surface which was formally anoxic, is then exposed (Fig. 7.3c). In this situation, colloidal carbohydrates are less available and remaining bacteria and infauna may utilise the less energetic, bulk carbohydrates. Diatom cells are deposited on the new surface from the water column and from surrounding sediments (Underwood and Paterson 1993). However, it may take several tidal cycles before colloidal carbohydrates reach previous concentrations. In addition, it would take longer for colloidal carbohydrate concentrations to recover after an erosion event than following a depositional event, since deposition merely covers the existing biofilm. Furthermore, bacterial activity would be affected by depositional and erosion events through the change in carbohydrate and oxygen concentrations. Therefore, greater bacterial numbers may be supported in an established biofilm than on a frequently disturbed bed. However, it is important to note that this conceptual model represents only one possible scenario and it does not match all the field data.

7.7 Future studies

A large scale (500 m^2 grid size) mathematical model showed sediment surface temperatures to be crucial in the prediction of microphytobenthic productivity (Guarini *et al.* 1997). However, little is known of microscale variation of sediment temperatures. Such information may provide a greater insight into EPS production (and colloidal carbohydrate concentrations), since temperature directly affects photosynthetic rate and diatom locomotion (Hopkins 1963, Rasmussen *et al.* 1983, Guarini *et al.* 1997). Furthermore, the enzymatic breakdown processes which govern the turnover of colloidal carbohydrates are highly temperature sensitive. The fluctuations of surface temperatures are likely to be influenced by the size of sediment particles, since light (and therefore, heat energy), penetrates deeper into sandy sediments than muddy sediments (Revsbech 1989a, Kühl and Jørgensen 1994). Therefore, the development of technology capable of microscale temperature measurement would provide a wealth of important information for studies of biostabilisation, heterotrophy and enzyme activity in surface sediments.

Estimates of net oxygen production and measurements of gross photosynthesis, in diatom biofilms, suggest that respiration rates increase with light intensity (Chapter 5), this has been reported in the literature and was thought to be photorespiration (Revsbech 1989a). This process accounts for 1/6 of carbon fixed by C3 plants being rapidly released as CO_2 under high oxygen concentrations (Hipkins 1984). Since 10 % of photoassimulates are directly channelled into colloidal carbohydrates (and more from intracellular glucan stores, Smith and Underwood *in review*) there is a need to assess the possible implications of photorespiration on colloidal carbohydrate concentrations.

EPS was observed to become increasingly 'sticky' with biofilm age (Sutherland 1996), a phenomenon which biochemical studies may provide answers to. As yet, there are few published studies on the influence of environmental conditions, such as salinity, nutrient loading, heavy metal concentration or sediment type on the composition and rheology of epipelic diatom EPS or colloidal carbohydrates (de *Winder et al. submitted*). Such information may provide greater insight into the mechanism of biostabilisation. It would also have significant implications for the rheology of surface sediments and the water column and for sediment transport predictions. Therefore, biochemical carbohydrate analysis of sediment carbohydrate fractions should continue to be an important part of biostabilisation studies.

7.8 Conclusions

The major findings of this study were that ;

1) Colloidal and bulk carbohydrates and chlorophyll *a* vary on a microscale with sediment depth. Colloidal carbohydrates were concentrated in the surface 200 μm and therefore, influence interface processes such as diffusion and erosion.
2) Colloidal carbohydrate concentrations were positively correlated to chlorophyll *a* in both laboratory and field studies. Both co-varied with sediment depth, however, the relationship broke down at low biomass levels.
3) There was an inverse correlation between the vertical distribution of colloidal and bulk carbohydrates.

4) The gradient at which colloidal carbohydrate concentrations decrease with depth varies unpredictably with season, tidal height and estuary.

5) There were significant spatial variations in colloidal carbohydrate concentrations at 1 m, 10 m and > 1000 m scales. This variability does not increase with scale in all cases. Field studies show tidal level, sediment moisture content and sediment particle size to influence large-scale spatial variation in colloidal carbohydrate concentrations. In addition, any topographical features which resulted in the variation of these factors, would influence the spatial distribution of colloidal carbohydrates.

6) Under extreme environmental conditions, the colloidal carbohydrate pool may be enhanced through cell death and intracellular leakage.

7) *In situ* concentrations of colloidal carbohydrates varied significantly over a short time-scale, partly due to changes in epipelic diatom biomass and in the rate of enzymatic hydrolysis and photosynthetic production. Bulk sediment carbohydrates did not vary over a diurnal time-scale and are less representative of diatom EPS production.

8) Significant microscale variations in sediment density were visualised using LTSEM and quantified for the first time, following fine-scale sediment sectioning. These increases were found at each site and reflected post-depositional compaction.

9) The monosaccharide composition of *in situ* carbohydrate fractions were characterised for the first time. Although the monosaccharide composition of colloidal and bulk carbohydrate fractions were the same, consisting mainly of; glucose, galactose, rhamnose, mannose, xylose and fucose, the proportion of sugars varied markedly. Glucose accounted for 82 % of the colloidal fraction and 37 % of the bulk fraction.

Colloidal carbohydrates represent a dynamic pool of monomers and polymers, being produced and degraded in areas where diatoms exist. A conceptual model allowed the dynamics of colloidal carbohydrates to be predicted under different hydrodynamic conditions on intertidal mud flats. This model

represents only one of many possible dynamic situations and requires verification from studies which combine biological and physical measurements.

Colloidal carbohydrates from intertidal surface sediments are energetically rich and diatoms can be easily cultured in the laboratory. These properties and the non-toxic nature of algal carbohydrates may mean that they could be used commercially. Alginates from macroalgae (*Phaeophyceae*) are currently exploited in the food industry to create gels and thickeners. The rheology of algal polymers can be easily altered through the addition or removal of Ca^{2+} (which binds to carboxyl groups on the polymer), to meet particular requirements. The polymeric component of colloidal carbohydrates consists of transparent gels which combine elasticity and strength. Therefore, addition of colloidal carbohydrates may improve the stability of sediments, this would be particularly useful along riverbanks and coastlines that are subject to erosion. In addition, the flocculating properties of EPS could be used in water treatment systems and the chemical binding properties of the polymer could be used in the treatment of soils contaminated with heavy metals. There are numerous potential applications for this natural resource however, such projects require well-funded pilot studies and considerable inter-disciplinary effort.

Studies into carbohydrate secretions from epipelic diatoms are important for the understanding of bacterial metabolism (Ruddy *et al.* 1996). In addition, sediment carbohydrates are an important component of dissolved organic matter (DOM). Approximately 10^{18} g carbon are contained in the total DOM of the world's oceans, this is greater than the atmospheric pool of CO_2 (McCarthy *et al.* 1996) and 30 - 50 % of this carbon exists in a colloidal form (Wells 1998). Therefore, the information on the distribution of colloidal carbohydrates on intertidal sediments in this study may be useful for the estimation of estuary carbon fluxes and carbon burial in aquatic ecosystems.

APPENDIX 1.

Protocol for Clark Type Oxygen Microelectrode

Cathode construction

The cathode was constructed from a piece of platinum wire (approx. 5 cm long, 0.1 mm diameter, thermocouple wire). The wire was negatively charged (2 - 6 V) and dipped into a solution of positively charged, saturated potassium cyanide. This degraded the wire until it reached the desired diameter for the electrode (2 μm). The wire was then inserted into a funnel shaped, pre-drawn piece of Schotts green glass (8533, Revsbech 1989), which was heated to form a thin coating over the platinum wire. A heated wire was applied near the tip (under microscope observation), to melt the glass away and expose 3 - 4 μm of wire.

Schotts glass has a lower melting point than the glass pipettes and was heat-fused to a washed, pre-drawn pipette (0.5 mm) with a heated wire (18 V) to form the inner shaft.

A negative charge was again applied to the wire (0.1 - 0.2 V) and the tip was dipped in a positively charged gold solution ($HAuCl_4$), so that the wire was electroplated. The gold crystallises randomly and is porous, allowing oxygen diffusion. The wire was rinsed with water to displace excess gold ions.

Another Pasteur pipette was used for the outer casing and was heat-elongated to around 100 μm using a heating wire. The pipette was then suspended above a beaker and a heated platinum wire (charged with 12 amps) was placed against it until the glass became thin enough to snap and the pipette was caught in the beaker below. The tip of the pipette was broken open, to around 4 μm, where the glass was stronger and then narrowed to 2 μm with the heated wire. The tip was filled with silicone resin (excess was gently cleared with lens tissue) and left for 1 hour to dry. The inner shaft (gold cathode) was placed inside the glass pipette casing until 5-10 μm behind the silicone membrane, using a micromanipulator and sealed at the distal end with a mixture of epoxy gel, and left for 30 min to harden.

Construction of the silver guard cathode

A piece of silver wire was inserted into a glass pipette capillary tube (100-200 μm diameter, narrowed by heating) so that 4-5 cm of wire protruded from one end and 1 - 2 cm of wire at the other end was folded back on the outside of the capillary. The wire was held in place with epoxy resin. The tip of the silver wire was thinned using potassium cyanide (NB. a different potassium cyanide solution was used for silver or platinum wire etching) charged at 5 V, 6 V then rinsed and etched at 3 V and rinsed again. For the anode, a piece of silver wire (300 μm diameter) was positively charged and chlorinated in 0.1 M HCl.

The silver guard cathode was inserted between the outer case and the inner shaft and the anode was positioned in the same place at the same distance from the tip but on the other side of the electrode and then fixed with epoxy. The epoxy did not fill the shaft completely, to allow for the electrolyte solution to be added ($KHCO_3$ 2.25 g, KCO_3 5.18 g and KCl 18.6 g in 500 ml for bulk preparation). 200 μl was added to the tip, and the electrode shaken. A vacuum was applied to the electrolyte solution (to remove bubbles) and then the solution was added until it reached the top of the pipette shaft and sealed with epoxy. The performance of the electrode was then tested (Chapter 5).

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