UNIVERSITY OF SOUTHAMPTON

THE ENVIRONMENTAL AND PHOTO-PHYSIOLOGICAL
CONTROL OF MICROPHYTOBENTHOS PRIMARY
PRODUCTION ON AN INTERTIDAL MUDFLAT

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Microphytobenthos are important primary producers on tidal flats - ecosystems which are characterised by steep physical and chemical gradients. In this study some of the environmental factors that control the distribution and primary production of benthic microalgae were investigated in a series of field surveys conducted on Hythe intertidal mudflat, Southampton Water. The ecological and photo-physiological responses of microphytobenthos to changes in physical factors were studied in the field and in controlled laboratory experiments. Results from a weekly sample program at four stations along an intertidal transect were used to address the hypothesis that changes in incident irradiance defined by a combination of meteorological factors and the tidal cycle cause significant changes in the biomass of microphytobenthos. The light regime at each station was characterised by a model which combined the changes in daylight irradiance with the timing and duration of low tide. At the low shore stations total light availability during low tide was limiting in spring and autumn/winter, when daily photo-periods ranged between 0 and 6 hours. Microalgal biomass exhibited a strong seasonality with a distinct spring bloom in April which coincided with high irradiances at spring low tide. At the high shore station which was exposed to three times longer photo-periods light availability did not correlate with microalgal biomass at any time of the year. Biomass was three times higher at this station than at the three low shore stations and showed little seasonal variation. Taxonomic marker pigments were used to describe the changes in microphytobenthos composition. High correlation between chlorophyll $a$ and fucoxanthin on a temporal and spatial (horizontal and vertical) scale combined with taxonomic analysis gave evidence that diatoms were the main primary producers on Hythe intertidal mudflat.

Chlorophyll $a$ and $I_k$ (defined as $P_{\text{max}}^a/\alpha^a$) correlated strongly with incident irradiance at low tide, indicating that photoadaptation of benthic microalgae was occurring. A spatial comparison of photosynthetic parameters showed that benthic microalgae inhabiting the low shore compensated for reduced photoperiods by increasing their photosynthetic capacity ($P_{\text{max}}^a$) and efficiency ($\alpha^a$) and decreasing their $I_k$. Photosynthetic parameters of microphytobenthos significantly changed during the low tide period under controlled laboratory conditions, suggesting that an endogenous photosynthetic rhythm was present, which caused an increase in $P_{\text{max}}^a$ and $\alpha^a$ towards the middle of the photoperiod. Irradiance levels at low tide enhanced the short-term variation in photosynthetic parameters due to photo-adaptation. The change in rates of photosynthesis was accompanied by a significant alteration of the cellular pigment composition, as the photoprotectant xanthophyll diatoxanthin increased and diadinoxanthin decreased in proportion. Short-term changes in cellular pigment composition induced in the laboratory were greater than seasonal pigment adaptation of microphytobenthos isolated from the field. Variation of $P_{\text{max}}^a$ and $\alpha^a$ during one photoperiod occurred on a similar scale to the seasonal and spatial changes, suggesting that short-term photo-physiological adaptations are an important feature of microphytobenthos, aiding their successful survival in the intertidal environment, where strong gradients of incident irradiance occur on small temporal and spatial scales.
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Dr. W. Ide
*20.10.1899 †11.4.1986
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CHAPTER 1
INTRODUCTION

1.1 THE ECOLOGY OF BENTHIC MICROALGAE AND ENVIRONMENTAL FACTORS CONTROLLING THEIR DISTRIBUTION IN MARINE HABITATS

Microphytobenthos are unicellular algae that live on the surface of marine or fresh water sediments. Benthic microphytic assemblages are primarily made up of diatoms but also include cyanobacteria, euglenoids and flagellates (Barranguet et al., 1997; Brotas and Plantecuny; 1998; Klein and Riaux-Gobin, 1991; MacIntyre et al., 1996). Habitats range from intertidal mud and sandflats, beaches and lagoons to subtidal sediments. These environments are characterised by strong gradients in light, temperature, and chemicals such as oxygen, nutrients and toxic substances. The unstable nature of soft sediments inhibits the permanent colonisation of macrophytes, and microphytobenthos are therefore the main benthic producers in soft bottom estuarine and coastal habitats (Admiraal, 1984).

Benthic diatoms inhabiting marine sediments can be divided into three life forms according to their relationship with the substratum: Epipelic diatoms are free living and motile. They belong to the biraphid group of pennate diatoms and encompass the genera *Navicula* and the giant sigmoidal shaped genera *Gyrosigma* and *Pleurosigma* (Round, 1971). Episamnic diatoms are immobile and attach themselves firmly to sand grains. They are subjected to the transport and mixing of their substratum and as a consequence can be buried into deeper layers or be eroded and swept away by wave action. Diatoms exhibiting this life style belong mainly to Araphidinae or Monraphidinae families (Round, 1971). The third group of diatoms exploit a half planktonic, half benthic life style. Diatoms of this category belong to either the pennate or the centric form and include genera such as *Cylindrotheca* spp. and *Melosira* spp. This group inhabits the surface of intertidal and subtidal sediments, where it photosynthesises during tidal emersion and, depending on light penetration, during inundation. Being immobile, diatoms of this group are suspended in the water column during tidal mixing, where they continue their photosynthetic activity. Due to this cycle of suspension and deposition, they are continuously redistributed. Little is known about the proportion of benthic diatoms suspended into the water column, however its contribution to phytoplanktonic biomass can be significant (deJonge and vanBeusekom, 1992). The three life strategies should be regarded as a continuum of adaptations rather than strict categories.
THE DISTRIBUTION OF MICROPHYTOBENTHOS IN BENTHIC MARINE HABITATS

The horizontal distribution of microphytobenthos is constrained to marine sediments and substrates which receive sufficient light for photosynthesis, these include salt marshes and intertidal sediments and can extend into the subtidal zone. High tide and subtidal production depends on the absorbing and scattering processes in the overlying waterbody and is largely reduced in water containing high concentrations of suspended particulate matter. In clear water subtidal production of microphytobenthos can significantly contribute to total water column production (Cohoon and Cooke, 1992). By absorbing light phytoplankton can significantly alter the quality and quantity of light available to phytobenthos and a decline of benthic algal biomass during the occurrence of phytoplankton blooms has been reported (Cohoon and Cooke, 1992; Sundbäck and Jönsson. 1985).

The colonisation of intertidal and subtidal habitats requires very different strategies. Epipelic diatoms which inhabit silts and clays are adapted to the physical conditions of the intertidal zone: finer grain sized sediments are more resistant to desiccation and the ability of epipelon to migrate protects against resuspension and harmful UV light. The trade off for this particular life style is minimal primary production at high tide. Episammic diatoms on the other hand continue to photosynthesise during high tide (Varela and Penas, 1985) and are therefore arguably better adapted to the subtidal zone.

In general, the biomass of microphytobenthos is higher on sheltered mud flats than on exposed sandy habitats and subtidal areas (MacIntyre et. al, 1996). Rates of gross photosynthesis follow the same trend, however higher rates of respiration in the more sheltered and organically rich habitats counteract increased primary production (MacIntyre et. al, 1996).

VERTICAL ATTENUATION OF LIGHT THROUGH THE SEDIMENT AND ITS INFLUENCE ON MICROALGAL DISTRIBUTION

Attenuation of light through sediment is primarily controlled by the refractive properties of quartz and is enhanced in sediments of fine grain sizes. In silts (<63 μm) 50% of light is absorbed in the first 300 μm while in coarse sand (500-1000 μm) it is absorbed in the first 650μm (Fenchel and Straarup, 1971). The penetration of light is further influenced by the moisture content of sediment, as the relative refractive index of quartz is higher in air than in
water. The light quality changes vertically, as longer wavelengths penetrate deeper into the sediment than short ones (Fenchel and Straarup, 1971).

It has been shown from pigment analyses and microscopic studies that there is a differential vertical distribution of microalgae in sediments. The vertical position of phototrophs is determined by adaptations to the particular light field and tolerance limits to desiccation, toxic substances such as sulphate and other chemical gradients (Joint et al., 1982; Cariou-Gall and Blanchard, 1995). It is especially apparent in microbial mats that the vertical change in light quality is exploited by autotrophic organisms which possess pigments with different absorption spectra. Below a layer of diatoms and cyanobacteria Pierson et al. (1990) found a layer of photosynthesising bacteria close to the anoxic layer. These anoxicogenic phototrophs contain bacterial chlorophylls a, b and c, which absorb light in the near infrared. Microbial mats in themselves are an adaptation to the prevailing light conditions, because aggregated microalgae compete against the high refraction of quartz (Parsons et al., 1984).

**MIGRATORY BEHAVIOUR OF EPIPELIC DIATOMS**

Migration of epipelon occurs in the upper millimetre of the sediment. It is a response to photo-, geo- and chemotaxis in combination with an internal clock, which responds to tidal and light cycles. This life strategy allows epipelic diatoms to reposition themselves after burial into a vertical position, which has sufficient light penetration for photosynthesis. In addition it offers a protective mechanism against resuspension by the incoming tide and harmful radiation of UV light during tidal emersion. The migratory behaviour of epipelic diatoms has been detected with freeze sectioning (Palmer and Round, 1967), low temperature SEM (Paterson, 1986) and non-destructively by *in vivo* chlorophyll a fluorescence (Serodio et al. 1997). Variation of chlorophyll a fluorescence at the sediment surface has been shown by Serodio, *et al.* (1997) to vary by up to 400% and was synchronised with daytime periods of tidal emersion. There are taxonomic differences in the vertical position of epipelic diatoms, their migratory behaviour, and their speed of movement (Joint *et al.*, 1982; Paterson, 1986). When samples are brought back into the laboratory epipelic diatoms have been shown to continue migration in the absence of external stimuli. However, the tidal oscillation changes to a diurnal one, suggesting that an endogenous rhythm is present which is readjusted by environmental stimuli (Palmer and Round, 1967; Serodio *et al.*, 1997). Light overrides the rhythm and the absence of light inhibits upward migration and induces downward migration (Palmer and Round, 1967). Flooding of the sediment is further believed to force downwards migration (Holmes and Mahall, 1982). The migratory behaviour of pennate diatoms is an
important factor explaining short term variations in gross photosynthesis. Thus 52% of the variation in photosynthesis of epipelic diatoms has been reported between high tide and low tide and was attributed to their migratory behaviour (Pinckney and Zingmark, 1991).

**THE INFLUENCE OF NUTRIENTS ON THE DISTRIBUTION OF MICROPHYTOBENTHOS**

In the estuarine environment, nutrient supplies are high compared to oceanic habitats. Rivers transport inorganic nutrients derived from rock weathering and in addition estuarine and coastal habitats receive large concentrations of nitrogen and phosphorus from anthropogenic sources. Studies focusing on the influence of nutrient levels on the distribution of microphytobenthos are divided into positive correlation between nutrient concentration and chlorophyll a (Sundbäck and Jönsson, 1985; Admiraal, 1982) and no relationship between availability of nutrients and microalgal biomass (Varela and Penas 1985). Controlled experiments with nutrient enrichment also resulted in controversial outcomes. In muddy sediment the addition of phosphate and nitrate did not cause an enhancement of microalgal growth (Sundbäck et al., 1991), while in sand elevated levels of these nutrients resulted in a stimulation of growth, indicating that the ambient concentrations were growth limiting (Sundbäck and Graneli, 1988; Nielsson and Sundbäck, 1991). When total levels of nutrients are not limiting, the immediate micro-environment can show signs of depletion due to active uptake by the algae themselves. Thus even though high levels of silica are brought in by rivers through rock weathering, the concentration of silica in the soluble form can be a limiting factor in dense algal mats (Admiraal, 1984). Adaptations against local nutrient depletion are the ability to vertically migrate through the substrate and chemotaxis to localise areas of depletion and enrichment (Harper, 1977).

**THE INFLUENCE OF TEMPERATURE ON BIOMASS AND PRODUCTION OF BENTHIC MICROALGAE**

Temperature fluctuations experienced by benthic microalgae occur over three different time scales. Tidal and diurnal changes are short term and can be drastic in intertidal habitats, where algae are exposed to different air and water temperatures over short periods of time (Guarini et al. 1997). The lunar cycle which determines the occurrence of spring and neap tides has a further effect on temperature fluctuation on intertidal mudflats, as it determines the duration of the emersion period and the phase difference between the lunar and the solar cycle. It has been shown that cellular division rates of benthic microalgae inhabiting temperate mudflats are not inhibited by extreme temperatures such as below 0°C and up to 30°C, suggesting that microphytobenthos is more eurythermal than phytoplankton (Admiraal and Peletier, 1980). Rates of photosynthesis and cell divisions are closely related to temperature,
and laboratory experiments with benthic microalgae collected from the field have shown a $Q_{10}$ of gross primary production between 2 and 3.3 up to the optimum temperature (Colijn and van Buurt, 1975; Grant, 1986).

Long term seasonal changes in temperature are superimposed on diurnal and weekly fluctuations causing the temperature range experienced by microphytobenthos to vary throughout the year. The relationship between microalgal photosynthesis and temperature changes during the different seasons (Colijn and van Buurt, 1975; Grant, 1986; Rasmussen et al., 1983). Blanchard et al (1997) found a fourfold increase in $P_{\text{max}}$ (photosynthetic potential) from December to April, with the spring value being twice as high as in summer and autumn. The high photosynthetic capacity coincides with the initial increase in temperature, irradiance and duration of photoperiod and is a possible cause for the spring blooms observed in many seasonal studies (MacIntyre et al, 1996). Optimum temperatures on the other hand are stable throughout the year, although the range of temperatures experienced in the field are significantly lower in the winter and higher in the summer (Blanchard et al, 1997). Since daily temperature fluctuations are so extreme, it is possible that long term physiological adaptations of optimum temperature are not ecologically viable.

**THE EFFECT OF GRAZING ON THE ABUNDANCE OF MICROPHYTOBENTHOS**

Herbivory is an important control on the standing stock of benthic microalgae. A review by Duarte and Cebrian (1996) estimated that approximately 40% of the net primary production of marine microphytobenthos is transferred to higher trophic levels within the individual ecosystem. Most data on the impact of grazing on the standing stock of microphytobenthos derive from experimental manipulations, where the accumulation of microalgal biomass is compared in the presence and absence of grazers. The outcomes of these experiments are reviewed by Miller et al. (1996) and show that the reduction of chlorophyll $a$ in the presence of grazers varied between 8-30% and was dependent on the abundance and distribution of herbivores and the growth dynamics of microphytobenthos. The study of grazing control on benthic microalgae is complicated by the complex interactions between the primary producers and the herbivores. The functional response of macro- and meiofauna can increase by a factor of 5 during maximum abundance of benthic microalgae, but varies among species (Montagna et al, 1995). However the removal of algal cells by grazing can to some extent be offset by an increase in growth rates of microphytobenthos from populations of lower density (Admiraal et al. 1983). The stimulation of microalgal growth by grazers is not
only due to the disruption of competitive interactions but also because nutrients are recycled and the sediment surface is disturbed, thereby diminishing chemical micro-gradients.

**SEASONAL AND SPATIAL VARIATION OF MICROPHYTOBENTHOS BIOMASS AND PRIMARY PRODUCTION**

Although distinct blooms of microphytobenthos have been reported in many studies (see Table 1.1), seasonal variations of chlorophyll $a$ in intertidal sediments are often less pronounced than in the water column (Varela and Penas, 1985; Cadee and Hegeman, 1974). Oppenheim (1991) could not find repeatable seasonal cycles of diatom biomass variation in salt marshes and mudflats over several years, and no seasonality in sandflats at all.

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<td>72</td>
<td>55 - 120</td>
<td>May, April-May</td>
<td>(Pinckney &amp; Zingmark, 1993a)</td>
</tr>
<tr>
<td>Marennes-Oleron Bay France</td>
<td>intertidal mudflat</td>
<td>30.6</td>
<td>na</td>
<td>May, September, April-May</td>
<td>(Cariou-Le Gall &amp; Blanchard 1995)</td>
</tr>
<tr>
<td>Ría de Arosa, Spain</td>
<td>intertidal sandflat</td>
<td>na</td>
<td>27 - 103</td>
<td>Dec, March, July, October</td>
<td>(Varela &amp; Penas, 1985)</td>
</tr>
<tr>
<td>Onslow Bay N. Carolina</td>
<td>subtidal</td>
<td>27.4</td>
<td>16 - 62</td>
<td>April</td>
<td>(Cahoon &amp; Cooke, 1992)</td>
</tr>
<tr>
<td>Massachusetts Bay, USA</td>
<td>subtidal</td>
<td>39.8 $^1$</td>
<td>16 - 79</td>
<td>April</td>
<td>(Cahoon et al, 1993)</td>
</tr>
</tbody>
</table>

Table 1.1 Chlorophyll $a$ values and annual fluctuations in temporal intertidal and subtidal habitats.

Primary production studied over an annual cycle closely follows the variation of benthic microalgal biomass (MacIntyre *et al.*, 1996). Most studies show a positive correlation between chlorophyll $a$ and primary production, however the regression slopes and correlation coefficients vary, which is partially due to different methodologies and depth integration (Cadee and Hegeman, 1974; Davis and McIntire, 1983; Pinckney and Zingmark, 1993a). When production is normalised to chlorophyll $a$ to obtain biomass specific productivity, rates are more constant over a seasonal cycle (Pinckney and Zingmark, 1993a) and variations between samples can exceed temporal variation (Cadee and Hegeman, 1974).
THE RELATIVE CONTRIBUTION OF MICROPHYTOBENTHOS TO MARINE PRIMARY PRODUCTION

The relative contribution of microphytobenthos to marine primary production has been reviewed by Charpy-Roubaud and Sournia (1990) and is estimated to be approximately 1% of total marine primary production and 5% of production on the continental shelf. This includes sediment inhabiting microphytes, epiphytes and coral reefs.

The primary production of sediment-inhabiting microphytes and its contribution to local primary production varies from system to system. It has been shown in a number of studies that the contribution of microphytobenthos is significant in the intertidal zone and can exceed planktonic production. In the Dutch Wadden Sea production by microphytobenthos was a magnitude higher than phytoplankton production (Cadee and Hegeman 1974), while in the Westerschelde and Ems Dollard estuary benthic production was estimated to contribute 17-20% to total production (Kromkamp et al., 1995; Colijn, 1982). In the sublittoral zone the contribution of benthic microphytes to total production generally decreases, however contributions ranging from 6-50% have been reported (Cahoon and Cooke, 1992; Cahoon et al. 1993, Schreiber and Pennock, 1995). Although benthic microalgal biomass can greatly exceed that throughout the water column, photosynthesis is more light limited, resulting in lower rates of primary production in some cases (MacIntyre and Cullen, 1996).

To understand the ecological role of microphytobenthos and their relative contribution to the marine carbon cycle, comparative estimates of primary production are not sufficient - the fate of their production needs to be examined. In a comprehensive review by Duarte and Cebrian, (1996) the fate of photosynthetic carbon produced by the different marine autotrophs ranging from mangrove swamps to oceanic phytoplankton were compared on a global scale. Several generalisations about the fate of carbon produced by microphytobenthos were made and are summarised in fig. 1.2. The fraction of NPP (net primary production) of microphytobenthos which is decomposed within the system is 25% in contrast to 40-50% of other marine plants. Herbivory is in the order of 40% similar to other microalgae and comprises the largest proportion of their NPP. Microphytobenthos exports a larger fraction of its NPP than other microalgae and around 6% of its NPP is stored within the sediment (Fig. 1.2). These findings suggest that although most of the carbon produced by benthic microalgae is passed on to higher trophic levels, a large proportion is advected out of the system. Resuspension and subsequent transport out of the estuary is the likely path for exported...
microphytobenthos and a large proportion of benthic microalgae have been reported in pelagic systems (deJonge and vanBeusekom, 1992, Baillie and Welsh, 1980).
Fig. 1.1. Average carbon budgets for the different marine autotrophs. The budgets are derived from the average (±SE) percentage of net primary production decomposed within the system (D), consumed by herbivores (H), exported (E), and stored in the sediment (S). The width of the arrows denoting the fluxes are proportional to the fraction of net primary production they represent, with the sides of the boxes representing 100% of NPP. Because the estimates were derived by averaging independent estimates the budgets shown need not add up to 100% (from Duarte and Cebrian, 1996).
1.2 THE PHOTOSYNTHETIC PROCESS IN BENTHIC MICROALGAE

Benthic microalgae including cyanobacteria are oxygenic photoautotrophs and their photosynthetic process can be summarised with the formula (Falkowski and Raven, 1997):

\[
2H_2O + CO_2 + \text{light } \text{chlorophyll } a \rightarrow (CH_2O) + H_2O + O_2 \quad (1.1)
\]

The photosynthetic process in benthic microalgae can be broken down into the **light reaction** where light energy is used to extract electrons from water to form oxygen:

\[
2H_2O + \text{light } \text{chlorophyll } a \rightarrow 4H^+ + 4e^- + O_2 \quad (1.2)
\]

and the **dark reaction** where CO₂ is reduced and requires the formation of intermediate reduction agents:

\[
CO_2 + 4H^+ + 4e^- \rightarrow CH_2O + H_2O \quad (1.3)
\]

THE PHOTOSYNTHETIC APPARATUS

In diatoms and euglenoids which are eukaryotic algae the entire photosynthetic process occurs in the chloroplasts. The light reaction is localised in lipoprotein membranes called thylakoids, which form closed vesicles around aqueous intrathylakoid spaces. Surrounding the thylakoids is an aqueous phase called stroma, which hosts the dark reaction. In Cyanobacteria which are prokaryotes and lack specialised photosynthetic organelles the light reaction takes place in membranes which are organised in sheets or lamelli adjacent to the periplasm. The dark reaction occurs in the centre of the cell.

Pennate diatoms normally contain two large chloroplasts. The thylakoids are grouped in stacks of three, called grana. The photosynthetic light harvesting pigment-protein complexes and the catalysts involved in O₂ evolution and ATP synthesis are located within the thylakoid membranes. The light absorption/electron transport system consists of two subsystems called photosystem I (PSI) and II (PSII). Each photosystem contains a reaction centre and a light harvesting pigment-protein complex (LHC) with specific associated electron transport chains. The two photosystems are physically separated but interact with each other (Fig. 1.2).
Fig. 1.2 Schematic representation of organisation of photosystems in the thylakoids and of electron transfer from H₂O to NADP⁺ via PSI and PSII (from Jeffrey et al. 1997)

PHOTOSYNTHETIC PIGMENTS

Photosynthetic pigments absorb light between 400 and 700nm and can be grouped into three types: chlorophylls, carotenoids and biliproteins. Chlorophylls are cyclic tetrapyrrole compounds with a magnesium atom situated in the centre of the molecule. Chlorophyll a is the principal pigment converting light energy into chemical energy and is found universally in all plants. In addition diatoms contain chlorophyll c1c2 and euglenoids contain chlorophyll b (Jeffrey et al. 1997). The absorption of chlorophyll a is highest in the blue or Soret band and the red band and almost zero in the green region of the spectrum. Chlorophylls b and c1c2 extend the absorption further between 450-650nm (Kirk, 1994).

Carotenoids are C40 isoprenoid compounds and cause a further extension of absorption between 450-650nm and at the short wavelengths of the visible range. While β-carotene occurs in all algae but cryptophytes (Jeffrey et al. 1997) other carotenoids are specific to different algal classes and can therefore be used as taxonomic markers (Millie et al. 1993). Phycobilisomes are found in Cyanophyta, Cryptophyta and Rhodophyta (Jeffrey et al. 1997). These water soluble molecules are chromophores attached to biliproteins and can be grouped into phycocyanins, phycoerythrins, phycoerithrocyanins and allophycocyanins according to their absorption spectra (Kirk, 1994).

Chlorophylls and carotenoids occur in pigment protein complexes, whereby all protein complexes contain chlorophyll a and most have one or several different carotenoids. Some of the complexes occur in the photosystem I and II, but the majority are contained in the LHCs which collect light energy and transfer it to the reaction centres (Brown, 1988). PS1, PSII and their associated LHCs constitute one photosynthetic unit. Core complexes I and II contain chlorophyll a and β-carotene. Most accessory pigments in the form of pigment - protein
complexes are present in LHCII and it is believed that light is mainly absorbed by this light harvesting complex, feeding energy into PSII and subsequently transferring energy to PSI during *spillover* (Kirk, 1994).

**REACTION CENTRE SAND ENERGY TRANSFER**

In the reaction centre within a photosystem absorbed light energy is used to transfer an electron from a donor molecule to an acceptor. This is carried out by a specific chlorophyll *a* protein complex. Chlorophyll *a* is raised to an excited electronic state, and thus reduces the acceptor molecule (i.e. donates an electron), hence chlorophyll *a* withdraws an electron from a donor molecule to return to its original state. The loss of an electron is accompanied by a change in absorption spectrum, in PSI at 700nm and PSII at 680nm; the chlorophylls- protein complexes in the reaction centres of PSI and PSII are therefore also called P<sub>700</sub> and P<sub>680</sub>. The excitation of P<sub>700</sub> and P<sub>680</sub> takes place by direct absorption of photons or by the transferral of energy from other pigments situated in the antenna or LHCs by a process called *inductive resonance transfer* ( Förster, 1947 cited by Kirk, 1994). A photon of a specific energy state, i.e. wave length is absorbed by a pigment of that wave length, hence the captured energy is emitted as fluorescence and dissipated in vibrations, bringing the electron to its lowest excited state. These vibrations are in resonance with the upper vibrational levels of the excited state of another pigment, the energy is then transferred from one molecule to the other. For the transfer to occur the fluorescence emission peak of the donating molecule must overlap with the absorption of the acceptor molecule. All energy is finally transferred to chlorophyll *a*. As energy migration takes place in the direction of molecules absorbing at greater wavelengths, the second absorption peak of chlorophyll *a* is at the longest wavelength of all pigments (Jeffrey *et al.*, 1997).

**THE PHOTOSYNTHETIC PROCESS**

During the light reaction H is split from H<sub>2</sub>O and passed along H carriers to NADP to form NADPH<sub>2</sub> with the liberation of O<sub>2</sub> and an associated conversion of ADP to ATP. The light reactions takes place in two stages: NADP<sup>+</sup> is reduced in PSI and O<sub>2</sub> is released from H<sub>2</sub>O in PSII. Figure 1.3 summarises the pathways of the light reaction in PSI and PSII.

When P<sub>680</sub> loses an electron it is passed to an adjacent phaeophytin molecule, which then reduces plastoquinone (QA), the electron is transferred to a pool of plastoquinone, which in turn reduces cytochrome *f*. The oxidised P<sub>680</sub> is reduced from donor Z (tyrosyl residue in reaction centre) and the oxidised form of Z removes electrons from water, liberating oxygen. Meanwhile P<sub>700</sub> is acitivated by light and donates an electron to A<sub>b</sub>, the electron is passed to ferredoxin and via flavoprotein and ferredoxin-NADP reductase to NADP. P<sub>700</sub> is then reduced
by plastocyanin, which in turn is reduced by cytochrome f (Kirk, 1994). During the electron transfer an energy gradient is generated across the thylakoid membrane which drives ATP synthase to form ATP from ADP and phosphate on the stroma side of the membrane (Fig. 1.2).

![Redox potential, E_m(V)](image)

Fig. 1.3. The light phase of photosynthesis (after Kirk, 1994).
THE DARK REACTION

The dark reaction which occurs in the stroma is the conversion of CO$_2$ into carbohydrate with the use of ATP and NADPH$_2$, produced in the light reaction. The fate of six molecules of CO$_2$ in the Calvin cycle is shown in Fig. 1.4. Six molecules of CO$_2$ react with six molecules of the C5 compound ribulose biphosphate (RuBP) to form 12 C3 compounds called phosphoglyceric acid (PGA). PGA is phosphorylated by ATP and reduced by NADP to form phosphoglyceraldehyde (PGAL), which is another C3 compound. Five of the six PGALs are used to form new RuBP, while the sixth molecule is used for the synthesis of glucose.

Fig. 1.4. Schematic diagram of photosynthetic CO$_2$ fixation via the Calvin Cycle (after Keeton and Gould, 1986).
PHOTOSYNTHESIS AS A FUNCTION OF LIGHT

The rate of photosynthesis is not directly proportional to the capture of quanta and the study of the functional relationship between photosynthetic output and irradiance is used to investigate the adaptation of algal populations to their light environment (Platt et al., 1980). P vs. E curves can be constructed by relating the rate of photosynthesis as measured by O₂ production or the incorporation of ¹⁴C labelled CO₂ to a range of different irradiance levels (Platt et al., 1980). A theoretical P vs. E curve is shown in Fig. 1.5

![Fig. 1.5. Representation of a typical photosynthesis vs. irradiance curve. Terms are explained in the text (after Falkowski and Raven, 1997).](image)

The curve commences at the point where irradiance is high enough for photosynthesis (P) to exceed respiration (R) (i.e. P > compensation point, C₀). Subsequently the photosynthetic rate follows a linear increase with irradiance up to certain value and is dependent on the efficiency of light utilisation to drive photosynthetic reactions. The initial slope called α (or α² when normalised to chlorophyll a) is related to the maximum quantum yield (φₐ) of photosynthesis through light absorption and is often taken as its direct measure.

Following a further increase of E, the photosynthetic rate levels off and the algae are light saturated and have reached their photosynthetic capacity Pₘₐₓ. This parameter is related to the number of photosynthetic units (n) and their maximum turn over rate (1/τ)

\[ Pₘₐₓ = n \left( \frac{1}{τ} \right) \]

(1.2)

At light saturation 1/τ is at its maximum rate at which electrons are transferred from H₂O to the terminal electron acceptor (Falkowski and Raven, 1997).
The light saturating irradiance $E_k$ is the point of intersection between the extrapolated linear part of the curve and the horizontal line at $P_{\text{max}}^B$ (see Fig. 1.5).

$$E_k = \frac{P_{\text{max}}^B}{\alpha^B} \quad (1.3)$$

When $E < E_k$, the electron transport capacity exceeds the rate at which photons are absorbed and when $E > E_k$ the rate of photon absorption exceeds the capacity of electron transport (Falkowski and Raven, 1997).

**VARIATIONS IN N, CROSS SECTION AND 1/T**

There are several processes that can alter the number of photosynthetic units ($n$), the absorption cross section (i.e. the area available for light harvesting), and their maximum turnover rate ($1/\tau$). These adjustments are state transition, xanthophyll cycling and long-term photoadaptation.

**State transition:** State transition alters the effective absorption cross section of photosystems by coupling and decoupling light harvesting antenna with the reaction centres (Fork and Satoh, 1986).

**Xanthophyll cycling:** The three xanthophylls zeaxanthin, violaxanthin and antheraxthin in higher plants and chlorophytes participate in xanthophyll cycling. Violaxanthin contains an epoxide that can be depoxidized at high irradiances by enzymatic activity to form zeaxanthin (Fig. 1.6). The conversion can take place within 30 minutes (Yamamoto et al. 1963). An analogous xanthophyll cycle has been found in chromophytes from diadinoxanthin to diatoxanthin (Fig. 1.6). Xanthophyll cycling does not occur in phycobilisome-containing classes such as cyanobacteria.

Demers et al. (1991) found a correlation between non photosynthetic quenching (NPQ) and the xanthophyll cycle as a protection from overexcitation of PSII. Normally carotenoids pass excitation energy to chlorophyll $a$. During NPQ the reverse occurs and excess energy is dissipated from the antenna of PSII as heat, decreasing the absorption cross section.
Fig. 1.6. The xanthophyll cycle in a). chromophytes and b). higher plants, phaeophytes and chlorophytes (after Falkowski and Raven, 1997).
**Long-term photoadaptation:** Both processes described above change the absorptive cross section within minutes. Long-term adaptations take place when the organism is exposed to a given light regime for a longer duration of time. An adaptation to a low light environment is the increase of the cellular chlorophyll content (Falkowski, 1980). Although this increases the maximum quantum yield, the absorptive cross section is decreased due to selfshading. At high irradiances algae possess relatively high carotenoid to chlorophyll a ratios. B-carotene and zeaxanthin do not transfer energy to chlorophyll a and therefore act as a screen from excess light, some other carotenoids transfer light with reduced efficiency. Hence at high irradiances algae possess lower maximum quantum yields. Long-term photoadaptation occurs in two ways: a). the number of reaction centres can be changed, while the functional absorptive cross section of each individual one remains constant or b). the functional size of the antennae complexes is changed while the number of the reaction centres remains constant (Falkowski, 1980).
1.3 THE INFLUENCE OF MICROPHYTOBENTHOS ON BIOGEOCHEMICAL CYCLING AND GEOMORPHOLOGY OF MARINE SEDIMENTS

IMPORTANCE OF MICROPHYTOBENTHOS AS A FOOD SOURCE FOR SEDIMENT INHABITING FAUNA

In estuarine sediments up to 70% of particulate organic carbon available to invertebrate grazers derives from the primary production of benthic microalgae (Currin et al., 1995). Due to a high content of structural carbohydrates and inhibiting phenolic compounds in macrophytes, benthic microalgae are a preferred choice of food for sediment inhabiting fauna (Miller et al., 1996). In the mudflats of the bay of St. Michel (France) the dominating deposit feeders such as Corophium volutator and Ovatella bidentata had a diet which consisted only of benthic diatoms (Creach et al., 1996). Resuspension of benthic algal cells makes this nutrition available for suspension feeders as well as deposit feeders (DeJonge and van Beusekom, 1992).

INFLUENCE OF MICROPHYTOBENTHOS ON THE BENTHIC-PELAGIC NUTRIENT EXCHANGE

In the estuarine ecosystem nutrient remineralisation occurs primarily within the sediment after deposition of faecal pellets and senescent phytoplankton cells, resulting in a net efflux from the sediment water interface into the overlying water column. Microphytobenthos inhabiting the sediment water interface have a direct and an indirect influence on the release of nutrients into the water column (Fig. 1.2). By using nutrients solubilised in the pore water of the sediment, benthic microalgae act as a direct filter within the benthic - pelagic exchange (Sundbäck and Graneli, 1988). During photosynthesis Sundbäck et al. (1991) observed an enhanced removal of NH₄, NO₃, PO₄ and SiO₄, which caused a depletion in the uppermost layer of the sediment compared to the subsurface layer and the overlying water column. Silicon fluxes were reversed during illumination of the sediment, indicating that nearly all of the soluble Si is assimilated by benthic diatoms (Sundbäck et al., 1991). In sediments of a hypertrophic marine pond, 90% of all ammonium was taken up by benthic microalgae and therefore did not reach the water column (Krom, 1991). Benthic diatoms and cyanobacteria often form dense microbial mats, in which algal cells are interconnected with mucoid polysaccharides. These mats present a strong diffusive barrier between the sediment and the water column, restricting nutrient exchange even further (Höpner and Wonneberger, 1985).
The metabolic activity of benthic microalgae also has indirect effects on the nutrient flux of the sediment. Benthic diatom photosynthesis can exceed atmospheric diffusion as a source of oxygen production at low tide by an order of magnitude (Bailli, 1986). Photosynthesis causes a significant thickening of the oxic sediment layer by up to 50% (Wiltshire, 1993; Epping and Jørgensen, 1996). The volume of sediment participating in aerobic respiration is thus increased and strictly aerobic nitrifying bacteria are stimulated while the process of denitrification is inhibited. The extent to which the direct and indirect factors of microphytobenthos on nutrient exchange interact depends on microphytic abundance, organic carbon content of the sediment and oxygen diffusion. While Sundbäck et al. (1991) found that the production of NO₃ due to enhanced nitrification only dampened the effect of nutrient uptake by microalgae, Wiltshire (1993) reported a net flux of nitrate out of the sediment when microalgae were incubated in the light and a net flux into the sediment during dark incubations.

Fig. 1.7: Microphytobenthos and sediment nitrogen conversion processes (taken from Wiltshire, 1993).
THE EFFECT OF MICROPHYTOBENTHOS ON SEDIMENT STABILISATION

Microbial assemblages inhabiting intertidal mudflats have been shown to play an important role in the stabilisation of cohesive sediments (Holland et al. 1974; Paterson; 1994). Epipelic diatoms move through the sediment by secreting carbohydrate rich exopolymers (EPS: Decho, 1990), whereby EPS is secreted through the raphe system and binds to sediment particles. The secreted mucilage complex is displaced by actin generated force and then broken off to move the cell (Edgar and Picket-Heaps, 1984). On intertidal mudflats which are sensitive to erosion by tidal currents and wind induced waves the presence of EPS has a positive effect on the stabilisation of the sediment (Yallop et al. 1994; Underwood and Paterson; 1993; Paterson et al. 1994). The critical shear strength needed to initiate the erosion of sediment is reduced as EPS binds the sediment particles and thereby produces a matrix at the sediment surface (Paterson, 1994). The concentrations of both chlorophyll a and colloidal carbohydrate have been found to correlate with the critical shear strength to erode the sediment and serve as a good indicator for the bio-stabilisation of cohesive sediment (Underwood and Paterson, 1993).

Biogenic mediation of sediment stabilisation is, however, dependent on a number of physical and eco-physiological factors which have to be taken into account when relating benthic microalgal biomass to sediment properties. The water content of the sediment influences the binding properties of EPS as it affects the hydration state of the muco-poly saccharide, thus when mucilage dries out, its binding strength increases (Paterson, 1988). Biogenic sediment stabilisation further depends on the species composition, migratory behaviour and age of the microbial assemblage. The stability of the sediment increases during the course of low tide when epipelic diatoms migrate to the surface (Paterson et al. 1990). Experiments on the allocation of photoassimilates in epipelic diatoms have shown that the production of EPS de novo and the reallocation of glucan into EPS was closely linked to the tidal cycle. EPS production was maximised at the end of the photoperiod, which coincided with enhanced migratory activity (Smith and Underwood, 1998). EPS that is secreted during diatom migration remains within the sediment and can therefore have an accumulative effect on sediment stability (Paterson, 1989). Laboratory studies on benthic biofilms have shown that mature assemblages of epipelic diatoms in the stationary phase caused a continuing increase of EPS in the sediment while chlorophyll a concentrations remained stable (Sutherland et al. 1998a). High rates of primary production in dense microbial assemblages can be counteractive to sediment stability when oxygen bubbles are trapped within the EPS sediment matrix as they increase the buoyancy of the sediment surface layer (Sutherland et al., 1998b).
The above mentioned factors influencing biogenic mediation of sediment stability need to be studied and combined with measurements of chlorophyll $a$ and sediment erosion threshold to provide a better understanding of the microbial role in sediment stabilisation (Paterson, 1994). The production of EPS by epipelic diatoms not only influences sediment stability, but also serves as an important carbon source for bacteria, meiofauna and macrofauna, which further indicates its ecological significance in marine shallow water habitats (Decho, 1990).
Benthic microalgae play an important role in the trophic dynamics, sediment stabilisation and biogeochemical cycling of estuarine and shallow marine ecosystems. In order to understand their relative contribution to these processes, the biomass and primary production of microphytobenthos need to be accurately quantified and related to environmental variables. A number of previous studies have focused on the temporal and spatial variability of microphytobenthos at relatively coarse temporal and spatial scales. The extrapolation of short-term measurements to longer time scales is complicated by the physical nature of the environment. In turbid estuaries, benthic production is limited to periods of tidal emersion during low tide. The phase difference between the tidal and the solar cycle causes strong biweekly oscillations in the light environment of benthic microalgae, upon which seasonal variations in meteorological factors are superimposed.

The research described in this thesis aimed to identify the environmental factors that control the production of microalgal biomass on the intertidal mudflats of a turbid macrotidal estuary. The central hypothesis to this study was - that changes in incident irradiance defined by a combination of meteorological factors and the tidal cycle over critical time scales (ie. weekly) causes significant changes in the biomass and production of microphytobenthos. A second related hypothesis to be tested was - that photo-physiological changes of microphytobenthos over critical time scales are important for population maintenance within this dynamic light regime.

Specific objectives were:

- to relate the variation of benthic microalgal biomass and taxonomic composition, sampled at weekly intervals over a year, to seasonal and short term changes in irradiance, temperature and other meteorological factors in order to identify the forcing environmental controls on their growth. Four different stations were sampled along an intertidal transect, to give an insight into the ecological and physiological compromise of microphytobenthos between light availability, physical disturbances and environmental stress in the form of desiccation, elevated salinity and high irradiance levels.

- to measure the spatial distribution of photosynthetic pigments in sediment at high vertical resolution in order to relate physical and chemical variables to the input, storage and degradation of pigments in fine grained intertidal sediments.

- to examine the ecological and photo-physiological responses of microphytobenthos to seasonal and spatial variations in environmental variables. In particular to determine how
• to examine the ecological and photo-physiological responses of microphytobenthos to seasonal and spatial variations in environmental variables. In particular to determine how changes in community structure, photo-adaptation and cellular pigment composition can be related to physical parameters resulting in variations in primary production.

• to determine whether there are short-term photo-physiological responses which override the long term photo-adaptation and how their scales compare by studying the change of photosynthetic parameters during individual photo-periods. Furthermore to examine whether the cellular pigment composition of microphytobenthos is altered as a photoprotective response to high incident irradiances, both in situ and under controlled laboratory conditions, with special reference to the xanthophyll cycle.
CHAPTER 2

REVIEW OF METHODOLOGY

2.1 FIELD SAMPLING METHODS

DESCRIPTION OF STUDY SITE

The sampling site in this study was located on the mudflat bordering Hythe Saltmarsh in Southampton Water (Fig. 2.1 and 2.2). Hythe saltmarsh is located at the western side of Southampton Water, approximately 7 miles north of the estuary inlet (Fig. 2.2). Intertidal mudflats extend up to 200 metres seawards, bearing no macroalgae except on a narrow cockle bed along spring low water mark which was colonised by brown algae. Four stations along an intertidal transect were chosen for the sampling program (Fig. 2.1). Stations 1 to 3 were situated on the mudflat in intervals of 45 metres, comprising the mean low water, neap low water and mean high water mark. Station 4 was located at the head of a tidal channel which extended 80 metres into the saltmarsh. This station was only inundated during spring high tides.

Fig. 2.1. The four sample stations in relation to tidal height on Hythe saltmarsh and mudflat.
Fig. 2.2. Southampton Water and Hythe saltmarsh and tidal flat.
SAMPLING TIME TABLE

Table 2.1 gives a summary of the sampling programmes carried out in this study. Between February 1996 and April 1997 all four stations were sampled weekly for microalgal biomass, photosynthetic pigments and taxonomy. From December 1997 until May 1998 station 2 was sampled monthly for primary productivity, photosynthetic parameters, pigments and taxonomy. In March and April, 1998 these measurements were also carried out once at stations 1, 3 and 4. In March, July and August, 1998 benthic microalgae were isolated from surface sediments of station 2 for laboratory experiments on short-term changes in photosynthetic parameters.

<table>
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<th>Station</th>
<th>Samples</th>
<th>frequency</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
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<td>1-4</td>
<td>-upper 1 cm of sediment</td>
<td>once</td>
<td>- grain size analysis</td>
</tr>
<tr>
<td>February 1996-</td>
<td>1-4</td>
<td>-upper 5 mm of sediment</td>
<td>weekly</td>
<td>- species identification</td>
</tr>
<tr>
<td>April 1997</td>
<td></td>
<td></td>
<td></td>
<td>- photosynthetic parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- organic and water content of sediment</td>
</tr>
<tr>
<td>December 1997-</td>
<td>2</td>
<td>-sediment surface</td>
<td>monthly</td>
<td>- species identification</td>
</tr>
<tr>
<td>May 1998</td>
<td></td>
<td>-upper 1 mm of sediment</td>
<td></td>
<td>- pigment composition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-upper 10 mm of sediment</td>
<td></td>
<td>- oxygen production</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>- P vs. E parameters</td>
</tr>
<tr>
<td>24 March -</td>
<td>1-4</td>
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<td>once</td>
<td>- species identification</td>
</tr>
<tr>
<td>9 April 1998</td>
<td></td>
<td>-upper 1 mm of sediment</td>
<td></td>
<td>- pigment composition</td>
</tr>
<tr>
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<td></td>
<td>- P vs. E parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- vertical distribution of photosynthetic pigments</td>
</tr>
<tr>
<td>March 1998, July</td>
<td>2</td>
<td>-sediment surface</td>
<td>twice in</td>
<td>- short term changes of pigmenan composition and P vs. E parameters</td>
</tr>
<tr>
<td>August 1998</td>
<td></td>
<td></td>
<td>March</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>once in July and August</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1. Duration, location, frequency of sample programs and measurements carried out on Hythe saltmarsh and mudflat.

SAMPLING PROCEDURE FOR GRAIN SIZE ANALYSIS IN JANUARY 1996

In January, 1996 about 500g sediment samples of the upper 1cm were collected in triplicates with a spatula from each station and stored in plastic bags. Details for grain size analyses are given in section 2.2.
SAMPLING PROCEDURE BETWEEN FEBRUARY 1996-APRIL 1997

Details of sampling frequency and samples taken are summarised in Table 2.1. All four stations were sampled weekly at low tide. Field work commenced at station 4 one hour before low tide, followed by stations 3, 2 and 1, whereby station 1 was sampled at low tide. Sediment core samples of 5 cm depth were taken in triplicate using 2.75 cm diameter cut-off syringes. The lower 4.5 cm of the cores were cut off with a spatula and discarded. The top 0.5 cm sediment section was placed into a precooled petri dishes for pigment analysis. Approximately 20 ml of 0.5 cm thick surface scrapings were collected in duplicates using a spatula and placed in watertight 30 ml plastic vials for cell identification and determination of organic matter and water content. Temperature readings of the exposed sediment at 1 cm depth, shaded air and overlying water were taken at the edge of the marsh pool, using a mercury thermometer with precision of ± 0.5°C. The salinity of the overlying water was measured with a handheld refractometer. All sediment samples were stored in a coolbox and brought to the laboratory within 45 minutes. Subsamples intended for pigment analysis were stored in liquid nitrogen. Sediment samples for species identification were transferred to large petridishes and homogenised with a spatula. A thin layer of filtered sea water was added and the lid closed to avoid desiccation. The dishes were left to settle overnight.

SAMPLING PROCEDURE BETWEEN DECEMBER 1997- MAY 1998

Station 2 was sampled once a month at spring low tide. Stations 1 to 4 were sampled once between 23rd of March and the 9th of April, 1998. The details for the sampling program are summarised in Table 2.1. Four large cores (10cm diameter 15cm depth in black plastic) of undisturbed sediment were collected for pigment analysis and primary production measurements as described in sections 2.4 and 2.5. They were closed at both ends with petri dishes and placed into a cool box. The upper 1mm section of six cores (cut off syringe: 2.9 cm diameter) were divided into two petri dishes for the determination of photosynthetic parameters with 14C as described in section 2.5. Approximately 500g of 0.5 cm surface scrapings were collected with a spatula and stored in a plastic bag for the harvesting of motile microphytobenthos cells. For the dilution of the sediment suspension two litres of seawater were collected in a plastic bottle from the outgoing tide. All samples were transported in a cool box to the laboratory within 45 minutes.
In the laboratory samples for the individual measurements were stored and prepared in the following way:

- The large cores intended for oxygen micro-electrode measurements were stored in a dark sea water bath at 15°C and analysed within 48 hours of collection. After primary productivity measurements were completed, three subcores (cut off syringe: 2.9 cm diameter) were taken and sliced into five sections: 0-1mm, 1-2mm, 2-3mm, 3-5mm and 5-10mm. Each section was placed into a labelled cryo-vial and frozen in liquid nitrogen for later HPLC pigment analysis as described in section 2.4.

- The samples for 14CO2 uptake analysis were stored in the dark at 15°C and analysed within 24 hours as described in section 2.5.

- The surface scrapings were mixed with 0.3 l. of the seawater sample and poured out into a tray to settle for several hours. One hour before the onset of the next photoperiod two layers of lens-tissue were placed on top of the sediment. The upper layer was peeled off after three hours, placed into 0.5 litres of GF/F filtered sea water collected at the site and mixed gently to release the cells from the tissue. The water was poured through a 150μm mesh to retain the lens tissue. 10-30ml of the cell suspension was filtered onto 25mm GF/F filters in triplicate and frozen in liquid nitrogen for later HPLC pigment analysis. The remaining suspension of cells was used for species identification as described in section 2.3.
2.2 SEDIMENT ANALYSES

GRAIN SIZE ANALYSES

Three sediment subsamples of 100g were placed in a beaker with 200ml 0.1% calgon solution and ultrasonicated for 15 minutes to disperse the particles. Samples were wet sieved through a 63 μm mesh and both fractions were collected in preweighed beakers. They were dried in an oven at 80°C and then weighed. The <63 μm fraction was homogenised with mortar and pestle and a 2.5g subsample was mixed with 0.1% Calgon solution and placed in an ultrasonic bath for 15 minutes. The sample was then analysed in a Micromeritics sedigraph (5100) to measure grain size fractions smaller than 63 μm.

WATER AND ORGANIC CONTENT OF SEDIMENTS

Approximately 30g of wet sediment was placed into preweighed aluminium dishes and the exact weight recorded. The samples were dried in an oven for 48 hours at 80°C and reweighed. The organic content was determined by combustion of oven dried samples in a muffle furnace at 550°C for six hours.
2.3 SPECIES IDENTIFICATION

**Harvesting of Microphytobenthos for Species Identification:**

After the collected sediment had settled in petridishes overnight, 8 round coverslips were placed onto the sediment surface two hours before the following low tide and left for a minimum period of two hours (after Oppenheim, 1985). The coverslips were placed onto microscope slides and observed under a Leitz inverted microscope. The first 75 cells were identified and counted. This was repeated with one more coverslip from each station. In case of bloom conditions at least three different coverslips were counted. A bloom was defined as cell numbers > 50 in one viewing field using the 25X objective.

**Preparation of Permanent Slides:**

To facilitate diatom identification, some samples of diatom frustules were cleaned with nitric acid following the method of Oppenheim (1985). For this purpose a layer of lens tissue was placed between the sediment and the coverslips. After two hours the coverslips were placed into a 150ml Pyrex beaker. In a fume cupboard approximately 10 ml of concentrated nitric acid was added to each beaker and gently heated on a hot plate just below boiling point for 20 minutes. Once cooled the acid solution was transferred to 15 ml centrifuge tubes without the coverslips, sealed and centrifuged at 1000 rpm for 5 minutes. The supernatant was poured off and the pellet was resuspended in distilled water. The rinsing procedure was repeated 7 times.

Three drops of the cleaned material were placed onto a round coverslip; 1 ml of distilled water was added to disperse the material. The area was covered to avoid dust contamination and left until the water had evaporated. A drop of Naphrax diatom mount was placed onto a microscopic slide and warmed up on a hot plate. The coverslip was put on top, reheated and left to cool. The microscopic slides were observed under a Leitz inverted microscope as described above.
2.4 PIGMENT DETERMINATION: EXTRACTION AND SPECTROPHOTOMETRIC METHOD

EXTRACTION PROCEDURE FOR SEDIMENT SAMPLES

During the first sampling programme in 1996 wet sediment samples were extracted. A subsample of approximately 1g was weighed and placed into a glass centrifuge tube. The water content was determined beforehand and 100% HPLC grade acetone was added to make a final concentration of 90% acetone. In 1997/1998 the samples were freeze dried and then extracted. A sediment subsample of approximately 0.5g was weighed and extracted in 4ml of 90% HPLC grade acetone. All extracts were ultrasonicated for 30 seconds and centrifuged at 3000rpm for 15 minutes. The supernatant was filtered through a 0.2µm Nyalo membrane filter (Gelman). In 1996 all samples were analysed by HPLC and spectrophotometry. In 1997/1998 only HPLC analysis was carried out (For the comparison between spectrophotometer and HPLC data see Appendix I).

SPECTROPHOTOMETRIC DETERMINATION OF CHLOROPHYLL A AND PHAEOPIGMENTS IN SEDIMENTS

Chlorophyll a was determined spectrophotometrically after Lorenzen, 1967. Filtered pigment extracts were pipetted into 1cm glass cuvettes and the absorption was measured at 665 nm and 750 nm on a Cecil Ce1010 Spectrophotometer. At each wavelength the sample absorption was compared to a blank containing 90% acetone. Two drops of 10% HCl were added to the sample cuvette, mixed and the absorbance was remeasured at 665 nm and 750 nm. The 665 nm absorption value was corrected for turbidity by subtracting the value read at 750 nm. The concentrations of chlorophyll a (chl a) and phaeopigments (ppg) in µg per gram of sediment were calculated using the following formulae:

\[
\text{chl} a = \frac{26.7(E_{665B} - E_{665A})V}{Wd}
\]

(2.1)

\[
\text{ppg} = \frac{26.7(1.7E_{665B} - E_{665A})V}{Wd}
\]

(2.2)

where 26.7 is a constant (absorption coefficient of chlorophyll a in acetone), \(E_{665B}\) is the absorption value at 665nm minus that at 750nm, \(E_{665A}\) is the absorption at 665nm after
acidification minus that of 750nm, $V$ is the volume of the extract, $W$ is the weight of the sediment is grams and $d$ is the path length of the cuvette (1cm).
2.5 HPLC ANALYSES

BACKGROUND

The quantitative analysis of chlorophyll a is a universal tool in Oceanography to estimate the standing stock of microalgal biomass (Jeffrey et al., 1997). Conventional methods which measured chlorophyll a by absorbance (spectrophotometry) or by fluorescence (fluorometry) have led to inaccuracies because the absorption spectra of chlorophylls, their derivatives and degradation products overlap. Chlorophyll a was therefore wrongly predicted by up to 400% (Mantoura and Llewellyn 1983). To determine biomass more accurately, pigments in natural samples have to be chemically separated prior to detection. This is achieved by using high performance liquid chromatography (HPLC). HPLC not only provides a powerful tool to calculate chlorophyll a accurately, but by identifying and quantifying accessory pigments and degradation products, it further allows the determination of the taxonomic composition of algae present in the sample and adds information about their physiological status (Millie et al., 1993).

METHOD OUTLINE

Pigments were separated by ion pairing reverse phase HPLC as described by Mantoura and Llewellyn (1983) and modified by Barlow et. al. (1993a) using a Perkin Elmer C18 column (3μm particle size -3.5 * 0.45 cm) and a Thermoseparation HPLC system with an online vacuum degasser, a dual solvent pump (P2000), an autosampler (AS3000), a UV detector (UV1000), a fluorometer (FL3000), integrator (SN4000) and integration software PC1000. Ammonium acetate was used as an ion pairing reagent, because separation of the acidic compounds in the pigment mixture is poor under normal conditions due to the anionic character of the carboxyl group, which is dissociated at neutral pH. The ion pairing reagent prevents dissociation, thus allowing separation of pigments not possessing a phytol group (chlorophyllids and phaeophorbides) at pH 7 (Zapata et al., 1987). Pigment extracts were loaded into the autosampler which retained a temperature of 0°C. 500μl sample aliquots were mixed with 500μl 1mol ammonium acetate and 100μl of the mixture was injected onto the column. The mobile phase consisted of a binary eluant system with solvent A (80% methanol and 20% 1M ammonium acetate) and solvent B (60% methanol and 40% acetone). A linear gradient at a flow rate of 1 ml per minute was run from 0% to 100% B for 10 minutes and followed by an isocratic stop at 100% B for 7.5 minutes. A second gradient of 2.5 minutes was used to return to the initial condition of 100% solvent A. Carotenoids were detected by
absorbance at 440nm, chlorophylls and degradation products were detected by absorption at 440nm as well as by fluorescence with excitation at 410nm and emission at wavelengths > 670nm.

IDENTIFICATION OF CHLOROPHYLLS AND CAROTENOIDS

Chlorophyll a, chlorophyll b and β-carotene peaks were identified by injecting commercially available standards (Sigma) and noting the retention times. Chlorophyll a standards were run every 12 samples to monitor variation in retention times during sample analyses.

Three approaches were taken to identify accessory pigments accurately:

- At the beginning of the study, a set of sediment samples from Hythe mudflat was analysed by HPLC with an online photodiode array using the method described by Barlow et al. (1993a) at the Quintiles laboratory, Hereford. This produced absorption spectra for each individual peak which could then be matched to published spectra. The method allowed a positive identification of each pigment present in the sample and further clarified problems with overlapping peaks and compounds of similar retention times. It was important to obtain information about the absorptive properties of each peak in a Hythe sediment sample, since pigments can be chemically transformed in sediments to produce compounds with similar molecular characteristics and retention times (Repeta and Gagosian, 1982).

- Throughout the study filtered samples of reference algae, containing well documented pigment compositions were analysed. Their chromatograms were compared to published data, pigments identified and the retention times noted on the HPLC used in this study (see Table 2.2).

- At the end of the study the pigments standards chlorophyll c1c2, peridinin, fucoxanthin, 19’hexanoyloxyfucoxanthin, diadinoxanthin, zeaxanthin and lutein were obtained from VKI Denmark, injected and their retention time noted.
Table 2.2 shows pigment retention times, reference cultures and commercial standards used in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Algal group</th>
<th>Pigments</th>
<th>Retention time in this study</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All species</td>
<td></td>
<td>chlorophyll a</td>
<td>10.3 min</td>
<td>Standard injection</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>Diatom</td>
<td>Chlorophyll c1c2</td>
<td>2.2 min</td>
<td>deProenca, 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fucoxanthin</td>
<td>4.3 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diadinoxanthin</td>
<td>5.75 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoxanthin</td>
<td>6.7 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b carotene</td>
<td>12.1 min</td>
<td></td>
</tr>
<tr>
<td>Syneecoccus</td>
<td>Cyano bacteria</td>
<td>Zeaxanthin b carotene</td>
<td>7 min</td>
<td>Wright et al, 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.1 min</td>
<td></td>
</tr>
<tr>
<td>Amphidinium carterae</td>
<td>Dino flagellate</td>
<td>Chlorophyll c1c2</td>
<td>2.2 min</td>
<td>Wright et al, 1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peridinin</td>
<td>2.9 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diadinoxanthin</td>
<td>5.75 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diatoxanthin</td>
<td>6.7 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>b carotene</td>
<td>12.1 min</td>
<td></td>
</tr>
<tr>
<td>Tertraselmis suecica</td>
<td>Green algae</td>
<td>Diadinoxanthin chlorophyll b b carotene</td>
<td>5.75 min</td>
<td>Barlow et al, 1993a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.3 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.1 min</td>
<td></td>
</tr>
<tr>
<td>Emiliania huxleyi</td>
<td>Coccolithophore</td>
<td>Chlorophyll c3</td>
<td>1.3 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19'hexanoyloxy</td>
<td>4.83 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fucoxanthin</td>
<td>2.1 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ß carotene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>lutein</td>
<td>7 min as zeaxanthin</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2. Identification of the main photosynthetic pigments, their retention times and reference cultures they occur in, pigments that were also obtained as commercial standards are printed in bold.
Quantification of chlorophylls and carotenoids

Standard solutions of chlorophyll a and chlorophyll b (Sigma) were made up in 90% HPLC grade acetone with concentrations ranging from 250\mu g l\(^{-1}\) to 2 mg l\(^{-1}\). The chlorophyll concentration in each solution was determined spectrophotometrically according to Jeffrey and Humphrey (1975). Absorption values at 630nm, 647nm and 664nm were measured and corrected for turbidity by subtracting the reading at 750nm. Chlorophyll a and b concentrations in mg l\(^{-1}\) were calculated using following formula:

\[
C(\text{Chl } a) = 11.85 E_{664} - 1.54 E_{647} - 0.08 E_{630} \tag{2.3}
\]

\[
C(\text{Chl } b) = 21.03 E_{647} - 5.43 E_{664} - 2.66 E_{630} \tag{2.4}
\]

where \(E_{664}\) is the absorption value at 664nm minus that of 750nm, \(E_{647}\) is the absorption value at 647nm minus that of 750nm and \(E_{630}\) is the absorption value at 630nm minus that of 750nm.

Carotenoid standard solutions from VKI were supplied in ethanol with predetermined concentrations. Owing to the cost and small volume (2.5 ml) supplied, only one dilution was carried out. B-carotene from Sigma was dissolved in 90% acetone and its concentrations in g l\(^{-1}\) was determined using the formula given in Jeffrey et al., 1997:

\[
C(\text{\beta-carotene}) = A_{\lambda_{\text{max}}} (0.1 E^1_{1\%} 1cm d)^{-1} \tag{2.5}
\]

where \(A_{\lambda_{\text{max}}}\) is the absorbance at \(\lambda_{\text{max}}\), \(E^1_{1\%} 1cm\) is the 1% extinction coefficient published in Jeffrey et al., 1997) and \(d\) is the cuvette path length (1 cm).

Response factors \(f_p\) for each pigment were calculated by converting standard concentrations from mg l\(^{-1}\) to ng per column and plotting them against peak area:

\[
f_p = \frac{\Delta A_p}{\Delta W_p} \tag{2.6}
\]

where \(A_p\) is the peak area detected at 440nm and \(W_p\) is the weight of pigment on the column in ng.
After the analysis of samples with HPLC pigment concentrations in µg per gram of sediment were calculated using following equation:

\[ C = \frac{A_p V}{W f_p B 100} \]  

(2.7)

where \( A_p \) is the peak area detected at 440nm, \( V \) is the extract volume in ml, \( W \) is the dry weight of sediment in gram, \( f_p \) is the response factor and \( B \) is the buffer dilution factor (0.5).

**IDENTIFICATION OF PHAEOPIGMENTS**

To identify phaeopigments, faecal pellets from *Artemia salina* cultures, fed on *Tertraselmis svecica* and *Phaeodactylum tricornutum*, were filtered onto 2.5 cm GF/F filters, extracted in 90% acetone, sonicated, analysed by HPLC and detected by online fluorescence. Phaeophorbides eluted prior to chlorophyll a and phaeophytins after chlorophyll a (Barlow, 1993b). Since several phaeophorbide-like and phaeophytin-like components were present in the sample they were numbered in order of elution. Table 2.3 shows the phaeopigments and their retention times found in this study.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Retention time</th>
<th>Identification source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>chlorophyllide a</td>
<td>1.3 min</td>
<td>copepod faecal pellet</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophorbide a1</td>
<td>3.1 min</td>
<td>copepod faecal pellet</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophorbide a2</td>
<td>4.1 min</td>
<td>copepod faecal pellet</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophytin a1</td>
<td>11.7 min</td>
<td>copepod faecal pellet</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophytin a2</td>
<td>12.5 min</td>
<td>copepod faecal pellet</td>
<td>Barlow et al., 1993b</td>
</tr>
</tbody>
</table>

Table 2.3. Identification of phaeopigments, their source and their retention times from fecal pellets of *Artemia salina* cultures.
QUANTIFICATION OF PHAEOPIGMENTS

The fluorometer was calibrated against the UV detector by analysing four different concentrations of faecal pellets at an absorbance setting of 670nm and measuring their simultaneous absorbance and fluorescence. Hence the extended Beer Lambert law was used with extinction coefficients presented in Table 2.5 to calculate the concentration for each phaeopigment in μg per gram of sediment:

\[ C = \frac{A_f V f_i}{W B E_{670}} \]  \hspace{1cm} (2.8)

where \( A_f \) is the fluorescence peak area divided by the response factor of the fluorometer as shown in Table 2.5, \( V \) is the volume of extract in ml, \( f_i \) is the response factor for the UV detector, \( W \) is the weight of dry sediment in grams, \( B \) is the dilution factor (0.5) and \( E_{670} \) is the specific extinction coefficient as shown in Table 2.4.

<table>
<thead>
<tr>
<th>Phaeopigment</th>
<th>Response factor for fluorometer</th>
<th>( E_{670} )</th>
<th>Refernce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaeophorbide ( a1 )</td>
<td>15.531</td>
<td>69.8</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophorbide ( a2 )</td>
<td>12.501</td>
<td>69.8</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophytin ( a1 )</td>
<td>14.831</td>
<td>49.5</td>
<td>Barlow et al., 1993b</td>
</tr>
<tr>
<td>Phaeophorbide ( a2 )</td>
<td>13.203</td>
<td>49.5</td>
<td>Barlow et al., 1993b</td>
</tr>
</tbody>
</table>

Table 2.4. Response factors and extinction coefficients for phaeopigments used in this study.
2.6 MEASUREMENT OF OXYGEN PRODUCTION USING OXYGEN MICROELECTRODES

PRINCIPLE OF METHOD

Oxygen microsensors with a tip diameter of a few μm have been developed to monitor oxygen production in sediments at a high spatial resolution (Revsbech et al., 1981). Vertical profiles of photosynthetic activity are measured at a resolution of 100 μm. The method is based on the following assumptions:

- The steady state oxygen concentration in sediments during light incubation is a balance between the production of oxygen by phototrophic and autotrophic organisms, the consumption of oxygen due to respiration and the net diffusion of oxygen into other layers of the sediment.
- The rates of respiration and diffusion do not change in the first few seconds after darkening the sediment.
- The decrease in oxygen concentration in the first few seconds after darkening the sediment is only due to the cessation of photosynthesis.

The rate of gross photosynthesis in mmol of O₂ m⁻² h⁻¹ can therefore be expressed as the negative rate of oxygen decrease in the first seconds after darkening the sediment. O₂ measurements were carried out with an O₂ measuring system from MasCom Analytical Instruments, Bremen. The experimental setup and technical specifications are summarised in Fig. 2.3.
CALIBRATION OF $O_2$ MICRO-ELECTRODES

Seawater of the same salinity as the collected sediment was filtered through GF/F and poured into a semi-enclosed calibration beaker containing an aeration stone. The water was temperature equilibrated at 15°C and aerated for 30 minutes. The exact temperature and salinity was measured with a salinity and temperature probe (WTW Multiline P4 and TetraCon 325 sensor). The saturated oxygen concentration was then measured using the electrodes. Oxygen concentration in mmol per kg of water was determined according to Garcia and Gordon (1992):

$$\mu\text{mol} \ O_2 \ \text{kg} \ H_2O^{-1} = (5.80818 + 3.20684 \ * \ T + 4.1189 \ * \ \ln(298.15-T) \ ^3 \ * \ \ln(298.15-T) + 4.93845 \ * \ \frac{1}{(273.15+T)^2} + 4.93845 \ * \ \ln(298.15-T) \ * \ \frac{1}{(273.15+T)^2} + 1.01567 \ * \ \ln(298.15-T) \ * \ \frac{1}{(273.15+T)^4} + 1.41575 \ * \ \ln(298.15-T) \ * \ \frac{1}{(273.15+T)^5} ) \ * \ Sal$$

(2.9)

Where $T$ is the temperature and $Sal$ is the salinity of the water.

The anaerobic layer of the sediment was used as a zero oxygen concentration baseline.
**MICROELECTRODE SET UP**

**Fig. 2.3.** O₂ micro-electrode setup and technical specifications.
**Vertical Profiles of Oxygen Concentration:**

Two hours before the next daytime low tide, sediment cores were placed into a water bath and temperature and light equilibrated for 30 minutes (15°C, 550 μEm²s⁻¹ of white light). The electrode was lowered with a micro-manipulator to the surface of the sediment, as determined with a magnifying glass. Data were collected at a rate of one reading per 100 milliseconds. A 5-second integrated reading was taken at each 100μm interval. The integrated value in μmol O₂ kg H₂O⁻¹ consisted of the average value during 3 seconds (30 datapoints) between the 3rd and the 5th second.

**Vertical Profiles of Photosynthesis**

Depth profiles of gross photosynthesis were determined by the light-dark shift method (Revsbech and Jørgensen, 1983). Sediment cores were temperature and light equilibrated as described above. Oxygen concentrations at 100 μm intervals were measured for 15 seconds after which the sediment was darkened for 15 seconds by covering the light source. The micro electrodes used in this study had a 90% response time of <2 seconds. For this reason the rate of gross photosynthesis was taken as the negative rate of oxygen decrease between the 3rd and the 4th second after darkening the sediment. It has been experimentally shown that respiration and diffusion rates do not change in the first 4.6 seconds after darkening the sediment, when gross photosynthesis is integrated over all depths (Glud et al., 1992). However, within the first three seconds the vertical distribution of photosynthesis is strongly affected by changes in the diffusion fluxes. Due to the comparatively slow response time of the electrodes used in this study it was decided to integrate production rates over all depths (always <1 mm) and not to present vertical profiles of photosynthesis. Three photosynthetic profiles in each of two sediment cores were measured, depth integrated and averaged. The noise of the slope approximated to 100 nmols O₂ (kg H₂O)⁻¹, using a signal to noise ratio of 2 to 1, only slope readings above 200 nmols O₂ (kg H₂O)⁻¹ were included in the depth integration. Following the production measurements, subcores for vertical profiles of pigment concentration were taken at 1 mm intervals as described in 2.2. The core sections were weighed before and after freeze drying to determine the water content of each section.
Gross photosynthesis in mmol O\textsubscript{2} m\textsuperscript{-2} h\textsuperscript{-1} for the upper 1 mm of sediment was calculated according to following formula:

\[ GP = 36\left( \sum \frac{P_{100}H}{10} \right) \]  \hspace{1cm} (2.10)

where \( P_{100} \) is the production of \( \mu \)mols O\textsubscript{2} kg H\textsubscript{2}O\textsuperscript{-1} 100 msec\textsuperscript{-1} in 100\mu m sediment sections and \( H \) is kg of H\textsubscript{2}O m\textsuperscript{-2} in the upper 1 mm; the multiplication by 36 is derived from the conversion of 100 msec to hour and the conversion of \( \mu \)mols O\textsubscript{2} to mmol O\textsubscript{2}. The H\textsubscript{2}O content in 1 m\textsuperscript{3} of 1mm depth was calculated by determining the water content of 1mm core sections (cut off syringe: 2.9 cm diameter) in triplicate as described in section 2.2 and scaling it to 1m\textsuperscript{2}. 

44
2.7 DETERMINATION OF PHOTOSYNTHETIC PARAMETERS IN SEDIMENT USING $^{14}$C LABELLED SODIUM BICARBONATE

OVERVIEW

$P$ vs. $E$ curves were constructed for the upper 1 mm of the sediment after measuring the $^{14}$CO$_2$ uptake of microphytobenthos in a photosynthetron (Lewis and Smith, 1983) according to MacIntyre and Cullen (1995). During each month of the 1997/98 sampling program at station 2 and for each station in March/April 1998 duplicate $P$ vs. $E$ incubations were carried out in sequence whereby the first incubation was carried out 30 minutes before the next daytime low tide and the second incubation at time of low tide.

PROTOCOL

The upper 1 millimetre sections of three sediment cores (cut off syringe, diameter: 2.95cm) were mixed with 150ml of GF/F filtered sea water collected at the site and suspended with a magnetic stirrer. 30ml of the suspension were transferred into a smaller beaker with a glass bulb pipette for $^{14}$C incubation. A further three 10ml subsamples were taken with a glass volumetric pipette, filtered onto 4.7 cm GF/F filters and frozen in liquid nitrogen for later HPLC pigment analysis. The remaining solution was filtered through GF/F and used for alkalinity measurements.

ALKALINTITY MEASUREMENTS

To measure total CO$_2$ content of the sample, a one point acid titration was carried out as described in Parsons et al. (1984). Salinity of the sample was measured with a salinity probe (WTW Multiline P4 and TetraCon 325 sensor). Three 20ml samples were pipetted into 100ml tall glass beakers using a glass volumetric pipette. The initial pH and temperature were measured in the samples with a Mettler Delta 350 pH metre. 7ml of 0.01N HCl (diluted from 0.1N Sigma standard) was added to each beaker using an automatic Metrohm 665 dosimat with a 10ml burette. pH and temperature were measured again after the addition of acid. Results were entered into a spreadsheet developed by D. W. Crawford to calculate total CO$_2$ content in sea water.
While stirring in subdued light, 100 μl of sodium $^{14}$C bicarbonate was added to the 30ml solution to give a final activity of 0.5 μCu ml$^{-1}$. 1ml aliquots were dispensed into 27 30ml glass scintillation vials. A photosynthetron with 25 light levels was constructed with a metal grid, which contained LEE neutral density light filters, ranging between 6.6 and 69% transmission. The photosynthetron was placed in a 15°C water bath that was illuminated from below by white fluorescent tubes. Irradiance levels in μEm$^{-2}$s$^{-1}$ were determined with a Licor light meter and ranged between 6 and 2000 μEm$^{-2}$s$^{-1}$. The incubation setup is shown in Fig. 2.4. Two samples were used as zero time and fixed immediately after dispersion. 25 samples including two dark ones were incubated at known light levels for 30 minutes at 15°C. Total activity was measured by subsampling 100μl of slurry in triplicates into scintillation vials and adding 100μl of phenylethylamine and 10ml of Ultima Gold XR scintillation cocktail (Packard). Following the incubation, inorganic carbon was expelled from each vial by adding 0.25ml of 6N hydrochloride acid and leaving the vials open in a fume cupboard for 5-6 hours. Prior to counting, the samples were neutralised with 0.25 ml of 6N NaOH. 10ml of scintillation cocktail was added and the vials were counted for 60 seconds on a Wallac 1411 scintillation counter. Counts were corrected for quenching. $^{14}$C counting efficiency was above 85% at all times.

![Fig. 2.4. Experimental setup for $^{14}$C incubation.](image-url)
CONSTRUCTION OF PHOTOSYNTHESIS VERSUS IRRADIANCE CURVES

Photosynthetic rate in μg C ml⁻¹h⁻¹ was calculated according to Parsons et al. (1984) from:

\[ P_C = \frac{(R_t - R_b) W}{R N} \]  \hspace{1cm} (2.11)

where \( R \) is the total activity of bicarbonate added to each sample, i.e. dpm of subsample *10, \( N \) is the total hours of incubation (0.5), \( R_s \) is the sample count (dpm) corrected for quenching, \( R_b \) is the average dpm count of two dark incubations, \( W \) is the weight of total CO₂ present in mg C as determined by the expression \( W = 12/1000 \text{TC} \) where \( \text{TC} \) is total carbon dioxide in μmol of CO₂.

Photosynthetic rate per ml slurry was converted into μg C per μg of chlorophyll \( a \) and plotted against irradiance using Sigma Plot. A non-linear regression was used to obtain \( \alpha^B \) and \( P_{max}^B \) according to Platt et al. (1980) where:

\[ f = P_{max}^B \times (1 \times \exp(-x \times \alpha^B / P_{max}^B)) \]  \hspace{1cm} (2.12)

where \( f \) is the rate of photosynthesis in μg C ml⁻¹h⁻¹, \( \exp \) is the exponential and \( x \) is irradiance in μEm⁻²s⁻¹.

DETERMINATION OF PHOTOSYNTHETIC RATE IN THE FIRST 20 MINUTES OF ILLUMINATION

To examine the change in photosynthetic rate during the first 20 minutes of exposure to saturating irradiance, 30 subsamples of 1ml algal suspension containing 2 μCu of ^14CO₂ bicarobonate were left to equilibrate for 5 minutes in the dark. They were then incubated at 550 μE m⁻² s⁻¹ during the onset of the natural photoperiod. ^14CO₂ uptake was measured for the first 20 minutes, during which time 1 ml sub-samples were successively removed every 30 seconds for the first 5 minutes and every 60 seconds thereafter and preserved with 150 µl of formaldehyde (after MacIntyre and Geider, 1996).
EXPERIMENTAL DESIGN FOR THE DETERMINATION OF SHORT-TERM CHANGES IN PHOTOSYNTHETIC PARAMETERS AND CELLULAR PIGMENT COMPOSITION

The short-term changes in photosynthetic parameters and cellular pigment composition were examined with a series of laboratory experiments. Designs of the individual experiments are given in Chapter 6, prior to the presentation of results.
2.8 CULTURING METHODS

ISOLATION OF BENTHIC MICROALGAE

The three benthic diatom species Cylindrotheca closterium (Ehrenb.) Reinmann & Lewis, Navicula gregaria Donk. and Entomoneis paludosa W. Smith were isolated and cultured for the use of photo-physiological experiments. Benthic microalgae were harvested as described in 2.1 and suspended in autoclaved GF/F filtered sea water. A dilute sample was observed in a sterile petridish under a Leitz inverted microscope. With an autoclaved micro glass pipette made from a pasteur pipette several cells from one species were collected and transferred into a second petridish. The process was repeated and the cells were diluted with a few drops of autoclaved sea water during each transferral. Isolated cells were checked for purity, immersed in more autoclaved sea water and placed into a culture incubator at 15°C at 150μEm²s⁻¹. After approximately 10 days the cells were observed under the microscope and the process of isolation was repeated using sterile petrides. Growth and isolation procedure was continued for three months until three monocultures of the species Cylindrotheca closterium, Navicula gregaria and Entomoneis paludosa were obtained.

MAINTENANCE OF CULTURES

The five planktonic species Phaeodactylum tricornutum Bohlin., Amphidinium carterae Hulburt., Tetraselmis suecica (Kylin) Butcher., Emiliania huxleyi (Lohmann) Hay & Mohler. and Synecoccus sp. were kept in culture as reference cultures for HPLC pigment chromatograms. Cultures were incubated at 15°C at 150μEm²s⁻¹ on a light/ dark cycle of 12/12 hours. The media recipe for the cultures was based on Keller’s recipe (Stein, 1975). All chemicals were sterile filtered through a 0.5μm millipore filter. Cells were subcultured every 4 weeks in a sterile hood under sterile conditions.
2.9 STATISTICAL ANALYSIS

Table 2.5 summarises the statistical tests applied in this study. Data were normally distributed or log10 or ln transformed prior to statistical analysis. Further details on the transformations of data and verifications of the tests are given in the relevant result sections. All tests were performed in Systat or Sigma Stat.

<table>
<thead>
<tr>
<th>Chapter and section</th>
<th>Null hypothesis</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3</td>
<td>no significant differences in chl a concentrations between stations and seasons</td>
<td>two way ANOVA followed by a Tukey multiple comparison of means</td>
</tr>
<tr>
<td></td>
<td>no significant interaction between station and season in determining the physical factors that control chl a concentrations</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td></td>
<td>no environmental factors controlled chl a concentrations at different stations and seasons</td>
<td>Backwards stepwise multiple regression</td>
</tr>
<tr>
<td></td>
<td>the spatial and temporal distribution of accessory pigments and pheopigments was not dependent on chlorophyll a</td>
<td>Linear regression</td>
</tr>
<tr>
<td>4.2 - 3</td>
<td>no significant differences in pigment concentrations with depth of sediment</td>
<td>One Way ANOVA + Bonferroni multiple comparisons</td>
</tr>
<tr>
<td></td>
<td>the depth distribution of accessory pigments and pheopigments was not dependent on chlorophyll a</td>
<td>Linear regression</td>
</tr>
<tr>
<td>5.2 - 3</td>
<td>no significant differences in chl a concentration, pigment composition, O₂ production, chl a normalised O₂ production, P_b max and a_b between the months or between the four stations</td>
<td>One Way ANOVA + Bonferroni</td>
</tr>
<tr>
<td></td>
<td>the spatial and temporal variations of chl a concentration, O₂ production, chl a normalised O₂ production, P_b max, a_b and I_b were not dependent on temperature, irradiance or photoperiod</td>
<td>Linear regression</td>
</tr>
<tr>
<td></td>
<td>there was no correlation between the chl a normalised O₂ production and P_b max measured by ^14CO₂ uptake</td>
<td>Pearson product-moment correlation</td>
</tr>
<tr>
<td>6.2</td>
<td>no significant differences in chl a concentration, pigment composition, P_b max and a_b during one photoperiod</td>
<td>One Way ANOVA + Bonferroni</td>
</tr>
<tr>
<td></td>
<td>no significant differences in pigment composition with time and irradiance</td>
<td>Two way repeated measurement ANOVA + Bonferroni</td>
</tr>
</tbody>
</table>

Table 2.5. Statistical tests performed in this thesis. Data were normally distributed or log10 or ln transformed. Further details are given in the relevant result sections.
CHAPTER 3

COMPOSITION AND BIOMASS VARIATION OF MICROPHYTOBENTHOS
ALONG AN INTERTIDAL TRANSECT ON HYTHE MUDFLAT

3.1 INTRODUCTION

Benthic microalgae inhabiting the intertidal zone are exposed to physical and chemical gradients, which fluctuate strongly on both temporal and spatial scales. Accordingly, biomass of microphytobenthos is highly variable on both scales, as has been demonstrated by a large number of studies (see review of MacIntyre et al., 1996). Our understanding of the factors that control the dynamics of benthic microalgal biomass in intertidal habitats is, however, limited because the majority of seasonal studies to date have used comparatively large time intervals (eg. monthly) between sampling. This infrequent sampling has restricted analysis of microalgal biomass fluctuations in relation to long term seasonal changes in physical factors and has excluded fluctuations over shorter time periods.

In this chapter results from a seasonal study are presented to show the influence of environmental factors on microalgal biomass on a mudflat in a macrotidal estuary. Samples were collected weekly for a year to examine the influence of seasonal changes in irradiance, temperature and other meteorological factors. A weekly sampling interval was chosen to investigate the influence of the short-term changes in light availability and length of photoperiod depending on the tidal cycle. For this purpose a simple tidal model including hours of tidal emersion, daylength and incident irradiance was constructed. Four different stations were sampled along an intertidal transect to give an insight into the compromise between light availability, physical disturbances and environmental stresses in the form of desiccation, elevated salinity and high irradiance levels. Photosynthetic pigment determination by HPLC in conjunction with taxonomic analysis were used to examine seasonal and spatial changes in the composition of microphytobenthos.
3.2 VARIATION OF ENVIRONMENTAL FACTORS ON HYTHE MUDFLAT, 1996

ANNUAL TEMPERATURE VARIATION ON HYTHE MUDFLAT

Maximum and minimum temperatures showed a seasonal trend with strong fluctuations on a daily basis (Fig. 3.1.a). The annual average air temperature measured on sample dates on Hythe mudflat was 13.9°C. The same values were obtained for the average water temperatures of water pools at station 4 and the mudflat itself. The average sediment temperatures were slightly higher, being 14.0°C on the mudflat and 14.1°C in the marsh pool. Maximum temperatures on sample dates at Hythe were recorded on the 13th of June with 26°C air temperature and 27°C water temperature. The sediment on the marsh reached 30°C on the same day. Minimum temperatures occurred on the 29th of February being 5°C in the air and 6°C in the water (Fig. 3.1.a).

ANNUAL IRRADIANCE VARIATION

Global solar radiation data in W m²d⁻¹ were obtained from the Meteorological Office in Everton (50.74°, -1.75°; 12 miles south west of Hythe mudflat) for the year 1996. Seasonal variation of irradiance is shown in Figure 3.1.b. Maximum irradiance on sample dates coincided with the temperature maximum on June 13th (Fig. 3.1.a). The minimum values occurred on March the 20th.
Figure 3.1. (a) Daily maximum and minimum air temperatures at Mayflower Park, Southampton 1996 (obtained from the Meteorological Office) with superimposed air, water, marshpool and sediment temperatures measured on sampling dates at Hythe. Sediment temperatures were measured at a depth between 0-1 cm, air temperature was measured with a shaded thermometer, (b) Daily solar radiation levels in Everton, 50.74°, -1.57° (nearest available source) in W m⁻²s⁻¹, measured from 9am - 9 am; * = sampling date (data obtained from the Meteorological Office).
TIDAL PATTERN IN SOUTHAMPTON WATER

Southampton Water is exposed to a very complex tidal pattern, exhibiting a double high tide, a young flood stand and rapid ebb flows (Webber, 1980). These patterns are a result of the prevailing tidal regime in the English Channel and the sheltering effect of the Isle of Wight. The English Channel can be seen as a stationary wave compartment undergoing a natural period of oscillation, whereby the dominant force is the semidiurnal tide of the North Atlantic. High tide at Land's End coincides with low tide in Dover and vice versa. Half way through the channel, i.e. at the centre of its major axis theoretically no variations in water height should be expected. However a significant decrease in channel depth at this point due to the Cherbourg peninsula and the Isle of Wight causes a resistance in tidal flow resulting in a quarter diurnal oscillation. Both oscillations combine causing a double high tide within the area bound by LeHavre, Cherbourg, Littlehampton and Portland. Within the Solent the cotidal lines lie very close together and since the western entrance to the Solent is close to the main axis of the channel, its tidal range is only half that of the eastern entrance. As high water at Nab head (eastern entrance) is only one hour later than at the Needles (western entrance) there is a current flowing in the eastern direction at low tide. The flow is reversed when the tide at Nab head overtakes the tide at the Needles, causing a slackening of water three hours after low tide (Webber, 1980). This phenomena is called a young flood stand and lasts for about 2 hours after which flood currents flow for a further two hours. A seven hour flood tide combined with a double high water of two hours result in an ebb tide of only 3.5 hours duration which means that the ebb tidal currents have twice the velocity of the flood currents. Tidal range varies from 1.98 m during neap tides to 4.98m during spring tides (Fig. 3.2.a) (data from ABP tidal prediction table).

TIDAL MODEL FOR HYTHE MUDFLAT

To examine the effect of incident irradiance during tidal exposure on benthic microalgal biomass variation a simple model was constructed which included the emersion times at the four stations, length of daylight and incident irradiance.

Tidal emersion at the four stations: The tidal amplitude curve for Southampton Water expressed in mean tidal height was obtained from the program “Tidal Synthesis and Prediction” by Nunes Vaz, 1998. The four stations at Hythe mudflat were levelled and height
was converted from Ordnance Datum to mean tidal height (ABP Southampton Tide Table, 1996). Fig. 3.2.b shows the profile of the mudflat in relation to mean tidal height.

**Length of daylight and incident irradiance:** To model daylight, daily variation of the solar declination angle $\delta$ was calculated using the following formula (Spencer, 1971):

$$
\delta = 0.39637 - 22.9133 \cos \psi + 4.02543 \sin \psi - 0.3872 \cos 2\psi + 0.052 \sin 2\psi
$$

(3.2)

where $\psi$ is the date expressed as an angle ($\psi = 360^\circ d/365$; $d =$ day number ranging from January 1st to December 31st).

The hourly variation of solar elevation $\sin \beta$ was calculated from the following formula (Kirk, 1994):

$$
\sin \beta = \sin \gamma \sin \delta - \cos \gamma \cos \delta \cos \tau
$$

(3.3)

where $\gamma$ is the latitude and $\tau$ is $360^\circ t/24$ ($t$ being the time elapsed since 00.00h in hours).

During daytime $\sin \beta$ is $> 0$ and during night time $\sin \beta < 0$. When $\sin \beta$ was $> 0$, hourly average values of solar radiation during daylight were calculated by dividing the daily values of solar radiation obtained from Everton by the number of daylight hours per day. The number of daylight hours per day ($DL$) were calculated according to Sharples (1999):

$$
DL = 12 + 4 \sin (0.0172 t - 1.377)
$$

(3.4)

where $t$ is the number of day (1 - 365).

The model was run at hourly intervals for the year 1996. Hours were counted when tidal height $\leq$ station height and $\sin \beta \geq 0$. Hours of daylight exposure 24 hours prior to sample dates ranged from 0 to 8 at Stations 1 and 2, 2 to 11 at station 3 and 6 to 18 hours at station 4 (Fig 3.2.c). The annual average of daylight exposure in 24 hours was 3 hours at Station 1; 3.8 hours at Station 2; 5.6 hours and 11.3 hours at station 3 and 4 respectively.
Figure 3.2(a) Mean neap and spring tide in Southampton Water relative to mean tidal height (ABP Southampton tide time table); (b) vertical profile of Hythe saltmarsh and mudflat relative to mean tidal height, \( * \) = levelling points, \( o \) = levelled station positions; (c) hours of daylight exposure during 24 hours prior to sampling dates at the four stations of Hythe mudflat, generated by the tidal model.
ANNUAL SALINITY VARIATION ON HYTHE MUDFLAT

Southampton Waters is classified as a partially mixed estuary (Dyer, 1973). In the region of the estuary bordering Hythe mudflat, salinity ranged between 29.7 and 33.3 in 1996, as shown in Figure 3.3.a (SONUS nutrient data from Hydes and Wright, 1999). The intertidal zone of Hythe mudflat was exposed to additional fluctuations in salinity due to tidal inundation and emersion. Aerial exposed sediment experienced a decrease in salinity during periods of precipitation and an increase during periods of evaporation. The annual average salinity on sampling dates, measured with a hand-held refractometer, was 29 on Hythe mudflat and 30 in the marshpool at station 4. Maximum salinities between 34 and 40 were recorded from June until August and coincided with high air temperatures and periods of low precipitation. During periods of high precipitation (Fig. 3.3.a), salinity decreased to give minimal values of 10 on the mudflat and 20 in the marshpool (Fig. 3.3.b).

NUTRIENT DATA

Nutrient data for Southampton Water in 1996 was obtained from the SONUS data set (Hydes and Wright, 1999). NO$_3^-$, NO$_2^-$, NH$_4^+$, Si(OH)$_4$ and PO$_4^{3-}$ concentrations in Southampton Water between Weston and Hound (estuary section adjoining the saltmarsh) were selected. Si(OH)$_4$, NO$_3^-$ and NH$_4^+$ showed strong seasonal variations. Maximum concentrations occurred between January and March, followed by a decrease in the summer months and a further increase in October (Fig. 3.4).
Figure 3.3 (a) Daily rain fall measured at Mayflower Park, Southampton, 1996 (obtained from the Meterological Office). ● = sample dates; (b) salinity in Southampton Water between Weston and Hound measured at monthly intervals (SONUS data set, Hydes and Wright, 1999) and on Hythe mudflat and marshpool measured on sampling dates in 1996 in surface pools of the sediment.
Figure 3.4: Monthly nutrient concentrations sampled between Weston and Hound, Southampton Water, 1996, * = Nutrient concentration in μM, • = salinity (obtained from the SONUS data set, Hydes and Wright, 1999).
GRAIN SIZE AND PATTERN OF SEDIMENTATION

The substratum of Southampton Water is dominated by mud and sandy mud, with some peat, sands and gravel from a relict gravel terrace (Dyer, 1980). Cohesive sediment extends into the intertidal zone giving rise to extensive mudflats on the west side of Southampton Water and the eastern part excluding sites of local erosion which result in the exposure of clay and gravel phases. The sediment type at all four stations of Hythe intertidal mudflat could be classified as silty clay (Fig.3.5.a). The proportion of particles > 63 μm did was not comprised of sand but shell fragments from an adjacent cockle bed.

WATER CONTENT OF SEDIMENT

Average values of water content in sediment decreased landwards with values of 60.5%, 58.2%, 55.9% and 55.4% for stations 1, 2, 3 and 4 respectively. High water content was detected in sediments of station 1 and 2, where concentrations exceeded 70% on several occasions(Fig. 3.5.b). Low concentrations were noted in sediment samples of station 4 with only 40%. Station 3 showed the least fluctuations in water content.

ORGANIC CONTENT IN THE SEDIMENT

Organic content per dry sediment fluctuated between 5.5 and 13.5%. Average values of 10.7% were recorded for station 4, 9% for station 1 and 8.6% for stations 2 and 3. A clear seasonal trend was not detectable (Fig. 3.5.c).
3.5.a) Grain size distribution of sediment at stations 1-4 of Hythe intertidal mudflat, January 1996.

3.5.b) Water content determined by weight loss of oven dried sediment.

3.5.c) Organic content of sediment determined by weight loss of combusted sediment collected from the four stations at Hythe intertidal mudflat during 1996.

Figure 3.5(a) Grain size distribution of sediment at stations 1-4 of Hythe intertidal mudflat, January 1996; (b) water content determined by weight loss of oven dried sediment and (c) organic content of sediment determined by weight loss of combusted sediment collected from the four stations at Hythe intertidal mudflat during 1996.
Seasonal Variation of Chlorophyll a at the Four Stations

The annual average and seasonal variation of chlorophyll a in the upper 5 mm of the sediment differed greatly between the four stations. At Station 1 microalgal biomass exhibited a strong seasonality (Fig. 3.6). In April chlorophyll a concentrations increased tenfold within four weeks to an annual maximum of 346 mg m⁻². Values then decreased but remained above the annual average (77 mg m⁻²) until the middle of July. The chlorophyll a spring peak at station 2 and 3 coincided with Station 1, but with maximum chlorophyll values of 209 mg m⁻² at station 2 and 212 mg m⁻² at station 3 the spring bloom was less pronounced. At station 3 chlorophyll a concentration remained elevated throughout October and then fell below the annual average in the remaining months of the study. A one way ANOVA of pooled log₁₀ transformed data (see section 2.10 for details) comparing chlorophyll a concentrations at the four stations revealed that there was no significant difference between the three mudflat stations, but all three stations differed significantly from station 4, which was situated in the marshpool.

The annual average of chlorophyll a concentration was four times higher at station 4, however the coefficient of variation was half that at the mudflat stations, indicating that microalgal biomass was more abundant, but less variable throughout the year (Table 3.1). The seasonal variation also differed at this station. Throughout the spring and early summer months chlorophyll a values ranged between 100 and 240 mg m⁻². In the late summer, chlorophyll a concentrations increased to an annual maximum of 481 mg m⁻² and remained elevated for two months.

<table>
<thead>
<tr>
<th>Station</th>
<th>All</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>98.39</td>
<td>76.8</td>
<td>57.8</td>
<td>69.6</td>
<td>204.3</td>
</tr>
<tr>
<td>CV</td>
<td>1.01</td>
<td>1.08</td>
<td>0.93</td>
<td>0.86</td>
<td>0.51</td>
</tr>
<tr>
<td>Range</td>
<td>83.3-346.8</td>
<td>6.7-208.9</td>
<td>13.3-211.6</td>
<td>22.1-517</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1. Summary statistics of chlorophyll a concentrations in mg m⁻² at the four stations between February 1996-March 1997, CV= coefficient of variation.
To test whether there was a significant interaction between the four stations and sample dates a two way ANOVA was performed on log10 transformed chlorophyll a data. Results of ANOVA showed no significant interaction effect between season and station (F = 2.46, df 15; P = 0.0814). The individual factors can therefore not be interpreted separately. The ANOVA results were that were the four stations and seasons (Table 3.2). A Tukey multiple comparison of means was carried out across stations and seasons (Table 3.3). There was no significant difference between the seasonal and at stations 1 and 2. Station 1 showed the strongest seasonal differences. There were significant differences between spring and summer. There were differences between the four stations, but during the spring there was a significant difference between stations 1 and 2, stations 3 and 4, and stations 3 and 4 in the summer.

Figure 3.6. Seasonal variation of chlorophyll a in the upper 5mm of sediment at the four stations on Hythe mudflat, 1996 - 1997. Error bars show the standard error of three replicates.
To test whether there was a significant interaction between the four stations and sample dates a two way ANOVA was performed on log10 transformed chlorophyll $a$ data. Results of ANOVA showed that there was a significant interaction effect between season and station (F-ratio of season * station = 7.4, $p=0.001$). The individual factors can therefore not be interpreted in isolation. For this purpose dates were grouped into the four seasons and season and station were the fixed factors (Table 3.2). A Tukey multiple comparison of means was carried out to find significant differences between station and seasons (Table 3.3). There was no significant difference between the seasons at station 4, however station 4 was different to the other three stations during all four seasons, except for winter at station 4 and spring at station 1. At station 3 there was also no significant difference between the seasons; and at station 2 only spring differed from autumn. Station 1 showed the strongest seasonal differences. Spring and summer were both significantly different to autumn and winter. There were differences between the stations during the four seasons, but during the spring bloom no significant difference could be found between the three mudflat stations.
Table 3.2. Key to station and season means in following comparison matrices; S refers to station.

<table>
<thead>
<tr>
<th>SEASON</th>
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<th>ROW</th>
<th>S</th>
<th>ROW</th>
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<td>5</td>
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<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Spring</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Summer</td>
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<td>2</td>
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<td>4</td>
<td>15</td>
</tr>
<tr>
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<td>1</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td>3</td>
<td>12</td>
<td>4</td>
<td>16</td>
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</tbody>
</table>

Table 3.3. Matrix of pairwise comparison probabilities of seasons and stations (key shown in Table 3.2), as obtained by a Tukey multiple comparison. Probabilities <0.1 are printed in bold, probabilities <0.05 are printed in bold and underlined; probabilities are rounded to two decimal places, so when p is given as 0.00, then p <0.005.
Environmental factors controlling biomass variation

Chlorophyll *a* concentrations in the upper 5 mm of the sediment were correlated with incident irradiance during tidal emersion, air temperature and salinity measured at the sampling date and with sediment water content to determine the dominating environmental control on microalgal growth.

Prior to the multiple regression analysis, the cumulative effect of irradiance during tidal exposure on microalgal biomass was examined. For this purpose hourly average values of solar radiation during tidal emersion were counted for 1, 2, 3, 5 and 7 days before sampling dates at each station. Linear regression analysis was performed with exposure duration as the independent factor and chlorophyll *a* (log10 transformed) as the dependent factor to find the closest fit. The correlation was strongest when 7 days prior to sampling were taken into account ($r^2 = 0.33$ p< 0.001; fig. 3.7.a).

Backward stepwise regression analysis was then carried out with chlorophyll concentration as the dependent variable. Independent variables included incident irradiance during tidal exposure for 7 days as generated by the tidal model, sediment temperature on the mudflat and the marshpool, salinity on the mudflat and the marshpool, and water content of the sediment at each station. It was decided to exclude nutrient concentrations in Southampton Water from the analysis as sample intervals for the nutrient survey were monthly to bimonthly and preliminary results indicated non-significant correlations with chlorophyll *a*. The four stations on Hythe mudflat were treated separately. Prior to the regression analysis, analysis of covariance (ANCOVA) was carried out which showed that there was a significant interaction between season and irradiance during tidal exposure (F-ratio season*exposure = 9.2, p = 0.01). Due to this result dates were divided into three seasons, spring (up to June), summer (June-August) and autumn (September-November) and tested separately. Winter was not included in the analysis, because the sampling commenced at the end of February and solar radiation data was not available from December 1996 onwards. Results from the backward stepwise regression were verified by single and multiple regression. Data were log10 transformed when the assumptions of normality and homoscedasticity were not met in the raw data. The confidence limit was 95%. Linear regression plots where a single physical factor accounted for most of the chlorophyll *a* variation are shown in fig 3.7b-f.

- **Spring:** At station 1 incident irradiance during tidal exposure integrated over 7 days, mudflat salinity and water content accounted for 86% of chlorophyll *a* variation (n=30, p<
0.001 for irradiance during exposure, p=0.001 for salinity and p=0.008 for water content). At station 2 incident irradiance during tidal exposure integrated over 7 days and salinity explained 33% of the variation (n=30, p=0.005 for incident irradiance during tidal exposure and 0.02 for salinity). At station 3 incident irradiance during tidal exposure integrated over 7 days explained 33% of the chlorophyll \(a\) variation in the upper 5 mm of the sediment (n=30, p=0.001). At station 4 no environmental variable significantly correlated with chlorophyll \(a\) concentrations.

- **Summer**: At stations 1, 2 and 3 no environmental variable significantly correlated with chlorophyll \(a\) concentrations. At station 4 salinity explained 36% of chlorophyll \(a\) variation (n=33, p<0.001).

- **Autumn**: Both at station 1 and 2 irradiance during tidal exposure accounted for 33% (n=30, p=0.002 at both stations). At station 3 no environmental variable significantly correlated with chlorophyll \(a\) concentrations and at station 4 water content in sediment accounted for 29% of chlorophyll variation (n=33, p=0.002).

When salinity correlated with chlorophyll \(a\) the relationship was always negative, suggesting that high salinity due to evaporation limited micro-algal growth rather than low salinity due to precipitation. At station 4 the relationship between water content and chlorophyll \(a\) was also negative (fig. 3.7.c).
Fig. 3.7.a) Scatter diagrams of linear regressions with chlorophyll $a$ at all stations during all seasons as the dependent factor and the incident irradiance during tidal emersion accumulated over 7 days as the independent factor; b-f) scatter diagrams of linear regressions where chlorophyll $a$ was the dependent factor and the environmental variable was the independent factor; environmental variables are presented when one physical factor significantly accounted for most of the chlorophyll $a$ variation at one station during one season, dashed lines are the 95% confidence limits for the regression line and the population.
SEASONAL VARIATION OF PHOTOSYNTHETIC PIGMENTS ALONG THE INTERTIDAL TRANSECT

Results from HPLC chromatograms revealed the presence of chlorophyll \( c_{1+2} \), chlorophyll \( b \), the carotenoids fucoxanthin, peridinin, diadino- and diatoxanthin and \( \beta \) carotene in the upper 5mm of the sediment. Lutein and zeaxanthin could not be successfully separated with the method used and were grouped together in the analysis. The listed pigments were present at all four stations throughout the year but their concentrations and relative proportions changed temporally and spatially. Small traces of 19' hexanoyloxyfucoxanthin, alloxanthin, violaxanthin and 4 other unidentified carotenoids were found in some samples but not included in the following data analysis.

Fucoxanthin was the most abundant taxonomic marker pigment at all four stations indicating that diatoms dominated the microphytic assemblages at each site of the intertidal mudflat and the marsh pool (Table 3.4 and 3.5). There was a strong correlation between fucoxanthin concentrations and chlorophyll \( a \) at the three mudflat stations (Table 3.6) and during peaks of chlorophyll \( a \) in April, fucoxanthin values increased concurrently at all three stations to reach maximum values of 132 mg m\(^{-2}\) (Fig. 3.8). At station 4 concentrations of fucoxanthin showed less annual variation and correlated only weakly with chlorophyll \( a \) (Table 3.6). Chlorophyll \( c_{1+2} \) occurred in lower concentrations than fucoxanthin, however the seasonal variation and the correlation to chlorophyll \( a \) were similar to those of fucoxanthin (Table 3.6).

Low concentrations of peridinin were found at all four stations. There was only a weak correlation between peridinin and chlorophyll \( a \) at all four stations and the coefficient of variation of the peridinin to chlorophyll \( a \) ratio was the highest of all pigments (Table 3.5). On the mudflat, annual average of absolute peridinin values and its ratio to chlorophyll \( a \) decreased landwards. At stations 1 and 2 peridinin concentrations showed a strong seasonality with maximum values of 26 to 29 mg m\(^{-2}\) in April and the beginning of June (Fig. 3.8). Peridinin levels exhibited less seasonal variation at station 4 and although the absolute values were higher than on the mudflat, the peridinin to chlorophyll \( a \) ratio was only half that of the mudflat stations.

Concentrations of zeaxanthin-lutein and chlorophyll \( b \) increased landwards (Table 3.4). There were no seasonal trends detectable for both pigments and the coefficient of variation was low compared to fucoxanthin, chlorophyll \( c_{1+2} \) and peridinin, suggesting that values were more stable throughout the year (Fig. 3.8). The correlation coefficients of both
pigments to chlorophyll \(a\) were weak and with the exception of station 2 chlorophyll \(b\) did not significantly correlate with chlorophyll \(a\) (Table 3.6).

**PIGMENT RATIOS**

On the mudflat stations fucoxanthin and peridinin ratios to chlorophyll \(a\) increased in spring and summer during bloom time and decreased over the summer, indicating that the relative contribution of diatoms and dinoflagellates to total algal biomass was highest during the spring months (Fig. 3.9). The ratios of zeaxanthin-lutein and chlorophyll \(b\) to chlorophyll \(a\) showed the opposite trend. They were high in winter and decreased during March, April and May; in the summer months they increase again. This trend decreased landwards with very stable pigment to chlorophyll \(a\) ratios throughout the year at station 4.
### Table 3.4. Annual average concentrations of accessory pigments at the four stations in mg m$^{-2}$ and their coefficients of variation (CV), S1-4 refers to station 1-4.

<table>
<thead>
<tr>
<th>Station 1</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $c_{r2}$</td>
<td>9.2</td>
<td>5.4</td>
<td>5.3</td>
<td>14.1</td>
</tr>
<tr>
<td>CV</td>
<td>1.6</td>
<td>1.5</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Peridinin</td>
<td>5.0</td>
<td>3.8</td>
<td>2.0</td>
<td>6.5</td>
</tr>
<tr>
<td>CV</td>
<td>1.9</td>
<td>2.0</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>23.7</td>
<td>15.9</td>
<td>16.3</td>
<td>48.4</td>
</tr>
<tr>
<td>CV</td>
<td>1.4</td>
<td>1.3</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Zeaxanthin-lutein</td>
<td>7.3</td>
<td>6.5</td>
<td>8.9</td>
<td>12.7</td>
</tr>
<tr>
<td>CV</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>9.4</td>
<td>8.6</td>
<td>11.5</td>
<td>15.9</td>
</tr>
<tr>
<td>CV</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>β-carotene</td>
<td>18.6</td>
<td>18.0</td>
<td>23.5</td>
<td>39.2</td>
</tr>
<tr>
<td>CV</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

### Table 3.5. Annual averages of accessory pigment to chlorophyll a ratio at the four stations in mg m$^{-2}$ and their coefficients of variation (CV), S1-4 refers to station 1-4.

<table>
<thead>
<tr>
<th>Station 1</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>peridinin to chl a ratio</td>
<td>0.06</td>
<td>0.06</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>CV</td>
<td>1.1</td>
<td>1.2</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>fucoxanthin to chl a ratio</td>
<td>0.24</td>
<td>0.23</td>
<td>0.2</td>
<td>0.25</td>
</tr>
<tr>
<td>CV</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>zeaxanthin-lutein to chl a ratio</td>
<td>0.17</td>
<td>0.16</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>CV</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Chl b to chl a ratio</td>
<td>0.22</td>
<td>0.21</td>
<td>0.23</td>
<td>0.1</td>
</tr>
<tr>
<td>CV</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Table 3.6. Results of linear regression analysis with pigment as dependent factor and chlorophyll a as independent factor; $r^2$ values > 0.5 are printed in bold, $r^2$ values > 0.75 are underlined; probabilities are rounded to two decimal places, so when $p$ is given as 0.00, then $p < 0.005$.

<table>
<thead>
<tr>
<th>Station 1</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $c_{r2}$</td>
<td>0.91 (p=0.00)</td>
<td>0.8 (p=0.00)</td>
<td>0.7 (p=0.00)</td>
<td>0.35 (p=0.00)</td>
</tr>
<tr>
<td>Peridinin</td>
<td>0.22 (p=0.00)</td>
<td>0.15 (p=0.00)</td>
<td>0.32 (p=0.00)</td>
<td>0.01 (p=0.00)</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>0.93 (p=0.00)</td>
<td>0.95 (p=0.00)</td>
<td>0.75 (p=0.00)</td>
<td>0.29 (p=0.00)</td>
</tr>
<tr>
<td>Zeaxanthin-lutein</td>
<td>0.05 (p=0.00)</td>
<td>0.34 (p=0.00)</td>
<td>0.2 (p=0.00)</td>
<td>0.28 (p=0.00)</td>
</tr>
<tr>
<td>Chlorophyll b</td>
<td>0.03 (p=0.10)</td>
<td>0.09 (p=0.00)</td>
<td>0.16 (p=0.001)</td>
<td>0.35 (p=0.05)</td>
</tr>
<tr>
<td>β-carotene</td>
<td>0.2 (p=0.00)</td>
<td>0.4 (p=0.00)</td>
<td>0.43 (p=0.00)</td>
<td>0.7 (p=0.00)</td>
</tr>
</tbody>
</table>
Figure 3.8. Seasonal variation of taxonomic marker pigments at the four stations on Hythe intertidal mudflat, 1996-1997 in mg m$^{-2}$, S1-4 refers to stations 1-4. Error bars are the standard errors of three replicate cores. Note the different axis scale for fucoxanthin.
Figure 3.9. Pigment to chlorophyll $a$ ratios of the four marker pigments fucoxanthin, peridinin, zeaxanthin-lutein and chlorophyll $b$ at the four stations of Hythe intertidal mudflat, 1996-1997 (S1-4 refers to stations 1-4).
THE DISTRIBUTION OF DIADINOXANTHIN AND DIATOXANTHIN

To evaluate the role of the photoprotectant pigment diatoxanthin and the dark induced diadinoxanthin in diatoms and how it can be related to the seasonal cycle, the distribution of the two pigments were compared to each other. At each station the annual average of diadinoxanthin was approximately half of diatoxanthin, however the coefficient of variation was twice as high, except at station 4 (Table 3.7). Diadinoxanthin concentrations showed strong seasonal fluctuation with maximum values coinciding with chlorophyll $a$ (Fig. 3.10). The correlation coefficient between diadinoxanthin and chlorophyll $a$ was above 0.5 at all four stations with the closest linear fit at station 1 resulting in a correlation coefficient of 0.91 (Table 3.9). The strength of the correlation decreased from low shore to high shore. Diatoxanthin values showed less seasonal variation than diadinoxanthin and the correlation between diatoxanthin and chlorophyll $a$ was below 0.5 at all four stations (Fig. 3.10). The diadinoxanthin to chlorophyll $a$ ratio was very stable throughout the year while the ratio of diatoxanthin to chlorophyll $a$ behaved similarly to zeaxanthin-lutein, ie. high in winter, summer and autumn, but decreasing during the spring bloom (Table 3.8 and Fig. 3.11).
<table>
<thead>
<tr>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diadinoxanthin</td>
<td>3.68</td>
<td>2.84</td>
<td>3.16</td>
</tr>
<tr>
<td>CV</td>
<td>1.36</td>
<td>1.20</td>
<td>1.16</td>
</tr>
<tr>
<td>Diatoxanthin</td>
<td>6.54</td>
<td>6.19</td>
<td>6.44</td>
</tr>
<tr>
<td>CV</td>
<td>0.66</td>
<td>0.64</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Table 3.7. Annual averages of diadinoxanthin and diatoxanthin concentrations in mg m$^{-2}$ and the coefficients of variation (CV).

<table>
<thead>
<tr>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diadinoxanthin</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>CV</td>
<td>0.4</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Diatoxanthin</td>
<td>0.13</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>CV</td>
<td>0.53</td>
<td>0.52</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Table 3.8. Annual averages of diadinoxanthin to chlorophyll $a$ ratios and diatoxanthin to chlorophyll $a$ ratios at the four stations and the coefficients of variation (CV).

<table>
<thead>
<tr>
<th>Station 1</th>
<th>Station 2</th>
<th>Station 3</th>
<th>Station 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>diadinoxanthin</td>
<td>0.91 ($p=0.00$)</td>
<td>0.89 ($p=0.00$)</td>
<td>0.73 ($p=0.00$)</td>
</tr>
<tr>
<td>diatoxanthin</td>
<td>0.34 ($p=0.00$)</td>
<td>0.34 ($p=0.00$)</td>
<td>0.42 ($p=0.00$)</td>
</tr>
<tr>
<td>diadino vs diatox</td>
<td>0.09 ($p=0.00$)</td>
<td>0.24 ($p=0.00$)</td>
<td>0.23 ($p=0.00$)</td>
</tr>
</tbody>
</table>

Table 3.9. Results of linear regression analysis with pigment as dependent factor and chlorophyll $a$ as independent factor and diadinoxanthin vs diatoxanthin in a Pearson product moment correlation; $r^2$ values > 0.5 are printed in bold, $r^2$ values > 0.75 are underlined; probabilities are rounded to two decimal places, so when $p$ is given as 0.00, then $p < 0.005$. 

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Figure 3.10: Seasonal variation of diadinoxanthin and diatoxanthin concentrations at the four stations of Hythe intertidal mudflat, 1996-1997, error bars refer to the standard error of three replicate cores (S1-4 refers to stations 1-4).
Figure 3.11. Diadinoxanthin and diatoxanthin to chlorophyll \(a\) ratios at the four stations of Hythe intertidal mudflat, 1996-1997 (S1-4 refers to stations 1-4).
THE SEASONAL VARIATION OF PHAEOPIGMENTS AT THE FOUR STATIONS OF HYTHE INTERTIDAL MUDFLAT

To evaluate the physiological state of microphytobenthos and the impact of grazing, phaeopigments were measured in conjunction with photosynthetic pigments. Four phaeopigments were found in the sediment samples; on the HPLC chromatograms two eluted before chlorophyll \( a \) and were called phaeophorbide \( a1 \) and phaeophorbide \( a2 \) and two eluted after chlorophyll \( a \) and were called phaeophytin \( a1 \) and phaeophytin \( a2 \). These four degradation products were found throughout the year at all four stations, but varied in absolute concentration and proportion. Chlorophyllide was not found in the samples. Phaeopigments were not analysed between March and April 1996.

Phaeophorbide \( a2 \) had the highest coefficient of variation (Table 3.10) and showed the strongest seasonal variation of the four phaeopigments. Maximum values at station 1 and 2 coincided with the second chlorophyll \( a \) maximum in the summer (Fig.3.12). The concentrations of both phaeophorbides decreased from low shore to high shore on the mudflat. At station 1 phaeophorbide \( a2 \) was the most abundant degradation product, at station 3 it was less than a third of phaeophytin \( a1 \) concentration (Table 3.10).

Although phaeopigment concentrations were highest at station 4, their ratios to chlorophyll \( a \) were comparatively low, indicating that degradation products were relatively less abundant. The seasonal variation of degradation products did not follow the same trend as variation in chlorophyll \( a \). Chlorophyll \( a \) reached its maximum concentration in the autumn months and phaeopigments decreased at the same time. Linear regression analysis showed that the correlation between chlorophyll \( a \) and each phaeopigment decreased landwards (Table 3.11). At station 1 and 2 all correlations were significant (except for phaeophytin \( a2 \) at station 2) and the correlation coefficient for phaeophorbide \( a1 \) was above 0.5 at both stations and above 0.7 for phaeophytin \( a1 \) at station 1. At station 4 correlation coefficients did not exceed 0.4 and none of the correlations were significant.
Table 3.10. Annual averages of phaeopigments at the four stations in mg m\(^{-2}\) and their coefficients of variation (CV), S1-4 refers to station 1-4.

<table>
<thead>
<tr>
<th>Station 1</th>
<th>SI</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu) Phaeophorbide (a1,2)</td>
<td>47.7</td>
<td>25.4</td>
<td>11.4</td>
<td>68.1</td>
</tr>
<tr>
<td>CV</td>
<td>1.75</td>
<td>1</td>
<td>0.34</td>
<td>0.88</td>
</tr>
<tr>
<td>(\mu) Phaeophorbide (a2,1)</td>
<td>155.6</td>
<td>125.3</td>
<td>55.9</td>
<td>81.9</td>
</tr>
<tr>
<td>CV</td>
<td>5.5</td>
<td>3.68</td>
<td>1.64</td>
<td>2.41</td>
</tr>
<tr>
<td>(\mu) Phaeophytin (a1)</td>
<td>145.9</td>
<td>110.7</td>
<td>178.3</td>
<td>347.2</td>
</tr>
<tr>
<td>CV</td>
<td>2.73</td>
<td>1.9</td>
<td>3.22</td>
<td>4.06</td>
</tr>
<tr>
<td>(\mu) Phaeophytin (a2)</td>
<td>107.2</td>
<td>90.7</td>
<td>148.6</td>
<td>192.5</td>
</tr>
<tr>
<td>CV</td>
<td>1.3</td>
<td>1.7</td>
<td>2.72</td>
<td>2.38</td>
</tr>
</tbody>
</table>

Table 3.11. Results of linear regression analysis with phaeopigment as dependent factor and chlorophyll \(a\) as independent factor; \(r^2\) values > 0.5 are printed in bold, \(r^2\) values > 0.75 are underlined; probabilities are rounded to two decimal places, so when \(p\) is given as 0.00, then \(p < 0.005\), S1-4 refers to station 1-4.

<table>
<thead>
<tr>
<th>Station 1</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phaeophorbide (a1)</td>
<td>0.58 ((p=0.00))</td>
<td>0.56 ((p=0.00))</td>
<td>0.12 ((p=0.06))</td>
<td>0.4 ((p=0.27))</td>
</tr>
<tr>
<td>Phaeophorbide (a2)</td>
<td>0.49 ((p=0.00))</td>
<td>0.42 ((p=0.00))</td>
<td>0.08 ((p=0.13))</td>
<td>0.06 ((p=0.17))</td>
</tr>
<tr>
<td>Phaeophytin (a1)</td>
<td>0.72 ((p=0.00))</td>
<td>0.36 ((p=0.00))</td>
<td>0.2 ((p=0.01))</td>
<td>0.01 ((p=0.53))</td>
</tr>
<tr>
<td>Phaeophytin (a2)</td>
<td>0.35 ((p=0.00))</td>
<td>0.08 ((p=0.14))</td>
<td>0.13 ((p=0.04))</td>
<td>0.02 ((p=0.41))</td>
</tr>
</tbody>
</table>

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Figure 3.12. Seasonal distribution of phaeopigments at the four stations of Hythe intertidal mudflat (— = chlorophyll a concentration in mg m⁻²). Bars and error bars are the averages and standard errors of three replicate cores, S1-4 refers to stations 1-4.
3.4 SEASONAL VARIATION OF MICROALGAL COMPOSITION AT THE FOUR STATIONS

THE DISTRIBUTION OF MICROALGAL GROUPS

Taxonomic analysis of benthic microalgae, collected onto the underside of coverslips, was carried out between April and December 1996. Algal groups were divided into the classes Bacillariophyta, Euglenophyta and Cyanophyta. Members of the class Bacillariophyta showed highest relative abundance and were therefore subdivided into the most common genera *Nitzschia*, *Navicula*, *Gyrosigma* and *Pleurosigma*. The category <other diatoms> encompassed the genera *Cylindrotheca*, *Amphiprora*, *Amphora*, *Diploneis*, *Suriella*, *Paralia*, *Melosira* and unidentified species.

At the mudflat stations 1, 2 and 3 the relative abundance of cyanobacteria and euglenoids was low. With the exception of the 4th of July at station 1 where cyanobacteria made up 15% of counted numbers, the proportion of cyanobacteria remained below 5% at all times. Cyanobacteria appeared only in the summer months between the end of May and the beginning of September. Individual euglenoids were recorded sporadically, their contribution to total numbers of individuals was below 1.5% at any time or station (Fig. 3.13). At station 4, the salt marsh pool, the average contribution of cyanobacteria to total numbers of individuals was 30%. Cyanobacteria were detected throughout the year with maximum values of 77% at the end of May and July (Fig. 3.13).

THE DISTRIBUTION OF DIATOMS AT THE FOUR STATIONS

At all four stations, most individuals counted belonged to the genus *Navicula*. This genus comprised 40% of all cells from station 1 and 3, 38% from station 2 and 43% from station 4. The species composition differed among the stations; at the mudflat stations 1, 2 and 3 *Navicula gregaria* was the most common naviculoid species (Fig. 3.14). On single sample dates the contribution to total counted individuals was as high as 70% at stations 1 and 3 and 80% at station 2. At the marsh pool station 4 this species was found in low numbers. *Fallacia pygmeae* which was rarely detected on the mudflat contributed up to 63% to total numbers of individuals in the marsh pool on single sample dates. The small species *Navicula cryptocephala* occurred in high numbers at station 3 with values of 60%, while it never exceeded 25% at the remaining stations (Fig. 3.14).
Nitzschia comprised 30%, 33%, 20% and 7% of total cells counted at station 1, 2, 3 and 4 respectively. The genus included the large species Nitzschia sigma, which was found in high numbers on the mudflat stations 1, 2 and 3 (Fig. 3.14). On the mudflat the proportion of Gyrosigma and Pleurosigma increased towards high shore with annual average values of 11% at low shore, 18% at mid shore and 23% at high shore. Only 1% of individuals recorded at the marsh pool belong to these genera. Gyrosigma attenuatum was the most abundant species of this genus. It was found in high numbers at all three mudflat stations, contributing up to 97% of all individuals at station 2 on single sample dates (Fig. 3.15). Pleurosigma aestuarii was abundant at station 3 with maximum values of 69% (Fig. 3.15). Other species in this genus included Pleurosigma angulatum and Gyrosigma fasciola. Numbers recorded for these species did not exceed 8% at any station or time.

Other diatoms recorded belonged to the genera of Amphiprora and Cylindrotheca at stations 1, 2 and 3 and to Amphora, Diploneis and Cylindrotheca at station 4. The species Diploneis didyma and Cylindrotheca gracilis were almost exclusively found at station 4, while Cylindrotheca fusiform was found in equal numbers at all four stations (Fig. 3.15).

**Seasonal Succession of Diatom Species**

At stations 1, 2 and 3 a seasonal succession of diatoms was detectable. Navicula gregaria showed a high relative abundance in spring and early summer (Fig. 3.14). The proportions of Gyrosigma and Pleurosigma and other diatoms gradually increased towards December. This trend was caused by the seasonal behaviour of Gyrosigma attenuatum and Pleurosigma aestuarii which were abundant in September, October and November (Fig. 3.15). Total numbers of Amphiprora also increased towards autumn. The proportion of Nitzschia remained stable throughout the year at stations 1 and 2. However species within this genus showed seasonal variations. Cylindrotheca closterium was abundant during the spring months at all four stations; numbers declined in June and in autumn this species was almost absent (Fig. 3.14). The abundance of Nitzschia sigma increased in late summer at station 1 and 2.

At station 4 there was no seasonal variation detectable on a genus level. Species belonging to Navicula were most abundant throughout the year, with the exception of April, when high numbers of Cylindrotheca gracilis were found. Fallacia pygmaea was very common in May, numbers decreased during the summer months and increased again towards the end of September (Fig. 3.14). The small naviculoid species including Navicula cryptocephala increased in the summer months and declined towards winter.
dydima, *Amphora ovalis* and *Amphora lineolata* were most abundant during the autumn months. Appendix II gives a full species list.
Figure 3.13. Distribution of major algal groups at the four stations of Hythe mudflat in % of 100 to 200 cells, identification of species was carried out between April and December 1996.
Figure 3.14. Relative abundance of major *Nitzschia* and *Navicula* species found at the four stations of Hythe mudflat, 1996 in % of 100 to 200 counted cells.
Figure 3.15. Relative abundance of other major diatom species and cyanobacteria found at the four stations of Hythe mudflat, 1996 in % of 100 to 200 counted cells.
3.5 DISCUSSION

Spatial distribution of algal taxa on Hythe mudflat

On Hythe mudflat a seasonal succession of algal groups and species were found in the sampling period of 1995-1996. In addition some strong spatial trends were detectable, whereby species distribution overlapped on the three mudflat stations. The salt marsh station 4 contained a very different species assemblage, indicating that this station represented a distinct habitat. Station four was the only habitat colonised by large numbers of cyanobacteria, whose relative abundance comprised up to 85%. In addition several diatom species were almost exclusively found at this station. *Fallacia pygmaea* was one of the most abundant species at station four (up to 60%) while only individual cells were found at the mudflat stations (<3%). This species has been shown to have a high abundance on salt marshes as opposed to mud and sandflats at Berrow Flats, Somerset, U.K. (Oppenheim, 1988, 1991) but is not exclusive to saltmarshes (Admiraal et al, 1982). *Diploneis didyma*, *Amphora ovalis*, *Amphora lineolata* and *Cylindrotheca gracilis* were also found in high abundance in the marsh creek but not on the mudflat, while *Nitzschia sigma*, *Gyrosigma fasciola* and *Gyrosigma attenuatum* were almost exclusive to the mudflat stations.

Other species showed a more widespread occurrence; *Cylindrotheca closterium* was equally distributed at all four stations. This species has been reported as widespread in different types of sediment habitats, ranging from sandflats (Asmus and Bauerfeind, 1994) and mudflats (Underwood, 1994) to salt marshes (Oppenheim, 1988, 1991). *Navicula gregaria* and *Navicula cryptocephala* are also widely distributed as shown by this and other studies. Due to their prevalence on the Berrow Flats, Somerset, U.K the distribution of both species could not be correlated to specific physical factors (Oppenheim, 1988, 1991).

Seasonal succession of species on Hythe mudflat

A seasonal succession of diatom species was found at all four stations, with the majority of species showing high temporal variations in relative abundance. Only a few species such as *Cylindrotheca fusiformis* were found in equal numbers throughout the sampling programme. Species could be grouped into spring/early summer species or autumn species: *Cylindrotheca closterium* and *Cylindrotheca gracilis* were abundant in spring and rapidly declined towards the summer. Similar results were reported for the Severn Estuary (Underwood, 1994), the Berrow flats (Oppenheim, 1991) and Königshafen (Asmus and Bauerfeind, 1994). *Nitzschia gregaria* which was the most abundant species in this study
also bloomed in spring. However, although its relative abundance declined in mid summer it persisted until November. Typical late summer/autumn species were *Gyrosigma fasciola*, *Gyrosigma attenuatum*, *Pleurosigma angulatum* and *Pleurosigma aestuarii*, which showed similar temporal distributions in other studies (Admiraal, 1984; Colijn and Dijkema, 1981; Oppenheim, 1991; Underwood, 1994). *Nitzschia sigma* also increased in proportion in autumn, similar to findings by Oppenheim (1991) as did *Navicula cryptocephala* which agrees with the findings of Admiraal et al. (1982), but not Colijn and Dijkema (1981). In summary it can thus be concluded that the temporal and spatial distribution pattern of many of the more common diatom species agrees with similar studies in temporal habitats. Repeating patterns are important to note as they allow to draw conclusions about the ecological niches of the common species. The approach to combine floristic studies with controlled experiments of natural populations as was taken by Admiraal (1984) would further increase our understanding of the ecological strategies of epipelic diatoms in such a variable environment. As many species also show distinct distribution patterns in response to nutrient enrichment (Underwood, 1998) and toxic compounds (Admiraal and Peletier, 1980), an extensive compilation of the ecological occurrence of epipelic diatoms would further allow to select indicator species for different sources of pollution.

*Pigment composition in sediments of Hythe mudflat:*

Algal pigments found in sediments of the intertidal zone are potentially derived from three different sources: benthic microalgae present within the sediment, phytoplankton cells deposited from the water column and macrophytic detritus derived from the salt marsh due to erosion and deposition. Faecal pellets from planktonic and benthic grazers contain chemically transformed pigment products and are derived either from in situ grazing or water column grazing and subsequent deposition.

Fucoxanthin was the most abundant accessory pigment at each station of the mudflat and the marsh pool. This carotenoid is found in Prymnesiophytes, dinophytes and bacillariophytes (Jeffrey et al., 1997). Since levels of 19′hexanoyloxyfucoxanthin were negligible and peridinin only correlated weakly with fucoxanthin ($r^2=0.29$, $p<0.001$) it can be assumed that microphytobenthos was dominated by diatoms. This agrees with the majority of studies carried out on intertidal sediments (MacIntyre et al., 1996). HPLC data were supported by cell identification. With the exception of the summer months at station 4 diatoms dominated the microphytic assemblage by more than 90%. Fucoxanthin, chlorophyll c1+2 and diadinoxanthin correlated strongly with chlorophyll a, suggesting that the variation of microalgal biomass was mainly due to the growth dynamics of benthic diatoms.

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The Mantoura and Llewellyn HPLC method (1983), used in this study did not allow the separation of the two carotenoids lutein and zeaxanthin. Low zeaxanthin-lutein concentrations were found at all stations with highest levels at station 4. Zeaxanthin is found in cyanobacteria and macrophytes (Jeffrey et al., 1997). Taxonomic analysis revealed large numbers of cyanobacteria at station 4 and in late summer dark green patches consisting of Oscillatoria sp. and Anabaena sp. were seen on the surface of the marshpool sediment. A significant correlation between cell counts and zeaxanthin to chlorophyll a ratio was however not detected ($r^2=0.04$, $p=0.33$) in contrast to the study of Pinckney et al., 1995. The discrepancy between species identification and pigment concentrations suggests that there was an alternative source of zeaxanthin-lutein in the sediment of the marshpool and the mudflat stations. Macrophytes contain lutein, zeaxanthin and chlorophyll b and it is therefore likely that the sediment contained macrophytic detritus from the adjacent saltmarsh. Chlorophyll b correlated weakly with lutein-zeaxanthin ($r^2= 0.31$, $p<0.001$) suggesting that the input of these two pigments was not completely linked. Cell counts revealed the presence of euglenoids in small numbers, which suggests that some of the chlorophyll b derived from this algal group. The presence of euglenoids in intertidal sediments, detected by HPLC and microscopy has been reported in similar studies (Klein and Riaux Gobin, 1991; Brotas and Plante-Cuny, 1998).

It can be concluded that taxonomic and HPLC analysis of sediment from Hythe mudflat and marshpool confirmed the dominant presence of benthic diatoms, with minor contributions from euglenoids and cyanobacteria and some organic input of macrophytic detritus and phytoplankton deposits. There were several problems associated with relating taxonomic data to HPLC analysis. The method that was chosen to harvest cells for species identification favoured motile species and therefore immobile microphytes present in the top 5mm of the sediment were not taken into account. Furthermore, photosynthetic pigments have different degradation rates (Repeta and Gagosian, 1982). Chlorophyll a is labile with a half life of less than 3 weeks in oxic sediments (Abele-Oeschger, 1991) and fucoxanthin degrades even faster than chlorophyll a (Klein and Riaux Gobin, 1991). Other pigments such as zeaxanthin, lutein and β-carotene are more stable and persist in sediments for long periods of time (Hurley and Armstrong, 1990). Results suggest that Hythe intertidal sediment contained high background levels of stable carotenoids, which bear little relation to the short term fluctuations of microalgal abundance. This would explain the independent behaviour of lutein-zeaxanthin and β carotene to chlorophyll a.
Biomass at the four stations

On Hythe mudflat annual average values of chlorophyll $a$ were in the same range as those reported for intertidal mudflats in the Ems Estuary (deJong and deJonge, 1995) and the North inlet estuary in South Carolina (Pinckney and Zingmark, 1993a) but only half of those reported for the Wester Schelde (deJong and Colijn, 1994) and the Dutch Wadden Sea (Colijn and Dijkema, 1981).

The average concentration of chlorophyll $a$ was approximately three times higher in the marshpool than on the mudflat stations and apart from the spring bloom microalgae had a higher abundance on the marshpool throughout the year. In fine grained sediments of turbid estuaries epipelic diatoms migrate to the surface of the sediment at low tide and photosynthesis during the period of aerial emersion (Pinckney and Zingmark, 1991). The marsh pool was only inundated during spring high tides and the tidal model showed that the average emersion period during daylight at this site was more than twice as long as on the mudflat. Longer photoperiods could therefore explain the higher abundance of benthic microalgae at station 4. The site was also sheltered from wave action and it can be assumed that less erosion and resuspension of microalgae facilitated the establishment of mature microphytic assemblages. There was no significant difference between the annual averaged chlorophyll $a$ concentrations of the three mudflat stations, although the tidal emersion period increased by a factor of two from station 1 to 3. Similar studies have shown a positive correlation between the abundance of microphytes and tidal height (Underwood and Paterson, 1993; Santos et al., 1997), but this is not universal (Guarini et al., 1998).

Seasonal variation of biomass at the four stations

The seasonality of microalgal biomass on Hythe intertidal mudflat varied among the four stations. At stations 1 and 2 microphytobenthos exhibited a pronounced spring bloom, which agrees with results of Pinckney and Zingmark (1993a) and Underwood and Paterson, (1993) (see also review of MacIntyre et al., 1996). At high shore chlorophyll $a$ variation exhibited a different seasonality with a weak increase in microalgal biomass in autumn, which was similar to the seasonal behaviour of benthic microalgae reported for the Tagus estuary, Portugal (Brotas et al., 1995, Brotas and Plante-Cuny, 1998).

Previous studies have related chlorophyll $a$ concentrations in sediment to physical factors to explain spatial differences (Santos et al., 1997; Brotas et al. 1995, deJong and deJonge, 1995) and seasonal variation (Colijn and Djikema, 1981) in microalgal biomass, but
not both. The results of this chapter give statistical evidence that the controlling factors on chlorophyll \( a \) concentrations vary in space and time. In spring, incident irradiance during tidal emersion and salinity were the main controlling factors at station 1-3 which are located on the mudflat. In the summer no physical factor could be identified to control microalgal growth at low to high shore and in autumn irradiance regained importance in controlling chlorophyll \( a \) concentrations. Incident irradiance and the length of daylight increased in the early spring months, allowing longer time periods for photosynthesis at higher irradiances. The combined effect caused the population to rapidly increase. Once the density of microphytobenthos was high, biological factors such as competition and grazing were a possible cause for the subsequent decline of microalgal biomass. Between March and August 1996 a study was carried out to investigate the macrofaunal abundance at station 2 of Hythe mudflat (Good, 1996). Deposit feeding polychaetes such as Caullerella zetlandica, Pygospio elegans and Polydora ciliata dominated the fauna during this period at station 2. Biomass of macrofauna increased from 96 wet weight g m\(^{-2}\) on 27th of March to 735 g m\(^{-2}\) on 7th of May, one week after the annual chlorophyll \( a \) maximum at station 2 (Fig. 3.16). A second peak followed in the beginning of June, after which the numbers declined again.

![Graph](image)

Fig. 3.16: Chlorophyll \( a \) concentration and macrofaunal biomass at station 2 of Hythe mudflat, March-July 1996 (data of macrofaunal biomass obtained from Good, 1996).
Variation of macrofaunal biomass followed chlorophyll $a$ concentrations, but a significant correlation between chlorophyll $a$ and abundance of fauna was not found even when a lag period of 1 or 2 weeks was taken into account ($r^2 = 0.28$, $p=0.06$). Fig. 3.16 however clearly illustrates the increased grazing pressure on microalgal biomass at station 2. Nutrient levels in the watercolumn decreased in the summer months as well (Hydes and Wright, 1999), yet no external factors could be statistically isolated as the causes for microalgal biomass decline in the summer at these two stations.

At station 4 chlorophyll $a$ concentrations was more stable and less seasonally influenced. A significant correlation between chlorophyll $a$ and incident irradiance during tidal exposure could not be found at this station. As station 4 was located at the spring high tide mark, it received long exposure periods throughout the year. In the summer when temperature and irradiance were high, long periods of exposure possibly had a negative effect on growth. Thus there was a significant negative correlation between chlorophyll $a$ and salinity during the summer, suggesting that during periods of high evaporation biomass declined. However in autumn, sediment water content also correlated negatively with biomass suggesting that growth of microphytobenthos at station 4 was also sensitive to excess water. The water content of sediment has been shown to influence the migratory response and photosynthesis of epipelic diatoms (Holmes and Mahall, 1982; Hopkins, 1966).

Chlorophyll $a$ has been used as an indicator for the seasonal variation of microalgal biomass. However it has to be taken into consideration, that the ratio of chlorophyll $a$ to carbon changes due to variations in environmental conditions. Among benthic diatoms and cyanobacteria a significant variation within the annual cycle has been reported by de Jonge (1980) with carbon to chlorophyll $a$ ratios ranging from 10 to 150. Nutrient limitations cause an increase in carbon to chlorophyll $a$ ratio (Reynolds et al. 1997). The synthesis of carotenoids does not require nitrogen in contrast to chlorophyll $a$ synthesis, thus the composition of pigments also changes under nitrogen limiting conditions. Variations in carbon to chlorophyll $a$ ratio can also be attributed to changes in irradiance levels. Photosynthetic cells adapt to an increase in prevailing irradiance levels by decreasing their chlorophyll $a$ content. Gould and Gallagher (1990) reported that the carbon to chlorophyll $a$ ratio was highest during the spring bloom of benthic microalgae. It can therefore be
assumed that seasonal variation of microalgal biomass was even more pronounced than suggested from the chlorophyll \( a \) concentrations.

**THE DISTRIBUTION OF PHAEOPIGMENTS**

Since chlorophylls and carotenoids undergo chemical transformation in the guts of grazers, the distribution of certain phaeopigments can be used to evaluate grazing in a semiquantitative way (Barlow, 1993b; Repeta and Gagosian, 1982). Five degradation products of chlorophyll \( a \) have been separated with HPLC in this study and named chlorophyllide, phaeophorbide \( a1 \) and \( a2 \) for peaks eluting before chlorophyll \( a \) and phaeophytin \( a1 \) and \( a2 \) for peaks which eluted after chlorophyll \( a \). Most sediment samples contained undetectable or very low levels of chlorophyllide, indicating that artificial degradation was minimal. This agrees with similar studies where chlorophyllide levels in intertidal sediments were below 5% of chlorophyll \( a \) concentration (Plante-Cuny et al 1993; Riaux Gobin et al., 1993).

Since detection of phaeopigments was carried out using the HPLC fluorescence detector, absorption spectra were not available and identification was restricted to comparisons with other studies. Phaeophorbide \( a2 \) (Barlow 1993b; Cariou le Gall and Blanchard, 1995) was believed to be the same pigment as the phaeophorbide \( a \)-like compound named in other studies (Klein and Riaux Gobin, 1991; Riaux Gobin et al., 1993) due to its close elution with 19’hexanoyloxyfucoxanthin. Phaeophorbide \( a2 \) is found in the stomach of polychaetes, deposit feeders and molluscs (Hawkins et al., 1986) and is believed to be the main indicator pigment for grazing.

At station 1 phaeopigments correlated most strongly with chlorophyll \( a \) suggesting a close coupling between primary production and grazing. Phaeopigments were not analysed in April and early May when the spring bloom occurred due to the lack of a fluorescence detector, hence it was difficult to predict whether the peak of phaeophorbide \( a2 \) in late May was a lag response to elevated chlorophyll concentrations during April/May or a secondary peak. The strength of the correlation between chlorophyll \( a \) and phaeopigments decreased landwards and was insignificant at station 3 and 4. This could be due to the slow degradation of macrophytic detritus and the deposition of senescent phytoplankton cells and faecal pellets of zooplankton.

**Pigment Physiology in Diatoms**

Diatoxanthin and diadinoxanthin are present in diatoms, euglenoids and dinoflagellates. The cycling of these two xanthophylls occurs in a similar fashion to the...
zeaxanthin/violaxanthin cycling in higher plants and is believed to have a photo-protective role (Stransky and Hager, 1970). The two pigments are located in the light harvesting complex of the photosystem 2 (Brown, 1988). During light exposure diadinoxanthin is de-epoxidised to form diatoxanthin by enzyme activity and the concentration of diatoxanthin rises with the increase in light intensity and duration of exposure (Moisan et al. 1998; Olaizola and Yamamoto, 1994). In the sediments of Hythe mudflat the two xanthophylls varied differently in relation to chlorophyll a levels. Diatoxanthin, the light induced protectant, was very stable throughout the year and correlated only weakly with chlorophyll a values, remaining constant even during bloom conditions. Diadinoxanthin levels were strongly correlated with chlorophyll a. In contrast diadinoxanthin concentrations were in general lower than diatoxanthin, however fluctuations followed those of chlorophyll a and during periods of bloom conditions exceeded levels of diatoxanthin.

Since these two pigments participate in xanthophyll cycling it was expected to find that they were inversely proportional to each other and that concentrations of both increased with total biomass. However no relationship was found between the two pigments and changes in diadinoxanthin were more closely related to chlorophyll a than changes in diatoxanthin. As elevated irradiances trigger the enzymatic conversion of diadinoxanthin to diatoxanthin (Demers et al., 1991), any changes in cellular pigment ratios would occur at the surface of the sediment. It can therefore be concluded that the sample resolution of 5 mm applied in this study was not high enough to follow any trends in xanthophyll cycling.
CHAPTER 4

VERTICAL DISTRIBUTION OF PHOTOSYNTHETIC PIGMENTS IN SEDIMENT OF HYTHE MUDFLAT

4.1 INTRODUCTION

It has been shown in Chapter 3 that most photosynthetic pigments found in intertidal sediments of Hythe mudflat derive from active phototrophs inhabiting the sediment, deposition of senescent phytoplankton and from macrophytic detritus from the adjacent saltmarsh. The sediment therefore acts as a sink for pigments of different sources and degradation rates. For this reason it can be argued that the analysis of samples from a single depth may not resolve dynamic processes of \textit{in situ} microphytic production. The work presented in this chapter focuses on the depth distribution of photosynthetic pigments and degradation products in the upper 1 cm of the sediment at a resolution of 1 mm, in order to differentiate between active surface processes, deposition and transport to deeper sediment layers and mechanisms of degradation. Between December 1997 and May 1998 sediment samples were collected each month at station 2 on Hythe mudflat. The sample period was chosen because it included the winter season when microalgal biomass was known to be low as well as spring, when a diatom bloom was expected to develop at the surface of the sediment and subsequently decline. To examine whether there were any spatial differences in the vertical pigment distribution, all four intertidal stations were once sampled and compared in March and April when maximum chlorophyll concentrations occurred.

Seasonal and spatial changes in vertical pigment concentrations and ratios are shown in contour plots. This form of presentation was chosen to illustrate two dimensional changes; it was not intended to extrapolate temporal or spatial changes between samples or stations.
4.2 MONTHLY CHANGE IN THE VERTICAL STRUCTURE OF MICROALGAL BIOMASS, ACCESSORY PIGMENTS AND DEGRADATION PRODUCTS

CHLOROPHYLL A

Concentrations and vertical distribution of chlorophyll a are shown in Fig. 4.1. Chlorophyll a was homogeneously distributed in the upper 1 cm of the sediment in December and then decreased with depth in January and February, however a one way ANOVA between the different depths showed no significant difference. In March, chlorophyll a values increased fivefold in the upper 1 mm of the sediment, but concentrations decreased rapidly with depth. The 1-2 mm section contained 30% and the 2-3 mm section only 13% of the surface value. By April there was a further increase in chlorophyll a in the upper 1 mm with a similar steep vertical gradient. During both months surface chlorophyll a concentrations were significantly different to values below 2 mm, and values below 3 mm did not significantly differ from each other. In May chlorophyll concentrations decreased at the surface, but levels increased in the deeper layers.

ACCESSORY PIGMENTS ASSOCIATED WITH DIATOMS:

Accessory pigments differed in their vertical distribution and seasonal variation. Concentrations of fucoxanthin and its ratio to chlorophyll a are shown in Figures 4.1 and 4.2. Fucoxanthin was the most abundant accessory pigment in the upper 3 mm at all times; its seasonal and vertical variation was very similar to chlorophyll a and the two pigments correlated strongly ($r^2 = 0.89$, $p < 0.0001$).

In the first three months the fucoxanthin to chlorophyll a ratio ranged between 0.36 and 0.42 (with one outlier of 0.52 in December). In March and April there was a decrease with depth to a minimum value of 0.14 in the deepest section in April. This was followed by a strong increase in May to above 0.5 between 1 and 5 mm. Chlorophyll $cI+2$ was less abundant than fucoxanthin, but its vertical distribution and monthly change was similar to that of fucoxanthin and it was also highly correlated to chlorophyll a ($r^2 = 0.82$, $p < 0.0001$). The chlorophyll $cI+2$ to chlorophyll a ratio was less variable than that of fucoxanthin to chlorophyll a, it showed a small increase with depth in the first three winter months and a small decrease in April (data not shown).

The vertical distributions of diadinoxanthin and diatoxanthin were examined to see whether a relationship between season, sediment depth and xanthophyll cycling could be
established. In the first three months the concentration of diatoxanthin and its ratio to chlorophyll $a$ was higher and than the concentration of diadinoxanthin and its ratio to chlorophyll $a$ (Fig. 4.3 & 4.4). In March and April when chlorophyll values increased, diadinoxanthin concentrations exceeded diatoxanthin concentrations in the upper mm of the sediment; but below the surface, diatoxanthin values and ratios to chlorophyll $a$ remained high (Fig. 4.3 & 4.4). While diadinoxanthin correlated strongly with chlorophyll $a$ ($r^2=0.88$, $p<0.0001$) there was only a weak relationship between chlorophyll $a$ and diatoxanthin ($r^2=0.3$, $p<0.0001$).
Fig. 4.1. Vertical distribution of chlorophyll a and fucoxanthin concentrations in µg g⁻¹ sediment in the upper 1 cm of sediment at station 2 of Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.001 and 0.98 for chlorophyll a and 0.04 and 0.84 for fucoxanthin.

Fig. 4.2. Vertical distribution of fucoxanthin to chlorophyll a ratio in the upper 1 cm of sediment at station 2 Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.01 and 1.
Fig. 4.3. Vertical distribution of diadinoxanthin and diatoxanthin concentrations in µg g⁻¹ sediment in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.13 and 0.8 for diadinoxanthin and 0.01 and 1 for diatoxanthin.

Fig. 4.4. Vertical distribution of diadinoxanthin and diatoxanthin to chlorophyll a ratios in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.05 and 0.74 for diadinoxanthin and 0.06 and 1 for diatoxanthin.
THE DISTRIBUTION OF OTHER MARKER PIGMENTS:

In the first three sample months peridinin concentrations and peridinin to chlorophyll a ratio increased with depth (Fig 4.5 and 4.6). From March onwards values in the top mm were highest, however there was still an increase of the peridinin to chlorophyll a ratio with depth. Surface concentrations increased towards May, suggesting increased deposition from the watercolumn and subsequent burial in late spring.

Zeaxanthin-lutein concentrations showed only small variations between months (Fig 4.5). There was an increase in concentration with depth, with highest concentrations found between 2 and 5mm, however a significant difference between the different depths could not be found on any sample date. The zeaxanthin-lutein to chlorophyll a ratio decreased towards spring and was always lower at the surface than in the deeper part of the sediment (Fig 4.6).

Values of chlorophyll b were low in the first three months of the sampling period with a slight increase with depth. In March concentrations increased to 2.7μg g⁻¹ at the surface. The structure of the depth profile changed in March and April with high values at the surface and the deepest section of the sediment (Fig 4.7). The ratio of chlorophyll b to chlorophyll a increased to >1 below 5mm (Fig 4.8). The depth profile of absolute concentrations and the increase of the chlorophyll b to chlorophyll a ratio with depth suggested that there were two sources of chlorophyll b in the sediment. The surface sediments apparently contained active phototrophs possessing chlorophyll b and in the deeper sediment chlorophyll b was not linked to chlorophyll a.
Fig. 4.5. Vertical distribution of peridinin and zeaxanthin-lutein concentrations in $\mu$g g$^{-1}$ sediment in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.01 and 1.4 for peridinin and 0.02 and 0.79 for zeaxanthin-lutein.

Fig. 4.6. Vertical distribution of peridinin and zeaxanthin-lutein to chlorophyll $a$ ratios in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.001 and 1.4 for peridinin and 0.03 and 0.63 for zeaxanthin-lutein.
Fig. 4.7. Vertical distribution of chlorophyll b concentrations in µg g⁻¹ sediment in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.006 and 1.3.

Fig. 4.8. Vertical distribution of chlorophyll b to chlorophyll a ratios in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.07 and 1.7.
VERTICAL DISTRIBUTION OF PHAEOPIGMENTS:

Four degradation products were found in the sediment and called phaeophorbide $a_1$, $a_2$ and phaeohytin $a_1$ and $a_2$ as described in Chapter 3. The distribution of phaeophorbides and phaeohytins and their ratios to chlorophyll $a$ are shown in Fig. 4.9 and Fig. 4.10. In the first three winter months phaeopigments were more concentrated than chlorophyll $a$ with phaeophorbide $a_2$ dominating the degradation products. During this time all four phaeopigments were evenly distributed in the top 1 cm of the sediment and their ratio to chlorophyll increased with depth. Concentration of chlorophyll $a$ started to rapidly increase in March and the surface value exceeded all degradation products. Both phaeophorbides showed lower values than in the previous 3 months and their concentrations decreased with depth. The decrease of phaeophorbide $a_2$ with depth was however less pronounced than chlorophyll $a$, resulting in an increase in its ratio to chlorophyll $a$ from 0.3 to 2 in the deepest layer. In March phaeohytin was more concentrated than phaeophorbide and although surface concentrations were also lower than chlorophyll $a$, phaeohytin became more concentrated in the 3-5$^{th}$ mm of the sediment and its ratio to chlorophyll $a$ increased by a factor of 10 within the top 1 cm of the sediment. In April, when chlorophyll $a$ concentration was at its maximum phaeohytins were again more concentrated than phaeophorbides and although they showed a strong decrease with depth their ratio to chlorophyll $a$ was still above 5 in the deeper layer of the sediment. Phaeophorbide $a_2$ was less concentrated than chlorophyll $a$ at the surface and continued to be so throughout the upper cm of the sediment. In May phaeohytin concentration exceeded chlorophyll $a$ even at the surface and stayed above 50$\mu$g g$^{-1}$ in the deeper layers. Phaeophorbide concentration showed an increase from the April values and exceeded chlorophyll concentrations below 1 mm.

DEPTH OF AEROBIC LAYER

The oxygenated depth of the sediment was measured with an O$_2$ micro-electrodes to examine the relationship between vertical pigment concentrations and oxic conditions in the sediment. The aerobic depth significantly changed over the 6 months period ($p=0.003$, $n=6$). When biomass and productivity increased in March, the oxic layer deepened from 1670$\mu$m to 1820$\mu$m and further increased in April to 1970$\mu$m. This was followed by a significant reduction in May to 1300$\mu$m (Fig. 4.11).
Fig. 4.9. Vertical distribution of phaeophorbides and phaeophytins concentrations in µg g\(^{-1}\) sediment in the upper 1 cm of sediment at station 2 on Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.005 and 0.7 for phaeophorbides and 0.02 and 1 for phaeophytins.

Fig. 4.10. Vertical distribution of phaeophorbides and phaeophytins to chlorophyll \(a\) ratios in the upper 1 cm of sediment at station 2 Hythe mudflat between December 1997 and May 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation between 0.03 and 0.83 for phaeophorbides and 0.07 and 1.26 for phaeophytins.
Fig. 4.11. Monthly changes of oxygenated sediment depth at station 2 between December 1997 and May 1998, determined with O₂ microelectrodes. Values and error bars are the averages and standard errors of six replicate depth profiles measured in two sediment cores.

where it showed a slight increase with depth, suggesting that anoxic layers throughout the depth of the sediment could be partially oxygenated by respiration. At station 3 and 4 the fucoxanthin to chlorophyll a ratio decreased with depth with a more than tenfold decline in station 2, indicating that other processes than diatom respiration were important in the deeper layers. At station 4 the diadinoxanthin to chlorophyll a ratio was lowest and showed no change in the deeper layers (Fig. 4.13). Diatoxanthin was more concentrated than diadinoxanthin down to the deepest part of the sediment at station 1 (Fig. 4.14). At all other stations diadinoxanthin dominated at the surface, but was exceeded by diatoxanthin at within the second (station 2 and 4) or the third layer. While the diadinoxanthin to chlorophyll a ratio was similar at each station and throughout the depth of sediment, there was a strong increase of the diatoxanthin ratio with depth at stations 2 and 4 (Fig. 4.13). The steady increase of diatoxanthin and its increase relative to chlorophyll a with depth suggests that the concentration of this pigment was not related to the amount because of selective respiration.

Small traces of peridinin were present at the very surface with highest concentration at station 4 (Fig. 4.16). Absolute values were very low, but its ratio to chlorophyll a increased below 2 mm depth. The depth variation in the pigments to chlorophyll a ratio for zeaxanthin - lutein and chlorophyll b was very similar (Figures 4.16-4.19).
4.3 SPATIAL COMPARISON OF THE VERTICAL STRUCTURE OF PIGMENTS AND DEGRADATION PRODUCTS

VERTICAL DISTRIBUTION OF MICROALGAL BIOMASS AND ACCESSORY PIGMENTS

The vertical distribution of photosynthetic pigments was measured once at each station between the 24th of March and the 9th of April, to examine spatial differences in vertical pigment distribution. Absolute concentrations and pigment ratios to chlorophyll $a$ at the four stations are shown in Figures 4.12 - 4.19. Chlorophyll $a$ concentrations in the top mm ranged from 103 to 153 $\mu g$ g$^{-1}$ with the highest value being found at station four (Fig. 4.12). At the three mudflat stations chlorophyll $a$ concentrations decreased exponentially with depth and only 2-12% of the surface values were found below 5mm. At station four the decrease with depth was less pronounced and 20% of the surface value was found in the 5-10mm section of the sediment.

Fucoxanthin, the indicator pigment for diatoms, showed a very similar depth distribution to chlorophyll $a$ (Fig.4.12). Its ratio to chlorophyll $a$ was highest at station 1, where it showed a slight increase with depth, suggesting that microalgal biomass throughout the depth of the sediment could be mainly attributed to diatoms. At station 2 and 3 the fucoxanthin to chlorophyll $a$ ratio decreased with depth with a more than twofold decline at station 2, indicating that other sources than diatoms contributed to biomass in the deeper layer. At station 4 the fucoxanthin to chlorophyll $a$ ratio was lowest and showed no change in the deeper layers (Fig.4.13). Diadinoxanthin was more concentrated than diatoxanthin down to the deepest part of the sediment at station 1 (Fig. 4.14). At all other stations diadinoxanthin dominated at the surface, but was exceeded by diatoxanthin in either the second (station 2 and 4) or the third mm. While the diadinoxanthin to chlorophyll $a$ ratio was similar at each station and throughout the depth of sediment, there was a very strong increase of the diatoxanthin ratio with depth at stations 2 and 3 (Fig.4.15). The depth distribution of diatoxanthin and its increase relative to chlorophyll $a$ with depth suggests that the concentration of this pigment was not related to the in situ biomass of microphytobenthos.

Small traces of peridinin were found at all four stations with highest concentration at station 4 (Fig. 4.16). Absolute values declined with depth, but its ratio to chlorophyll $a$ increased below 2mm depth. The depth distribution and pigment to chlorophyll $a$ ratio for zeaxanthin - lutein and chlorophyll $b$ were very similar (Figures 4.16-4.19). Highest
concentrations were found 2 mm below the surface at station 4. Elevated surface values were only found at station 2. The ratio to chlorophyll $a$ showed two strong trends: there was an increase with depth and a decrease towards the shore.
Fig. 4.12. Vertical distribution of chlorophyll $a$ and fucoxanthin concentrations in $\mu$g g$^{-1}$ sediment in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.02 and 1.2 for chlorophyll $a$ and 0.09 and 0.96 for fucoxanthin.

Fig. 4.13. Vertical distribution of fucoxanthin to chlorophyll $a$ ratios in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.02 and 0.7.
Fig. 4.14. Vertical distribution of diadinoxanthin and diatoxanthin concentrations in μg g\(^{-1}\) sediment in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show intervals of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.2 and 1.1 for diadinoxanthin and 0.1 and 1.4 for diatoxanthin.

Fig. 4.15. Vertical distribution of diadinoxanthin and diatoxanthin to chlorophyll a ratios in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.04 and 0.86 for diadinoxanthin and 0.07 and 0.84 for diatoxanthin.
Fig. 4.16. Vertical distribution of peridinin and zeaxanthin-lutein concentrations in $\mu$g g$^{-1}$ sediment in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.07 and 1.7 for peridinin and 0.19 and 1.6 for zeaxanthin-lutein.

Fig. 4.17. Vertical distribution of peridinin and zeaxanthin-lutein to chlorophyll $a$ ratios in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.06 and 1.7 for peridinin and 0.03 and 0.63 for zeaxanthin-lutein.
Fig. 4.18. Vertical distribution of chlorophyll b concentrations in μg g⁻¹ sediment in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.03 and 1.7.

Fig. 4.19. Vertical distribution of chlorophyll b to chl a ratios in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.07 and 1.7.
**PHAEOPIGMENT CONCENTRATIONS:**

The composition and depth distribution of phaeopigments and their ratio to chlorophyll a at the four stations is shown in Figures 4.20 and 4.21. Phaeopigments correlated strongly with chlorophyll a at each station with the highest $r^2$ value at station 1 ($r^2$ at S1= 0.96 p< 0.001). Phaeophorbides were the highest concentrated phaeopigments at station 1, whereas at station 2 the dominance shifted to phaeophytins. On the three mudflat stations phaeopigments were most concentrated in the upper 1 mm of the sediment, and although chlorophyll values were always higher in the surface layer, phaeopigment concentrations exceeded chlorophyll a in the 2nd or 3rd mm. The increase of phaeopigment ratios to chlorophyll a was strongest at station 3, where phaeophytin to chlorophyll a ratio increased to 19 in the deepest section.

At station 4 the maximum concentrations of phaeopigments were found in the 2-3 mm section. Relative to chlorophyll a phaeopigments were least concentrated at this station. The phaeophorbide to chlorophyll a ratio did not exceed 1 at any one depth and the highest phaeophytin to chlorophyll a ratio was 2.5 in the 2-3 mm section.

**CHANGE OF AEROBIC LAYER AT THE FOUR STATIONS**

The depth of the aerobic layer was similar at the three mudflat stations, ranging from 1680 to 1970μm (Fig. 4.22). The layer was deepest at station 2, which also had the highest $O_2$ production rate (see chapter 5). In comparison, the aerobic layer was very narrow at the marshpool (station 4) being only 920 μm, which was half the depth of those on the mudflat and significantly differed from the other 3 stations (p<0.0001, n=6).
Fig. 4.20. Vertical distribution of phaeophorbide and phaeophytin concentrations in μg g\(^{-1}\) sediment in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.06 and 1.3 for phaeophorbides and 0.09 and 1.5 for phaeophytins.

Fig. 4.21. Vertical distribution of phaeophorbide and phaeophytin to chlorophyll \(a\) ratios in the upper 1 cm of sediment at stations 1 - 4 on Hythe mudflat between March and April 1998. Crosses show the centre of sample sections. Data are the average of three replicates with a coefficient of variation ranging between 0.04 and 0.87 for phaeophorbides and 0.002 and 1.14 for phaeophytins.
Fig. 4.22. Change of oxygenated sediment depth at stations 1 - 4 in Hythe, March- April 1998, determined with $O_2$ microelectrodes. Values and error bars are the averages and standard errors of six replicate depth profiles.
4.4 DISCUSSION

The photozone in sediments and vertical distribution of microalgal biomass

The depth intertidal sediments that receives enough light for photosynthesis (photic zone) is known to depend on grain size and organic content (Fenchel and Straarup, 1971). In organically rich mud, similar to the sediment on Hythe mudflat, the photic zone was reported to be 0.4 mm (Jørgensen and des Marais, 1986). The depth over which sufficient light is available for photosynthesis is therefore very limited and a large proportion of viable algal biomass in Hythe mudflat was below the apparent sediment eutrophic zone. Similar results have been found in the majority of studies that have determined the depth distribution of chlorophyll in silty intertidal sediment (Fenchel and Straarup, 1971; Cariou-le Gall and Blanchard, 1995; Baranguet et al., 1997). Lucas and Holligan (1999) reported 14% of surface chlorophyll a values below 15mm in silty sediments of the Moolenplaat; in the Ems estuary 28% of surface chlorophyll a values was found between 0.5 and 2cm and chlorophyll was detected down to 10 cm (deJonge and Colijn, 1994). The distribution of viable algal biomass below the photic zone depends on the degree of sediment mixing, burial and bioturbation. The survival of microphytobenthos in the deeper layers is explained by their ability to migrate upwards (Palmer and Round, 1967) and a possible switch to heterotrophy (Admiraal, 1984). Compared to other investigations on similar sediment types, the decrease in chlorophyll a with depth was greater in Hythe sediment. During March and April, when chlorophyll a was at its maximum, less than 50% of the surface value was found in the second mm of the sediment and below 5mm less than 4% was found. These results suggest that there was comparatively little sediment mixing on Hythe mudflat.

The distribution of diatom-associated pigments

The only pigments that correlated strongly with chlorophyll a were indicative of diatoms, reaffirming the earlier conclusion that they were the main contributors to active microalgal biomass on Hythe mudflat. The vertical distribution of diadinoxanthin and diatoxanthin was investigated to assess whether xanthophyll cycling of epipelic diatoms was apparent in the sediment at a spatial resolution of 1mm. However, only diadinoxanthin correlated strongly with chlorophyll a. The depth distribution and the large increase in the diatoxanthin to chlorophyll a ratio with depth suggests that the concentration of this pigment was not linked to light-induced epoxidation at the sediment surface, and that any pigment cycling was overshadowed by large background levels of diatoxanthin in the sediment. A positive link between irradiance levels, sediment depth and xanthophyll cycling in epipelic
diatoms has not been established in situ, even when the effects of damaging UV radiation was studied on microbial mats (Sundbäck et al., 1996; Sundbäck et al., 1997; Underwood et al., 1999). There are several problems associated with tracing in situ xanthophyll cycling in sediments. Light attenuation occurs at a scale of micrometers and the sample resolution of millimetres might not be fine enough to trace light related pigment changes. The deposition of planktonic diatoms and dinoflagellates onto the sediment can mask in situ pigment conversions. Furthermore diadino and diatoxanthin have very different degradation rates (Hurley and Armstrong, 1990). Pigments containing the 5.6 epoxide such as fucoxanthin and diadinoxanthin undergo rapid discoloration in sediments, while depoxidated diatoxanthin, b-carotene and zeaxanthin are more stable (Repeta and Gagosian, 1987).

The distribution of other photosynthetic pigments

Peridinin concentrations, indicative of the deposition of planktonic dinoflagellates steadily increased between March and May; suggesting increased input of phytoplankton to the sediment in late spring. The ratio of peridinin to chlorophyll a increased every month with depth, suggesting that peridinin has a slower degradation rate than chlorophyll a although peridinin belongs to the group of pigments that are easily degraded due to their lack of the 5.6 epoxide (Repeta and Gagosian, 1987). It was surprising to find the highest concentrations of this pigment at station 4, which was the high shore station. The immersion period at this station was shortest, allowing less time for phytoplankton settlement, however wave action at this site was drastically reduced, which possibly facilitated deposition.

Lutein-zeaxanthin always showed highest concentrations in the 2nd to 5th mm of the sediment and no apparent input from the surface was evident, except at station 1. This leads to the conclusion that these pigments derived from macrophytic detritus, rather than cyanobacteria which is supported by the fact that chlorophyll b showed similar depth distributions with maximum concentrations below 2mm. In March and April chlorophyll b values were also high at the surface, suggesting that a second source of this pigment was present, which might have been active phototrophs such as euglenoids. However, taxonomic analysis did not reveal the presence of euglenoids at station 2 (details of taxonomy are shown in the following chapter).

Lutein - zeaxanthin is therefore not an ideal taxonomic marker in intertidal sediment, because it does not allow the differentiation between input from cyanobacteria and macrophytic detritus. Furthermore lutein and zeaxanthin are very stable carotenoids in
oxygenated and anoxic sediments (Abele-Oeschger, 1991) and their concentration can reflect the accumulation of input from the recent months. A potential taxonomic marker for cyanobacteria in sediments is mycoxanthophyll as this pigment has no other sources (Bianchi et al., 1993). A comparison between mycoxanthophyll, lutein-zeaxanthin and chlorophyll distribution would thus give a clearer picture about the input from detritus and blue green algae.

**Degradation processes of chlorophyll and other pigments**

In the first three winter months pigments deriving from diatom biomass were evenly distributed in the upper 1 cm of the sediment, indicating that degradation was slower than burial and sediment mixing. Phaeophorbide was the dominant phaeopigment present, which suggests that grazing by macrofauna was the most important process controlling chlorophyll transformation (Bianchi et al., 1988). The relatively high contribution of accessory pigment such as zeaxanthin-lutein suggest that degradation products did not only derive from microphytobenthos, but also consisted of detritus from the saltmarsh. In March when microalgal biomass increased there were two important changes: Phaeophytin was more concentrated than phaeophorbide suggesting that initial diatom mortality during the bloom was primarily due to senescence and microbial decomposition rather than grazing. The diatom associated pigments and chlorophyll degradation products showed a steeper slope with depth and values in the deeper layers of the sediment were lower than in winter, suggesting that decomposition was now faster than burial and sediment mixing. An increase in temperature could be one of the reasons for enhanced microbial activity and accelerated degradation of pigments as reported by Wassman et al. (1992). In April and May there was a strong increase of phaeophytin concentrations to the extent that this degradation product exceeded chlorophyll even at the surface in May.

The mineralisation of carbon from benthic diatoms is significantly reduced in anoxic sediment (Andersen, 1996). Pigments also degrade slowly in anoxic sediment and while chlorophyll $a$ is rapidly converted into phaeophytin in oxic and anoxic sediment, phaeophytin itself is very stable under anoxic conditions (Sun et al., 1993). From April to May there was a 34% reduction in the depth of the oxic layer which could explain the enhanced pigment preservation and higher surface values of phaeophytin. At station 4 the oxic layer was less than 1 mm thick, which was half the thickness of the mudflat stations. Concentrations of all photosynthetic pigments were higher throughout the analysed depth of sediment at station 4.
than of any other station. Although data suggest that microalgal biomass was more abundant at this station, it is likely that enhanced preservation of chlorophylls and carotenoids in an anoxic environment have contributed to the high concentrations of pigments.
CHAPTER 5

SEASONAL CHANGES IN PRIMARY PRODUCTION AND PHOTOADAPTATION OF MICROPHYTOBENTHOS

5.1 INTRODUCTION

Primary production of microphytobenthos on intertidal mudflats exhibits large variability, on both seasonal and short-term scales (Pinckney and Zingmark, 1993a; Kromkamp et al. 1995; Barranguet et al., 1998). The proportion of variation in production that can be explained by changes in chlorophyll ranges between 30% and 60% in most observations cited in the literature (see MacIntyre et al. 1996 for review). This suggests that a change in the photosynthetic response to environmental variables must account for some of the variability in primary production of microphytobenthos. The objective of the work described in this chapter was to examine the potential primary production and photo-adaptation of microphytobenthos and relate these to changes in environmental factors by: a.) determining the photosynthetic response of benthic microalgae to their variable environment and b.) to examine how much of the variability in primary production is due to a change in photosynthetic parameters.

The change in microphytobenthic community structure, photo-physiological adaptations and the photosynthetic efficiency from a seasonal and a spatial study at Hythe mudflat between December 1997 and May 1998 are reported and compared. The temporal study comprised a six months sample period at station 2 where primary production and photosynthetic parameters were measured using O₂ micro-electrodes and ¹⁴CO₂ uptake (in slurry), as well as the pigment composition and species identification of motile microphytobenthos. In the spatial survey the stations 1 to 4 along an intertidal transect were studied once for the same biological parameters during the spring bloom in March/April, 1998.
5.2 TEMPORAL CHANGES IN PHOTOSYNTHETIC CHARACTERISTICS

OVERVIEW: MONTHLY CHANGE OF BIOMASS AND PRIMARY PRODUCTION AT STATION 2, DECEMBER 1997-MAY 1998

Concentrations of chlorophyll a in the upper 1 mm of sediment at station 2 significantly increased in spring to reach a maximum value of 48 mg m⁻² in April (one way ANOVA, p<0.05 between March and April, n=5) and then decreased in May by 50% (Fig. 5.1a).

Oxygen production also increased towards April to its maximum of 21.1 mmol m⁻²h⁻¹ and decreased to 7.1 mmol m⁻²h⁻¹ in May (Fig. 5.1b). There was a significant difference between the months (p < 0.001) with April differing from January and February. Oxygen production was strongly correlated with biomass (r²=0.85, p=0.009) during the six month period. Biomass normalised productivity ranged between 2 and 14 mg O₂ mg chl a⁻¹ h⁻¹ with highest values being found in December and lowest in February (Fig. 5.1c). With the exception of the high December value there was a seasonal trend in biomass integrated production with an increase in April, however the differences between the months were not significant (p=0.137).

Photosynthetic capacity (P b max) and photosynthetic efficiency (α b) obtained from P vs E curves by ¹⁴CO₂ uptake both showed significant changes between the months (p=0.0003 for P b max, p=0.0015 for α b). P b max and α b showed highest values in December and decreased towards February (Fig. 5.2.a+b). There was an increase in April which coincided with the chlorophyll maximum. In May P b max decreased again while α b maintained the same value as in April. I s, the measure for photo acclimation, changed little in the first three months. There was a strong increase in March from 77.5 µE m⁻² s⁻¹ to 122.5 µE m⁻² s⁻¹ with a further rise in April to 144 µE m⁻² s⁻¹ (Fig. 5.2.c). The rise in I s coincided with increasing in situ irradiance in spring indicating that photo-acclimation to higher irradiances was occurring; in May I s decreased to 93 µE m⁻² s⁻¹ concurrently with the drop in microalgal biomass and P b max.
Fig. 5.1. Biomass and primary production of microphytobenthos at station 2, Hythe, December 1997-May 1998, a) chlorophyll \(a\) concentration in the upper 1 mm of the sediment; grey bars are the average of five replicate cores with standard errors, black bars are the average of the two cores in which \(O_2\) production was measured, b) gross production of oxygen measured with oxygen microelectrode at 550 \(\mu E m^{-2} s^{-1}\) in the upper 1 mm, bars and error bars are the averages and standard errors from six depth integrated production profiles of two cores, c) biomass integrated production, whereby \(O_2\) production was divided by chlorophyll \(a\) in the upper 1 mm of the same core, bars represent standard errors of six replicates.
Fig. 5.2. Photosynthetic characteristics of microphytobenthos at station 2, Hythe, December 1997-May 1998 as determined from $^{14}$CO$_2$ uptake experiments of slurry in the upper 1 mm, bars are the average and error bars the combined standard errors of two replicate measurements. a) Photosynthetic capacity ($P_{\text{max}}^b$) in the upper mm of the sediment, b) photosynthetic efficiency ($\alpha^b$) determined by the slope of the $P$ vs. $E$ curves, c) $I_k = P_{\text{max}}^b (\alpha^b)^{-1}$, index for photoacclimation.
TAXONOMY AND PIGMENT COMPOSITION OF MOBILE MICROPHYTOBENTHOS

To assess the change in community structure over the six months sample period, the mobile fraction of microphytobenthos was isolated from the sediment, identified and their pigments analysed. With the exception of a small proportion of cyanobacteria detected in February, all mobile phototrophs were benthic diatoms. The majority belonged to the genera *Nitzschia* and *Navicula*, with their relative contribution increasing in spring. *Navicula gregaria* was the most abundant species and reached 76% of cells counted during the chlorophyll *a* maximum in April (Figure: 5.3).

The pigment determination data corresponded closely with the taxonomic results (Fig. 5.4). Although small traces of chlorophyll *b* were found during some months, the only apparent accessory pigments present in the samples were chlorophyll *c1+c2*, fucoxanthin, diadino- and diatoxanthin and β-carotene, all of which occur in diatoms. There were temporal changes in the cellular pigments composition with significant differences in the fucoxanthin, diadinoxanthin and diatoxanthin to chlorophyll *a* ratios. The fucoxanthin to chlorophyll *a* ratio was highest in December (0.46) and then decreased to its lowest value in January. The fucoxanthin to chlorophyll *a* ratio of isolated diatom populations was very similar to the ratio found in the sediment throughout the entire depth during all the months apart from April, when a decrease in ratio occurred below 5mm (see chapter 4).

The proportion of diadinoxanthin fell from December to January and then increased towards the spring months with the highest ratio to chlorophyll *a* in May. The diatoxanthin to diadinoxanthin ratio peaked in January, but no seasonal trend was detectable. When these two pigments were added together to see whether the available pool for xanthophyll cycling was changing over the months, results showed that total xanthophyll content increased in spring with highest values in May. The total xanthophyll and accessory pigment ratio to chlorophyll *a* were related to photosynthetic parameters of the benthic diatoms at Hythe mudflat to investigate the role of photo-protection, but neither a relationship between accessory pigment/ chlorophyll *a* ratio to *P*<sub>max</sub>, α<sup>b</sup> and I<sub>k</sub> nor xanthophyll pigment/ chl *a* ratio to *P*<sub>max</sub>, α<sup>b</sup> and I<sub>k</sub> could be found.
Fig. 5.3. Distribution of algal groups and diatom species of the mobile fraction of microphytobenthos at station 2 of Hythe mudflat, December 1997 - May, 1998 collected via the lens tissue method. Values are expressed as percentage of 200 counted individuals.
Fig. 5.4. Variation of photosynthetic pigment composition in the motile fraction of microphytobenthos at station 2 of Hythe mudflat, December 1997-May, 1998. Values and errors are the average and standard errors of three replicates, note the different axis scales.
To assess the photo-adaptation of microphytobenthos to changes in environmental factors, chlorophyll \( a \) in the upper 1 mm of the sediment, primary production, measured with \( O_2 \) micro-electrodes and photosynthetic parameters, determined by \( ^{14}C \) P vs. E curves, were related to monthly changes in temperature, hours of exposure and light availability. For this purpose the tidal model, introduced in chapter 3, was run for the first 5 months of 1998 with slight modifications. For this year hourly values of solar radiation in \( \text{W m}^{-2}\text{h}^{-1} \) were available from Everton meteorological station. Hence instead of using daily averages, radiation during low tide period was selected, integrated over a 5 day period and correlated with production data and photosynthetic parameters by linear regression analysis.

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<td>0.9</td>
<td>10</td>
</tr>
<tr>
<td>vs ( I_t )</td>
<td>0.06</td>
<td>0.48</td>
<td>10</td>
</tr>
<tr>
<td>5 day Irradiance vs chl ( a )</td>
<td>0.63</td>
<td>(&lt;0.001)</td>
<td>25</td>
</tr>
<tr>
<td>vs ( O_2 ) production</td>
<td>0.5</td>
<td>(&lt;0.001)</td>
<td>30</td>
</tr>
<tr>
<td>vs ( O_2 ) chl ( a^{-1} ) (log10)</td>
<td>0.45</td>
<td>(&lt;0.001)</td>
<td>30</td>
</tr>
<tr>
<td>vs ( P^{\text{max}}_a )</td>
<td>0.009</td>
<td>0.78</td>
<td>10</td>
</tr>
<tr>
<td>vs ( \alpha^a )</td>
<td>0.12</td>
<td>0.33</td>
<td>10</td>
</tr>
<tr>
<td>vs ( I_t )</td>
<td>0.47</td>
<td>0.03</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 5.1: results of linear regression analysis with environmental variables as independent and biological variables as dependent factors; \( r^2 \geq 0.45 \) and \( p \leq 0.05 \) are printed in bold, \( r^2 \geq 0.75 \) and \( p \leq 0.001 \) are underlined.

Neither chlorophyll \( a \), primary production nor photosynthetic parameters correlated significantly with ambient temperature or hours of exposure. Irradiance at low tide integrated over five days had the strongest effect on biological parameters, with 63 % of microalgal biomass and 50% of \( O_2 \) production and 45% of \( O_2 \) production chl \( a^{-1} \) variation being explained by changes in irradiance (Table 5.1). Results also gave evidence for the occurrence of photo-acclimation as \( I_c \) correlated significantly with irradiance.
5.3 SPATIAL CHANGE OF PHOTOSYNTHETIC PARAMETERS OF MICROPHYTOBENTHOS AT THE FOUR STATIONS

CHLOROPHYLL A CONCENTRATIONS AND OXYGEN PRODUCTION:

Microalgal biomass, primary production and photosynthetic parameters were measured once at each of the four stations between March, 24th and April, 9th to examine whether microphytobenthos showed photo-adaptation to their different light environments. Chlorophyll a concentrations in the upper 1mm of the four stations ranged between 43-48 mg m$^{-2}$ and were not significantly different from each other (One way ANOVA, p=0.92, n=5). Oxygen production did not correlate with chlorophyll a ($r^2=0.02$, p=0.85) and station 2 showed the highest production rate with 21.1 mmol O$_2$ m$^{-2}$h$^{-1}$ (Fig. 5.5), differing significantly from the other stations (One way ANOVA, p=0.001, n=6). When production was biomass normalised, a spatial trend was apparent, with highest production per chlorophyll a at low shore station 1, decreasing landwards by more than 50%.

P VS. E PARAMETERS

A similar spatial trend was detectable for photosynthetic capacity ($P_{\text{max}}^b$) and photosynthetic efficiency ($\alpha^b$) whereby both parameters decreased towards high shore at station 4. $P_{\text{max}}^b$ declined from 11.5 mgC chl$^{-1}$ h$^{-1}$ at station 1 to 4.3 mgC chl$^{-1}$ h$^{-1}$ at station 4 with a twofold reduction between stations 3 and 4 (Fig. 5.6.a and 5.7); the change was significant between stations 1 and 2 and between stations 3 and 4 (one way ANOVA, p=0.001, n=2). $\alpha^b$ decreased fivefold from station 1 to station 4 (Fig. 5.6.b and 5.7), whereas $I_e$ the measure for photo-acclimation showed the opposite trend. $I_e$ increased from 121.5 $\mu$E m$^{-2}$s$^{-1}$ at station 1 to 190 $\mu$E m$^{-2}$s$^{-1}$ at station 3 and then decreased slightly to 177 $\mu$E m$^{-2}$s$^{-1}$ at station 4 (Fig. 5.6.c). Thus photo-adaptation seemed to be occurring with an increase of $I_e$ at the high shore stations, which are exposed to high irradiances at low tide for longer periods.
Fig. 5.5. Biomass and primary production of microphytobenthos at the four stations on Hythe mudflat, March–April 1998, a). chlorophyll a concentration in the upper 1 mm of the sediment; grey bars are the average of five replicate cores with standard errors, black bars are the average of the two cores in which O₂ production was measured, b). gross production of oxygen measured with oxygen micro-electrodes in the upper 1 mm at 550μEm⁻²s⁻¹, bars and error bars are the averages and standard errors of six depth integrated production profiles of two cores, c). biomass integrated production, whereby O₂ production was divided by chlorophyll a in the upper 1 mm of the same core; bars represent standard errors of six replicates.
Fig. 5.6. Photosynthetic characteristics of microphytobenthos at the four stations, Hythe, March-April 1998 as determined from $^{14}$CO$_2$ uptake in the upper 1mm of sediment, bars are the average and error bars the combined standard errors of two replicate measurements. a). Photosynthetic capacity ($P_{\text{max}}^b$) in the upper mm of the sediment, b). photosynthetic efficiency ($\alpha^b$) determined by the slope of the P.vs.E curves, c). $I_k = P_{\text{max}}^b (\alpha^b)^{-1}$, index for photoacclimation.
Fig. 5.7. Photosynthesis versus irradiance curves of microphytobenthos at the four stations on Hythe mudflat, March-April 1998, light circles & dashed lines and dark circles & solid lines represent individual samples from two replicate incubations.
TAXONOMIC COMPOSITION AND PHOTOSYNTHETIC CHARACTERISTICS:

Different populations of motile microphytobenthos were found at the four stations (Fig. 5.8) between March 24th and April 9th, 1998, with the three mudflat stations being exclusively inhabited by epipelic diatoms, while station 4 also contained 4% euglenoids and 1% cyanobacteria. The species composition of epipelic diatoms overlapped at the three mudflat stations and differed from the marshpool. *Navicula gregaria* was the most abundant species at all three stations at the time of sampling, ranging from 77 to 84% of all individuals counted, while only 0.5% were found at station 4. *Navicula cryptocephala* and *Fallacia pygmeae* were exclusive to station 4. The proportion of *Nitzschia* and *Navicula <20μm* increased landwards as did the contribution of other genera including *Amphora* and *Diploneis* but the latter contributed < 5% of cell counts at all stations. Thus the mudflat stations had overlapping populations with one dominating species, while station 4 had a larger diversity which consisted of smaller organisms.

PIGMENT COMPOSITION OF MOTILE MICROPHYTOBENHOS FRACTION

Slight changes in pigment composition between the four stations were detected with the most different pigment ratios at station 3 (Fig. 5.9). Chlorophyll c1+2 and fucoxanthin ratios to chlorophyll varied by less than 3% while the β-carotene to chl α ratio increased landwards by a third. The total xanthophyll pigment content also increased slightly, due to the relative increase of diadinoxanthin. The diatoxanthin to diadinoxanthin ratio decreased by more than 40%. A relationship between tidal height and relative content of photo-protectant or total accessory pigments could not be found.
Fig. 5.8. Relative abundance of algal groups and diatom species of the motile microphytobenthos fraction at the four stations of Hythe mudflat, March-April, 1998, percentages are from 200 counted cells.
Fig. 5.9. Variation of pigment ratios in the motile fraction of microphytobenthos at the four stations of Hythe mudflat, March-April 1998, values and errors are the averages and standard errors of three replicate samples, note the difference in axis scales.
Biomass, production and photosynthetic parameters in relation to changes of abiotic factors along the intertidal transect

The tidal model was applied again to calculate the difference in exposure duration and irradiance received by the four stations for 5 days prior to the individual sampling dates for each station. The results of the linear regression analysis between biological and environmental factors are shown in Table 5.2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$r^2$</th>
<th>$p$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>In situ temperature vs chl $a$</td>
<td>&lt;0.001</td>
<td>0.9</td>
<td>20</td>
</tr>
<tr>
<td>vs O$_2$ production</td>
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<td>0.18</td>
<td>24</td>
</tr>
<tr>
<td>vs O$_2$ chl $a^{-1}$</td>
<td>0.03</td>
<td>0.045</td>
<td>24</td>
</tr>
<tr>
<td>vs P$_{max}$</td>
<td>0.007</td>
<td>0.84</td>
<td>8</td>
</tr>
<tr>
<td>vs a</td>
<td>0.04</td>
<td>0.6</td>
<td>8</td>
</tr>
<tr>
<td>vs I$_k$</td>
<td>0.11</td>
<td>0.43</td>
<td>8</td>
</tr>
<tr>
<td>Hours of exposure vs chl $a$</td>
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<td>0.6</td>
<td>20</td>
</tr>
<tr>
<td>vs O$_2$ production</td>
<td>0.07</td>
<td>0.19</td>
<td>24</td>
</tr>
<tr>
<td>vs O$_2$ chl $a^{-1}$</td>
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<td>0.002</td>
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<tr>
<td>vs P$_{max}$</td>
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<td>&lt;0.001</td>
<td>8</td>
</tr>
<tr>
<td>vs $\alpha^b$</td>
<td>0.69</td>
<td><strong>0.01</strong></td>
<td>8</td>
</tr>
<tr>
<td>vs I$_k$</td>
<td>0.19</td>
<td>0.27</td>
<td>8</td>
</tr>
<tr>
<td>5 day Irradiance vs chl $a$</td>
<td>0.013</td>
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<td>20</td>
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<tr>
<td>vs O$_2$ production</td>
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<td>0.02</td>
<td>24</td>
</tr>
<tr>
<td>vs O$_2$ chl $a^{-1}$</td>
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</tr>
<tr>
<td>vs P$_{max}$</td>
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<td>0.01</td>
<td>8</td>
</tr>
<tr>
<td>vs $\alpha^b$ (sq root)</td>
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<td>&lt;0.001</td>
<td>8</td>
</tr>
<tr>
<td>vs I$_k$ (log10)</td>
<td><strong>0.56</strong></td>
<td>0.03</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 5.2. Results of linear regression analysis with environmental variables as independent and biological variables as dependent factors; $r^2 \geq 0.5$ and $p \leq 0.05$ are printed in bold, $r^2 \geq 0.75$ and $p \leq 0.01$ are underlined.

None of the biological parameters were related to temperature, which agreed with the results of the monthly data at station 2, described in the previous section (Table 5.2). Exposure duration and irradiance during exposure strongly influenced P$_{max}^b$. The relationship was negative, with P$_{max}^b$ decreasing with increasing exposure time and irradiance. A strong negative relationship with irradiance was also found for photosynthetic efficiency ($\alpha^b$) and for biomass integrated oxygen production, whereby the difference in irradiance accounted for 85% of variation in $\alpha^b$ and 50% of variation in O$_2$ production per chlorophyll $a$. In contrast I$_k$ was positively related to irradiance, giving further evidence that photo-acclimation occurred in the microalgal populations studied.
5.4 DISCUSSION

O$_2$ production vs. $^{14}$CO$_2$ uptake

Potential primary production in the form of $^{14}$CO$_2$ uptake and O$_2$ production of sediment samples collected at different stations and in different months was measured in the laboratory under constant conditions to focus on the photosynthetic response of microphytobenthos to changes in environmental factors. Only a few studies have used O$_2$ production estimated with micro electrodes and $^{14}$CO$_2$ uptake simultaneously to calculate production rates of benthic microalgae and comparisons between the two methods has shown some discrepancies (Revsbech et al. 1981, Barranguet et al. 1998 and Wolfstein and Hartig, 1998). In this study production measurements with both methods were in reasonably good agreement with a correlation coefficient of 0.69 (p=0.03, n=9).

Fig. 5.10: Scatter plot between $P_{\text{max}}^a$ determined by $^{14}$CO$_2$ uptake at 25 irradiance levels and O$_2$ production at 550$\mu$E m$^{-2}$ s$^{-1}$; $r = 0.69$, determined with a Pearson product moment correlation (p = 0.03 and n = 9). Values are the averages of 6 replicate measurements of O$_2$ production and 2 replicate measurements of $P_{\text{max}}^a$ as determined by $^{14}$CO$_2$ uptake.

The variation between the two methods was greater at the lower range of O$_2$ production measurements, ie <0.15mmol O$_2$ mg chl a$^{-1}$ (Fig. 5.10). The sensitivity of the O$_2$
measurements was lower than that of the $^{14}$CO$_2$ uptake technique. Thus the majority of O$_2$ production values were below the detection limit in December, January and February and while O$_2$ production could not be detected, P$_{\text{max}}^n$ estimated by $^{14}$CO$_2$ uptake was highest in December and January.

The average P$_{\text{max}}^n$ value ($\mu = 1.1$ mmol C mg chl $a$ h$^{-1}$) was 2.4 times higher than O$_2$ production ($\mu = 0.45$ mmol O$_2$ mg). There are a number of reasons why the results of the two methods differed: Both methods measured production at saturating irradiances (as $I_k$ never exceeded 200 $\mu$E m$^{-2}$s$^{-1}$), but O$_2$ production measured with micro-electrodes took the actual photic zone into account, while the $^{14}$C method measured the potential production within the upper 1 mm. In silty clay the photic zone is extremely limited and production depth profiles measured with O$_2$ electrodes revealed no production below 600$\mu$m. Chlorophyll $a$ however was found below the photic zone (see also chapter 4), which indicates the presence of potential primary producers. Furthermore chemical micro-gradients of nutrients and CO$_2$ are potentially eliminated when a sediment suspension is prepared for $^{14}$CO$_2$ uptake experiments and any limitations which could exist in situ are removed. A discrepancy between CO$_2$ and O$_2$ measurements with higher values for CO$_2$ as encountered in this study strongly suggests that the photic zone was significantly narrower than 1 mm and possibly that in situ nutrient limitations existed. Although a photosynthetic quotient of 1 is proposed for benthic microalgae in some studies (Barranguet et al. 1998; Hargrave et al. 1983), it is argued whether the quotient is not higher, such as 1.2 (Asmus, 1982; Wolfstein and Hartig, 1998) or 1.3 (Colijn et al., 1983). If the photosynthetic quotient is higher than 1, the difference between potential production measured by $^{14}$CO$_2$ uptake and the actual O$_2$ production in undisturbed sediment in this study was even greater.

Primary production on Hythe mudflat compared to other systems

Primary production values of microphytobenthos on Hythe mudflat lie within the upper range of values reported for comparable systems at similar latitudes (Barranguet et al., 1998, Pinckney and Zingmark, 1993a, MacIntyre et al., 1996) suggesting that the mudflat was potentially very productive. Biomass normalised production and photosynthetic potential were very similar to those reported for the Gironde estuary, France (Blanchard and Cariou-le Gall), 1994 and the Molenplat, Netherlands (Barranguet et al. 1998). However the efficiency to reach P$_{\text{max}}^n$ was higher than in other studies resulting in comparatively lower $I_k$ values. $I_k$ values for microphytobenthos range from 310 to 680 $\mu$Em$^{-2}$ s$^{-1}$ in the above cited literature, while in Hythe they were below 160 $\mu$Em$^{-2}$ s$^{-1}$ during the temporal study and between 120 and 190
\( \mu \text{Em}^2 \text{ s}^{-1} \) in the spatial comparison. These values were however in the same range as those reported for tidal flats of the German Wadden Sea (Hartig et al., 1998; Wolfstein and Hartig, 1998). The high photosynthetic efficiency and low \( I_k \) values suggest that microphytobenthos on Hythe mudflat were more shade adapted. The sediment consists of silty clay, which attenuates light rapidly, however it is not certain whether this directly influences the photo-acclimation of microphytobenthos. Different grain sizes did not seem to cause a difference in \( I_k \) values on the Moolenplat (Barranguet et al. 1998) and migration to the sediment surface at low tide should eliminate any light limitations within the sediment. The relationship between light attenuation in sediment, diatom migration and photo-acclimation needs to be investigated in more detail and new non-destructive techniques such as PAM (pulse-amplitude-modulated) fluorescence may provide this (Hartig et al., 1998).

**Temporal and spatial photo-adaptation**

When results from both the seasonal and spatial survey were combined and related to environmental factors by linear regression, chlorophyll \( a \) \( (r^2 = 0.74, p= 0.001 \) for log10 transformed chl \( a \) data), \( I_k \) \( (r^2 = 0.58, p<0.001) \), gross primary production \( (r^2 = 0.38, p<0.001) \) and \( \alpha^B \) \( (r^2=0.32, p=0.02) \) correlated significantly with irradiance during tidal exposure. This suggest that the mechanism regulating photo-acclimation and growth of microalgal biomass were similar during the temporal and spatial survey.

Biomass normalised production, measured with the \( \text{O}_2 \) micro-electrodes and \( P^B_{\text{max}} \) measured by \(^{14}\text{CO}_2\) incorporation, only correlated significantly with irradiance during tidal exposure when the results from the seasonal and spatial surveys were separated prior to linear regression analysis, as the relationship between biomass normalised production and irradiance was positive in the seasonal study and between irradiance and biomass normalised production and \( P^B_{\text{max}} \) negative in the spatial study. It can therefore be assumed that two types of photosynthetic adaptation to changes in irradiance were occurring. Results of the temporal study gave some evidence that long term photo-adaptation in microphytobenthos was occurring, which resulted in higher photosynthetic output when irradiance and length of photoperiod increased in spring.

In contrast, data from the spatial study suggested that microalgae at the low shore station compensate for the limited light availability with higher photosynthetic output (ie higher \( P^B_{\text{max}} \) and \( \alpha^B \)). Microalgae at station 1 had the highest photosynthetic potential and the highest efficiency to reach \( P^B_{\text{max}} \): there was a steady decline of these two parameters towards
high shore with low $P_{\text{max}}^b$ and $\alpha^b$ values at the marsh pool. As the light exposure increased towards high shore, microphytobenthos at Hythe mudflat increased their $I_6$ value which shows that they became more light adapted. Microphytobenthos inhabiting intertidal mudflats migrate to the surface of the sediment at low tide to photosynthesise and in turbid estuaries such as Southampton Water it be assumed that the duration of low tide during daylight exposure equals to the photoperiod of benthic microalgae. Along the intertidal transect emersion periods changed dramatically, for instance at the time sampled in this spatial study the hours of exposure integrated over a 5 day period ranged from 15 hours at station 1 to 63 hours at station 4. While the marsh pool contained a different community of phototrophs, the species composition on the three mudflat stations was almost identical, indicating that the photosynthetic response to changes in irradiance was a photo-physiological adaptation rather than an ecological one. This relationship has not been demonstrated to the same extent prior to this study. Results from comparable studies showed no differences in the photosynthetic potential of microalgae from stations of different tidal heights (Barranguet et al., 1998), or different light environments (Pinckney and Zingmark, 1993b). Photoacclimation occurs either by a change in the size or the number of photosynthetic units (Falkowski, 1981) and the nature of photoacclimation can be determined by observing changes of $P_{\text{max}}^a$ and $\alpha^a$. The size of the photosynthetic units are changed when $P_{\text{max}}^a$ is increased and $\alpha^a$ remains the same (Falkowski, 1981). Since $P_{\text{max}}^a$ was actually lower in the light-adapted algae in this study, it was not possible to identify the nature of photoacclimation of microphytobenthos on Hythe mudflat.
CHAPTER 6

SHORT-TERM CHANGES IN PHOTOSYNTHETIC PARAMETERS OF MICROPHYTObENTHOS

6.1 INTRODUCTION

During alternating periods of tidal immersion and exposure, benthic microalgae in the intertidal zone are exposed to rapid changes in irradiance and temperature. A migratory endogenous rhythm in tune with the tide has been established for many species of epipelic diatoms (Palmer and Round, 1967) and has been used to explain short term variation in rates of photosynthesis (Pinckney and Zingmark, 1991). A series of controlled experiments were conducted with cultured and field populations of benthic diatoms, collected from Hythe mudflat, to examine whether they show a periodicity in photosynthetic parameters. The influence of irradiance on photosynthetic parameters during one photoperiod was also investigated. The analysis of photosynthetic pigments was carried out concurrently to examine the relationship between irradiance, photosynthetic capacity ($P_{\text{max}}$) and efficiency ($\alpha$) and cellular pigment composition with special reference to the xanthophyll cycle.
6.2 RESULTS

EXPERIMENT 1: CHANGES IN PHOTOSYNTHETIC PARAMETERS OF NITZSCHIA CLOSTERIUM IN CULTURE DURING ONE PHOTOPERIOD

A culture of the diatom *Cylindrotheca closterium*, isolated from Hythe mudflat, was incubated at 50 μE m⁻² s⁻¹ on a dark/light cycle of 12/12 hours. When the culture reached its sub-saturating phase three P vs E curves with 25 irradiance levels were constructed: 1) at the onset of the photoperiod, 2) after six hours and 3) after twelve hours. This preliminary experiment was carried out to optimise experimental conditions and establish the existence of an endogenous rhythm in photosynthetic parameters. The photosynthetic capacity $P_{\text{max}}$ increased by 28% and the photosynthetic efficiency $\alpha^B$ by 44% after six hours of light exposure; both parameters decreased to their original values after 12 hours (Fig. 6.1). The changes of $P_{\text{max}}$ and $\alpha^B$ were both significant ($p=0.00$ for $P_{\text{max}}$ and $p=0.001$ for $\alpha^B$). $I_k$ showed a small decrease in the first half of the photoperiod from 13.2 to 11.8 μE m⁻² s⁻¹ but then increased to 15.4 μE m⁻² s⁻¹ after twelve hours (Table 6.1).

The cellular pigment composition of *Cylindrotheca closterium* also changed during the 12 hour light period (Fig. 6.2). Chlorophyll $a$ concentration in the culture did not change significantly over time ($p=0.6$). The ratios of chlorophyll $c_1+c_2$, fucoxanthin, diadinoxanthin and β-carotene to chlorophyll $a$ remained constant. There was a significant increase in the diatoxanthin to chlorophyll $a$ ratio ($p=0.02$) and the diatoxanthin to diadinoxanthin ratio ($p=0.01$) with time; both ratios increased almost threefold in 12 hours (Table 6.1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 hours</th>
<th>6 hours</th>
<th>12 hours</th>
<th>p (Anova)</th>
</tr>
</thead>
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<tr>
<td>$P_{\text{max}}$ (mg C mg chl $a$⁻¹ h⁻¹)</td>
<td>1.2±0.09</td>
<td>1.5±0.1</td>
<td>1.2±0.08</td>
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</tr>
<tr>
<td>$\alpha^B$ (mg C mg chl $a$⁻¹ h⁻¹ (μE m⁻² s⁻¹)⁻¹)</td>
<td>0.09±0.001</td>
<td>0.13±0.007</td>
<td>0.08±0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>$I_k$</td>
<td>13.2±1</td>
<td>11.8±3</td>
<td>15.4±3</td>
<td>-</td>
</tr>
<tr>
<td>chlorophyll $c_1+c_2$/chl $a$</td>
<td>0.159</td>
<td>0.153</td>
<td>0.163</td>
<td>0.4</td>
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<td>fucoxanthin/chl $a$</td>
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<td>0.351</td>
<td>0.359</td>
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<td>0.036</td>
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<td>0.0073</td>
<td>0.012</td>
<td>0.02</td>
</tr>
<tr>
<td>diatox/diadino</td>
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<td>0.198</td>
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<td>0.01</td>
</tr>
<tr>
<td>β-carotene</td>
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<td>0.026</td>
<td>0.028</td>
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</table>

Table 6.1. Photosynthetic characteristics and pigment ratios after 0, 6 and 12 hours incubation of *Cylindrotheca closterium* at 50 μE m⁻² s⁻¹ as determined by $^{14}$CO₂ uptake, errors are standard errors as determined by P vs E curve fitting and combined for $I_k$. Underlined values in columns 2-4 represent significantly different results from the Bonferroni pair wise comparisons, where $p<0.05$. 

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Fig. 6.1. Change of P vs E parameters of *Cylindrotheca closterium* incubated at 50µE m⁻² s⁻¹ during a 12 hour photoperiod; error bars are standard errors determined from P vs E curve fitting.

Fig. 6.2. Change of pigment ratios of *Cylindrotheca closterium* incubated at 50µE m⁻² s⁻¹ during a 12 hour photoperiod; values and errors are the average and standard error of three replicates.
EXPERIMENT 2: CHANGES IN PHOTOSYNTHETIC PARAMETERS OF AN ISOLATED FIELD
POPULATION OF EPIPELIC DIATOMS DURING ONE EMISSION PERIOD

Diatoms were collected from station 2 of Hythe mudflat at low tide in March, 1998 and isolated via the lens tissue method on return to the lab. After being kept in the dark the cell suspension was exposed to an irradiance of 250 μE m⁻² s⁻¹, 3 hours before the following low tide. Three P vs. E curves were constructed from ¹⁴CO₂ incorporation measurements at 25 irradiance levels after 0 hours, 3 hours and 6 hours of incubation at 250 μE m⁻² s⁻¹. $P_{\text{max}}^a$ increased by 76% and $\alpha^b$ by 50% in the first three hours (Fig. 6.3). At the end of the emersion period both parameters decreased to their original values. Changes of both parameters were significant (Table 6.2). $I_a$ increased after three hours from 100 to 117 μE m⁻² s⁻¹ and then decreased to 84 μE m⁻² s⁻¹ after six hours light incubation (Table 6.2).

The cellular pigment composition of the diatom field population changed significantly during the six hour light incubation (Table 6.2). The ratio of diadinoxanthin to chlorophyll $a$ decreased by 37%, the diatoxanthin to chlorophyll $a$ ratio increased almost twofold (Fig. 6.4). In addition the β-carotene to chlorophyll $a$ ratio underwent significant changes (Table 6.2).

To examine how long it took for the cells to reach maximum photosynthesis a subsample of the population was incubated at 550 μE m⁻² s⁻¹ at the onset of the photoperiod. ¹⁴CO₂ uptake was measured for the first 20 minutes, during which time 1 ml sub-samples were preserved with formaldehyde every 30 seconds for the first 5 minutes and every 60 seconds thereafter (after MacIntyre and Geider, 1996). Figure 6.5 shows the changes in the rate of photosynthesis at 550 μE m⁻² s⁻¹ for the 20 minutes duration. After an increase in the photosynthetic rate for the first 12 minutes, the curve reached a plateau around 4.5 mg C mg chl $a$⁻¹ h⁻¹.

<table>
<thead>
<tr>
<th>$P_{\text{max}}^a$ (mg C mg chl $a$⁻¹ h⁻¹)</th>
<th>0 hours</th>
<th>3 hours</th>
<th>6 hours</th>
<th>p (Anova)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[mg C mg chl $a$⁻¹ h⁻¹(μE m⁻² s⁻¹)]</td>
<td>5⁺⁻±0.2</td>
<td>8.8⁺⁻±0.3</td>
<td>4.2⁺⁻±0.2</td>
<td>0.000</td>
</tr>
<tr>
<td>$\alpha^b$</td>
<td>0.05⁺⁻±0.006</td>
<td>0.075⁺⁻±0.009</td>
<td>0.05⁺⁻±0.008</td>
<td>0.000</td>
</tr>
<tr>
<td>$I_a$ (μE m⁻² s⁻¹)</td>
<td>100⁺⁻±6</td>
<td>117⁺⁻±4</td>
<td>84⁺⁻±2</td>
<td>-</td>
</tr>
<tr>
<td>chlorophyll c $a$/ chl $a$</td>
<td>0.126</td>
<td>0.143</td>
<td>0.126</td>
<td>0.7</td>
</tr>
<tr>
<td>fucoxanthin/ chl $a$</td>
<td>0.42</td>
<td>0.43</td>
<td>0.44</td>
<td>0.265</td>
</tr>
<tr>
<td>diadinoxanthin/ chl $a$</td>
<td>0.073</td>
<td>0.049</td>
<td>0.046</td>
<td>0.000</td>
</tr>
<tr>
<td>diatoxanthin/ chl $a$</td>
<td>0.012</td>
<td>0.016</td>
<td>0.023</td>
<td>0.004</td>
</tr>
<tr>
<td>diatoxanthin/ diadinoxanthin</td>
<td>0.16</td>
<td>0.28</td>
<td>0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>β-carotene/ chl $a$</td>
<td>0.11</td>
<td>0.06</td>
<td>0.065</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 6.2. Photosynthetic characteristics and pigment ratios after 0, 3 and 6 hours incubation of isolated microphytobenthos field populations at 250 μE m⁻² s⁻¹ as determined by ¹⁴CO₂ uptake, errors are standard errors as determined by P vs E curve fitting and combined for $I_a$. Underlined values in columns 2-4 represent significantly different results from the Bonferroni pair-wise comparisons, where p<0.05.
P vs E curve after 0 hours of light

\[ P_{\text{max}}^B = 5.0 \quad \alpha^B = 0.05 \]

P vs E curve after 3 hours of light

\[ P_{\text{max}}^B = 8.8 \quad \alpha^B = 0.075 \]

P vs E curve after 6 hours of light

\[ P_{\text{max}}^B = 4.2 \quad \alpha^B = 0.05 \]

Fig. 6.3. Change of P vs E curves of field samples of benthic microalgae over a 6 hour photoperiod in March, 1998.
Fig. 6.4: Change of cellular pigment composition in a field population of benthic diatoms, incubated at 250μEm^{-2}s^{-1} during one photoperiod.

Fig. 6.5. Photosynthetic rate of isolated field population of microphytobenthos incubated at 550μE m^{-2} s^{-1} in the first 20 minutes.
 Experiment 3: Comparison between the variation of photosynthetic parameters of epipelic diatom populations incubated in the dark and the light during one photoperiod

To test whether the changes in photosynthetic parameters detected in experiment 1 and 2 were dependent on irradiance during the light incubation a third experiment was designed to compare changes in photosynthetic parameters of epipelic diatom populations incubated in the light and in the dark during one photoperiod.

A field population of epipelic diatoms was collected at station 2 of Hythe mudflat in August, 1998 as described for the previous experiment. The cell suspension was kept in the dark at 15°C. 2 hours before the following low tide 14CO2 uptake at 15 different irradiance levels was measured to construct the initial P vs. E curve. The suspension was then divided into two subsamples, one was kept in the dark at 15°C and one was incubated at 250 µEm-2 s-1 at 15°C. P vs. E curves with 15 irradiance levels were constructed from both samples after 2 hours and 4 hours.

The subpopulation which was incubated in the light showed a very strong increase in Pmax after 2 hours to 14.8 mg C mg chl a-1 h-1. Pmax after two hours of light incubation was significantly different to the Pmax reached after 2 hours of dark incubation, but also to 0 hours and 4 hours in the light and the dark (Table 6.4). The rise in Pmax was accompanied by an increase in α from 0.05 to 0.08 mg C mg chl a-1 h-1 (µEm-2 s-1)-1. After 4 hours both parameters decreased more than twofold. Ik also increased after two hours to 195 µEm-2 s-1 and then decreased to 131 µEm-2 s-1. Results indicate that although some internal rhythm was present, exposure to light apparently triggered adaptive mechanisms, which caused the photosynthetic potential and efficiency to increase. Ik increased under both incubation conditions in the initial two hours but large standard errors impede further conclusions.
Table 6.3. Photosynthetic characteristics after 0, 2 and 4 hours incubation of isolated field populations at 250 μE m⁻² s⁻¹ and in the dark as determined by ¹⁴CO₂ uptake. Errors are standard errors as determined by P vs E curve fitting and combined for Ik; p values were determined by a one way ANOVA for Pmax and RM ANOVA for αB.

<table>
<thead>
<tr>
<th></th>
<th>0 hours</th>
<th>2 hours light</th>
<th>2 hours dark</th>
<th>4 hours light</th>
<th>4 hours dark</th>
<th>p Anova</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pmax</td>
<td>4.8±0.2</td>
<td>14.8±0.8</td>
<td>6.9±1.1</td>
<td>5.9±0.3</td>
<td>3.8±0.1</td>
<td>0.000</td>
</tr>
<tr>
<td>αB</td>
<td>0.05±0.006</td>
<td>0.08±0.004</td>
<td>0.03±0.007</td>
<td>0.045±0.004</td>
<td>0.034±0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Iκ</td>
<td>96±13</td>
<td>195±11</td>
<td>230±63</td>
<td>131±15</td>
<td>112±9</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 6.4. Results of the post ANOVA pairwise comparison for Pmax and αB (Bonferroni) (s = significant difference as p<0.05, ns = not significant as p>0.05, nt = not tested).

Pigment composition changed over the four hour period in the light incubation. Fucoxanthin to chlorophyll a ratios remained at 0.32 throughout the incubation period. β-carotene to chlorophyll a ratios ranged between 0.054 and 0.057 with no differences between light and dark incubation, with one outlier at t0 (0.049). There was a steady and significant decrease of the diadinoxanthin to chlorophyll a ratio during the light incubation (p<0.001), while the ratio did not change in the population that was incubated in the dark. The ratio of diatoxanthin behaved more sporadically with no apparent relationship with duration of incubation or light level.

Table 6.5. Pigment ratios after 0, 2 and 4 hours incubation of isolated field populations at 250 μE m⁻² s⁻¹ and in the dark, values are the averages of three replicates. Underlined values represent significant different results from the Bonferroni pairwise comparisons, where p<0.05.
Fig. 6.6. The change of photosynthetic parameters of field populations of benthic diatoms collected from station 2 on Hythe mudflat and incubated at 250μEm⁻²s⁻¹ and in the dark during a 4 hour emersion period, August, 1998.
EXPERIMENT 4: COMPARISON OF SHORTTERM VARIATION IN PHOTOSYNTHETIC PIGMENT

COMPOSITION OF EPIPELIC DIATOM POPULATIONS INCUBATED AT DIFFERENT IRRADIANCE LEVELS

Results from the previous experiments in this chapter have shown that the cellular pigment composition in benthic diatoms changes during light incubation. A further experiment was therefore designed to focus on the changes in pigment ratios at different irradiance levels. Diatoms were collected in the field at low tide in June, 1998 and isolated via the lens tissue method on return to the lab and then incubated in the dark at 15°C. The following day at the time of low tide, the suspension was divided into four subsamples which were incubated at 0, 250, 500 and 2000 µE m⁻² s⁻¹. Samples were collected for HPLC pigment analysis every 20 minutes for the first 60 minutes and then after 100 and 140 minutes. A two way ANOVA with light and time as fixed factors was carried out for each pigment to test whether changes in the cellular pigment composition were significant. Ratios of chlorophyll c1+2, fucoxanthin and β-carotene to chlorophyll a did not significantly change with time and irradiance. Diadinoxanthin to chlorophyll a ratios of samples incubated at 500 and 2000 µE m⁻² s⁻¹ decreased by 40% after 140 minutes with the maximum change occurring after 100 minutes of incubation. Diatoxanthin to chlorophyll a ratios increased fivefold at 250 µE m⁻² s⁻¹ and ten fold at 500 and 2000 µE m⁻² s⁻¹ (Fig. 6.6). Maximum changes for the two high levels of irradiance occurred in the first 20 minutes and between 40 and 60 minutes in the sample incubated at 250 µE m⁻² s⁻¹. The diatoxanthin/diadinoxanthin ratio increased seven fold at 250, 20 fold at 500 and 17 fold at 2000 µE m⁻² s⁻¹ (Fig. 6.6).
Fig. 6.7. Change of cellular pigment ratios in an isolated field population of benthic diatoms collected at station 2 on Hythe mudflat, June 1998; incubated at four different irradiance levels.
6.3 DISCUSSION

Variations in the photosynthesis-irradiance relationship

A number of authors have reported short-term variations in biomass integrated photosynthesis of microphytobenthos in situ and attributed these to fluctuations of environmental variables. The main controlling factors have been shown to include irradiance (Blanchard and Cariou-le Gall, 1994), temperature (Barranguet et al., 1998; Blanchard et al. 1996)) and possibly CO₂ concentrations (Kromkamp et al., 1995). In situ short-term variations in primary production have not been consistently demonstrated and the existence of an endogenous photosynthetic rhythm has yet to be demonstrated (Kromkamp et al., 1998). In this laboratory based study, fluctuating physical factors were eliminated to concentrate on photosynthetic variation due to internal periodicity and the influence of irradiance. Results from experiments 1 and 2 suggest that an internal rhythm is present in both benthic diatoms maintained in culture and in field populations, as photosynthetic capacity and efficiency changed during the course of the photoperiod without changes in the external physical environment. While the increase in photosynthetic potential in the middle of the photoperiod could be attributed to light adaptation, the decrease towards the end of the photoperiod may be explained by cellular regulation as limitation by CO₂ or nutrient concentrations is unlikely.

Diel periodicity of photosynthetic parameters in phytoplankton cultures on light-dark cycles has been shown to occur in a number of species, but is not universal (Harding et al., 1981). *Cylindrotheca closterium*, which was tested by Harding et al., 1981 as well as in this study showed different amplitudes in the photosynthetic variation, which was possibly due to different incubation and measuring techniques. In natural populations of phytoplankton diurnal oscillations in photosynthetic parameters have also been shown, whereby the amplitude and the timing of maximum photosynthetic capacity changes with species composition and habitat (Erga and Skjoldal, 1990; Harding et al., 1982). This rhythm can persist under a constant light regime for several days (Legendre et al., 1988). Epipelic diatoms isolated from fresh water habitats were shown to undergo rhythmic diel changes in photosynthesis, whereby the photosynthetic periodicity was closely linked to their migratory behaviour (Brown et al. 1972). Maximum cell emergence to the sediment surface coincided with maximum photosynthetic rate (measured at one irradiance level) at mid-morning, after which there was a continuos
decrease of both activities until night. The initiation of increased photosynthesis and upward migration occurred during the night and thus was not triggered by phototaxis.

**Semi diurnal periodicity**

The results from experiment 2 suggest that the variation in photosynthetic parameters was not circadian but semidiurnal, as $P_{\text{max}}^b$ decreased to its original value after six hours of incubation. As a semidiurnal rhythm in migratory behaviour has been shown for some epipelic diatoms (Palmer and Round, 1967) and irradiance is strongly limiting at high tide, a photosynthetic rhythm linked to the tidal regime seems a plausible ecological adaptation in the intertidal habitat. It suggests that migration and photosynthesis are coupled and are triggered by the same external stimulus (Zeitgeber: Bunning, 1967). Evidence for a semidiurnal rhythm in $a^b$ and chlorophyll $a$ in estuarine phytoplankton has been given by Legendre et al., 1985; the rhythm was phased on the semidiurnal variation of vertical tidal mixing. Epipelic diatoms have shown photosynthetic periodicity in accordance with the tide when inhabiting the intertidal zone (Brown et al., 1972; Smith and Underwood, 1998) but not the subtidal zone (Blanchard and Montagna, 1992). Therefore comparing results from the literature and this study suggest that when irradiance changes induced by the tides are as strong as the diel variation in irradiance, photosynthesis shows a semidiurnal rather than a circadian rhythm.

**The effect of irradiance**

Field populations of epipelic diatoms in experiment 3 were shown to increase their photosynthetic capacity ($P_{\text{max}}^a$) and efficiency ($\alpha^b$) in the middle of the photoperiod with or without illumination. These results suggest that the endogenous photosynthetic rhythm persisted in epipelic diatoms in the dark, but that the amplitude of the photosynthetic variation was smaller. Two hours after the onset of the photoperiod, the photosynthetic capacity of samples incubated in the light was twice as high and the efficiency three times higher as in dark incubated samples, suggesting that photo-adaptation occurred in light incubated samples to optimise photosynthesis.

**Pigment ratios and xanthophyll cycling**

Chlorophyll $a$ concentrations did not change significantly during illuminated or darkened incubations in this study. Diel periodicity of cellular chlorophyll content has been demonstrated in pelagic diatoms, whereby maximum chlorophyll $a$ to carbon ratios occurred during the dark phase of a light/dark cycle (Owens et al., 1980). In this study only the
photoperiod has been investigated; therefore the existence of periodic changes in the cellular chlorophyll $a$ content over a complete diel cycle cannot be ruled out.

The cellular pigment composition of benthic diatoms changed during light incubation. These results show that while there were no short term changes in the ratios of chlorophyll $c_{1+2}$, fucoxanthin and $\beta$-carotene to chlorophyll $a$, the ratios of the two xanthophyll pigments were significantly altered. Experiment 4, which was designed to examine the effect of irradiance levels on cellular pigment composition showed that diadinoxanthin and diatoxanthin were inversely related to each other ($r=-0.7$, $p<0.001$) suggesting that cycling between the two pigments was occurring and that this was induced by light. The conversion was already triggered at an irradiance of 250 $\mu$E m$^{-2}$ s$^{-1}$ and the intensity of the change was dependent on irradiance levels, whereby samples at 500 and 2000 $\mu$E m$^{-2}$ s$^{-1}$ behaved very similarly. This suggests that there was a threshold between 250 and 500 $\mu$E m$^{-2}$ s$^{-1}$, beyond which changes were insignificant. Although there was a significant correlation between the two xanthophyll pigments, it can’t be classified as strong, which is due to the different responses of the two ratios with time. While the strongest decrease of the diadinoxanthin to chlorophyll $a$ ratio occurred after 100 minutes, the diatoxanthin to chlorophyll $a$ ratio increased significantly after 20 minutes at the higher irradiances. The reason for this discrepancy was the $de$ novo synthesis of diatoxanthin and possibly of diadinoxanthin. This was indicated by an increase of the xanthophyll pool during the period of incubation which ranged between 27% to 64% depending on light intensity. The synthesis of diatoxanthin $de$ novo has been shown to occur in *Phaeodactylum tricornutum* within 20-30 minutes following a shift from growth limiting irradiance (120$\mu$E m$^{-2}$ s$^{-1}$) to saturating light (750$\mu$E m$^{-2}$ s$^{-1}$) (Olaizola et al., 1994) and also in *Nitzschia palea* (Willemoe and Monas, 1991).

In experiment 1 pigment cycling could not be established, but an increase in diatoxanthin strongly suggests that this carotenoid was synthesised $de$ novo. Two reasons can explain why diatoxanthin was synthesised at lower irradiances in *Nitzschia closterium* than shown for *Phaeodactylum tricornutum* (Olaizola et al., 1994) and for *Nitzschia palea* (Willemoe and Monas, 1991). The culture of *Nitzschia closterium* was grown under a low light regime (50$\mu$E m$^{-2}$ s$^{-1}$) and the experiment was conducted when the culture was in its second exponential phase prior to saturation. During this phase and continuing into the senescent phase Klein et al. (1988) found an increase in diatoxanthin synthesis in batch cultures of *Nitzschia closterium.*
An increase in diatoxanthin levels can be directly correlated with an increase in non-photochemical quenching (Olaizola and Yamanoto, 1994; Moisan et al., 1998). Energy is dissipated as heat from the antennae complex and the reaction centre of the PSII (photosystem 2), rather than being absorbed, resulting in a decrease in photosynthetic efficiency (Olaizola and Yamanoto, 1994; Olaizola et al., 1994; Schubert et al., 1994). A short-term decrease in the effective absorption cross section of PSII can alter the photosynthesis irradiance curve, without necessarily affecting the maximum quantum efficiency (Olaizola et al., 1994). In experiments 1, 2 and 3 $P_{\text{max}}$ and $\alpha^*$ decreased at the end of the photoperiod, indicating that in this study both the effective absorption cross section of PSII and the maximum quantum efficiency were affected. It is likely that the increase in diatoxanthin caused a decrease in the photosynthetic potential and efficiency to photo-protect the cells as no photo-inhibition was observed. The role of xanthophyll cycling in the photo-protection of epipelic diatoms is virtually unexplored. Benthic diatoms have been noted for their lack of photo-inhibition (Barranguet et al. 1998; MacIntyre and Cullen, 1995; Rasmussen, 1983) and the experiments in this chapter have shown that xanthophyll cycling and synthesis de novo occur at comparatively low irradiance levels. This suggests that benthic diatoms which are inhabiting the intertidal zone possess efficient photo-physiological mechanisms to survive the dramatic changes of incident irradiance. Further experiments should be conducted to examine the photo-physiological status of benthic diatoms under various irradiances. Photosynthetic pigment analysis in conjunction with fluorescence measurements such as PAM (pulse amplitude modulated) fluorescence will provide a powerful tool to improve our knowledge on the photosynthetic regulation of benthic diatoms in such an extreme light regime.
CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The aim of the research conducted in this thesis was to examine the factors controlling microphytobenthos production on intertidal mudflats and to determine the physiological and ecological responses of benthic microalgae to environmental variables. In this final chapter the results obtained in this study are discussed in relation to the main hypothesis. The limitations of the research are summarised and further work is proposed.

The analysis of the temporal and spatial distribution of microphytobenthos along an intertidal transect sampled at weekly intervals for more than a year (Chapter 3) gave statistically significant evidence that a.) the seasonal variation of benthic microalgae differed among the stations and that b.) the dominating environmental factors controlling the standing stock of microphytobenthos biomass varied in time and space (Fig. 7.1). Station 4, the marshpool station, can be classified as a distinctly separate habitat; the temporal variation of microphytobenthos found at this station significantly differed from the three mudflat stations throughout the year. The microflora at this site consisted of very different species assemblages to those on the mudflat with few species common to both environments. It was also the only station that was shown not to be light limited throughout the whole year.

Biomass fluctuations of benthic microalgae at the three mudflat stations were not significantly different from each other, when the data of each station was pooled over the year. However, during separated seasons the stations showed marked differences in their temporal variability. Similar results were found by Rizzo and Wetzel (1983) for benthic micro-flora in Chesapeake bay and they concluded that: “the apparent lack of differences on an annual timescale was due to small scale variability and the rapid response time of benthic microflora; thus typical temporal sampling designs (once a month) do not resolve seasonal dynamics and seasonal plots appear as randomly generated points”. Similar conclusions were drawn by Schaffer and Onuf (1985) for primary production measurements of benthic microalgae. Although these observations were made more than a decade ago, the majority of studies to date still measure fluctuations in microphytobenthos biomass over monthly intervals (Barranguet, et al 1997; Cariou-le Gall and Blanchard, 1995, deJong and deJonge, 1995; deJonge and Colijn, 1994; Lucas and Holligan, 1999) very few have sampled bimonthly (Pinckney and Zingmark, 1993a; Brotas et al, 1995) and the exception sampled weekly (Santos et al. 1997).
Fig. 7.1. Temporal variation of chlorophyll $a$ (mg m$^{-2}$) and incident irradiance during low tide (accumulated over 7 day period prior to sampling) at the four stations on Hythe mudflat 1996. The data are divided into the three seasons Spring, Summer and Autumn. Arrows indicate environmental factors that significantly correlated with chlorophyll $a$ variation in multi-regression analysis for the seasons indicated (for details see chapter 3).
Results obtained from the weekly sampling program conducted during this study have helped to resolve some of the seasonal variability of microphytobenthos detected on Hythe mudflat and have allowed the testing of the central hypothesis of this thesis i.e. "changes in incident irradiance defined by a combination of meteorological factors and the tidal cycle over critical time-scales (ie. weekly) cause significant changes in the biomass and production of microphytobenthos".

Microalgal biomass was statistically shown to be light related at all three mudflat stations in spring and at the two low shore stations 1 and 2 in autumn. During these two seasons photoperiods ranged between 0 and 7 hours at stations 1 and 2 and between 2 and 8 hours at station 3. In the light limiting periods of the year the neap-spring tidal cycle exerted a major influence on microphytobenthos biomass, as it determined the phase difference between the tidal and the solar cycle. As a result an increase in hours of light exposure per day of up to six fold occurred within one week at stations 1 and 2. Figure 7.1 shows the incident irradiance during tidal emersion accumulated over 7 days prior to sampling and the variation of chlorophyll $a$ at each station. The spring bloom at station 1 during April/May was initiated when high daily irradiances coincided with spring low tides, resulting in a 3.5 times increase in solar radiation received by microphytobenthos in the week prior to sampling (April 11th, Fig. 7.1). Chlorophyll $a$ levels during the same week increased by a factor of three. Following the initial increase in algal biomass, the 7 day irradiance decreased, but accumulation of microalgal biomass continued for a further two weeks. MacIntyre (1999) has recently proposed a positive feedback mechanism for the regulation of microalgal biomass in subtidal habitats, whereby growth of microphytobenthos causes an increase in bio-stabilisation of the sediment, thus reducing resuspension and promoting further growth. Such a feedback mechanism might explain the continuing increase of microalgal biomass at station 1 during the spring bloom.

At stations 2 and 3 the spring bloom was less pronounced and biomass showed higher intra-monthly variability. These results have shown that microalgal biomass varied significantly over weekly time-scales, however only some of this variability could be attributed to changes in incident irradiance. Thus although benthic microalgal biomass showed large fluctuations on a weekly basis at station 3 in summer and autumn and at station 4 throughout the year, there was no significant correlation between incident irradiance and chlorophyll $a$ (Fig. 7.1). Other environmental factors which accompany the neap-spring tidal
cycle must therefore exert an important influence on chlorophyll $a$ levels in the sediment. The strength of tidal currents changes during the neap-spring tidal cycle (Baillie and Welsh, 1980) and it is likely that some of the detected biomass variability was due to resuspension. Variation of resuspension of benthic diatoms over tidal cycles has been reported in the deeper part of Southampton Water near Hythe mudflat (Lauria, 1998), however a difference in the degree of resuspension at the four intertidal stations was not investigated. Brotas et al. (1995) found a negative relationship between chlorophyll $a$ and daily quantum irradiance at a low shore intertidal station of the Tagus Estuary (Portugal) during her weekly sampling program. As biomass at the same station correlated positively with tidal height and emersion periods, she also concluded that other environmental factors related to the tidal cycle such as resuspension/erosion were controlling the standing stock of biomass. A similar conclusion was drawn by Santos, et al. 1997 from his biweekly sampling program of the intertidal mudflats of the Gironde Estuary (France) stating that the dynamics of chlorophyll $a$ was a function of primary production and resuspension/erosion related factors. Results from this thesis, Brotas et al. (1995) and Santos, et al. (1997) emphasise the importance of high frequency temporal sampling to include short-term changes in environmental variables induced by the neap-spring tidal cycle, when conducting seasonal surveys on microphytobenthos distribution.

A second hypothesis to be tested in this thesis was that photo-physiological changes of microphytobenthos over critical time scales are important for population maintenance within this dynamic light regime.

This study has revealed that primary production (derived from O$_2$ changes), photosynthetic parameters $P_{max}^b$, $O^b$ and $I_s$ (derived from $^{14}C$ P vs. E curves) and the cellular pigment composition of benthic microalgae from the Hythe tidal flat changed temporally and spatially. Combined results from the spatial and monthly comparisons have shown that the $I_s$ values correlated significantly with incident irradiance during low tide suggesting that microphytobenthos on the tidal flat optimised their photosynthetic responses to the ambient light environment. Results from the temporal study showed that changes in chlorophyll $a$ and primary production were also positively related to incident irradiance.

The photosynthetic response of microphytobenthos to changes in irradiance was clear in the spatial study as measurements of $^{14}CO_2$ uptake and O$_2$ production showed the same general trends. Production did not correlate with chlorophyll $a$ or incident irradiance, indicating that a change in the photosynthetic response of microalgae was occurring. This
could be explained by the changes in $P_{\text{max}}^a$, $\alpha^a$ and biomass normalised production which correlated significantly with irradiance, giving evidence that photosynthetic parameters were adjusted in response to a change in the light environment. Applying the tidal model has shown that due to lengthening of the photoperiod, light availability increased towards the high shore. Photosynthetic potential and efficiency however decreased towards the high shore, suggesting that benthic microalgae in the low shore region compensated for the light limitation with increased production, resulting in higher output per biomass. It can be suggested that the change of photosynthetic response of microphytobenthos was of physiological nature rather than ecological as the same species were present at the three mudflat stations 1 to 3 where the strongest changes in photosynthetic output per biomass occurred. This spatial relationship between incident irradiance and photosynthetic parameters has not been as clearly shown in previous studies (Barranguet et al, 1998, Pinckney and Zingmark, 1993b) and has important implications for primary production models of benthic microalgae.

In this study, photosynthetic parameters of microphytobenthos have also been shown to change on an hourly scale in a stable laboratory environment. These changes could be attributed to light adaptation and an internal photosynthetic rhythm. It is therefore expected that benthic microalgae in the field do not only vary production rates due to changes in external factors, such as was observed by Barranguet et al. (1998), Kromkamp et al. (1995) and Kromkamp et al. (1998) but also because their physiological responses to those factors change over a short temporal scales. Table 7.1 shows the range of values in photosynthetic parameters obtained from the seasonal, spatial and short-term study.

<table>
<thead>
<tr>
<th>Photosynthetic parameter</th>
<th>Monthly range at S2 (Chapter 5.1)</th>
<th>Spatial range S1-4 (Chapter 5.2)</th>
<th>Range in one photoperiod (Chapter 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{max}}^a$ (mgC mg chl a$^{-1}$ h$^{-1}$)</td>
<td>4.6 - 13.3</td>
<td>4.2 - 12</td>
<td>4.8 - 14.8</td>
</tr>
<tr>
<td>$a^a$ [(mgC mg chl a$^{-1}$ h$^{-1}$) (\mu\text{Em}^{-2}\text{h}^{-1})]</td>
<td>0.06 - 0.14</td>
<td>0.03 - 0.1</td>
<td>0.05 - 0.08</td>
</tr>
<tr>
<td>$I_s$ ((\mu\text{Em}^{-2}\text{h}^{-1}))</td>
<td>77.5 - 144</td>
<td>121.5 - 190</td>
<td>96 - 195</td>
</tr>
</tbody>
</table>

Table 7.1. Range of changes in photosynthetic parameters in the monthly, spatial and short-term comparison.

The ranges of change in the monthly, spatial and short-term comparison are on the same scale for $P_{\text{max}}^a$, and $I_s$, while it is slightly higher for $\alpha^a$ in the seasonal study. These results strongly suggest that short-term physiological adaptations are an important feature of microphytobenthos aiding their successful survival in the intertidal zone, where strong gradients in environmental parameters occur on an hourly time scale.
Short-term experiments under controlled conditions in the laboratory have given further evidence that the cellular pigment content of benthic diatom populations changed during exposure to light. The concentration of diatoxanthin, which is associated with non-photochemical quenching (Olaizola and Yamanoto, 1994) increased at irradiances as low as 50μEm⁻²s⁻¹ in low light adapted cultures of Nitzschia closterium and at 250μEm⁻²s⁻¹ in field populations of diatoms collected in March and June. The increase in the photo-protectant pigment was therefore occurring at irradiances which did not induce photo-inhibition in the cells, suggesting that benthic microalgae possess efficient mechanisms to avoid photo-damage. The absence of photo-inhibition has been generally noted in microphytobenthos inhabiting the intertidal and subtidal zone (Barranguet et al, 1998, MacIntyre and Cullen, 1996, Rasmussen et al., 1983), in contrast to phytoplankton (Kirk, 1994). To date xanthophyll cycling of epipelic diatoms has not been demonstrated in the sediment. Benthic diatoms have been reported to migrate downwards as a photoprotective mechanism against high irradiance (Serodio et al. 1997), and a new hypothesis is postulated by Kromkamp et al. 1998 that there is a vertical ‘micro’-migration of benthic microalgae in the upper mm of the sediment, where algae in the surface migrate to deeper layers to avoid photo-inhibition and are replaced by other cells. It is therefore possible that physiological photo-protection such as xanthophyll cycling only occurs when behavioural adaptation fails. The short-term changes of pigment ratios during illumination experiments were more pronounced than monthly or spatial changes. This suggests that long-term pigment adaptations are not ecologically viable in the intertidal zone, where strong light gradients are compounded by the daily changes of the tide.

The main conclusions from this thesis are that the growth of microalgae on an intertidal mudflat of a turbid macrotidal estuary is controlled by the incident irradiance as a function of meteorological factors and the tidal cycle and by the photosynthetic optimisation of the microalgae themselves. In contrast, the reduction of microalgal biomass is strongly influenced by grazing and export through resuspension. Benthic microalgae in this habitat have the physiological potential to optimise photosynthesis on the same time scale as changes occurring in environmental factors (ie. minutes to hours).

Limitations of this study and suggestions for further work:

a) Optimisation of the tidal model: The initial tidal model applied in chapter three used daily averages of solar radiation and therefore did not take the hourly variation due to changes in the sun elevation angle into account (as hourly radiation values were not available in the
proximity of the study site in 1996). These variations have been shown to have an important effect on microphytobenthos production (Pinckney and Zingmark, 1991) and an inclusion of hourly variation in chapter five revealed closer correlations between biomass and incident irradiance. The tidal model could be further improved to include a quantification of the change in tidal currents dependent on the neap-spring tidal cycle, in order to estimate the influence of resuspension on microalgal biomass at the four stations of Hythe mudflat.

b) Spatial and temporal changes of photosynthetic parameters: Although the spatial study showed some strong trends in the changes of photosynthetic response to variation in irradiance, it was difficult to differentiate between a short-term temporal response and spatial adaptation or a combination of the two, as it was not possible to conduct all measurements at the four stations simultaneously. The sample period covered a 14 days period and encompassed a complete neap-spring tidal cycle, during which time the light environment changed at each station. These changes have been taken into consideration by applying the tidal model which calculated the incident irradiance received by each station for 5 days prior to the individual sample dates. However, to accurately compare the changes in photosynthetic responses of microphytobenthos at the four stations, measurements need to be conducted simultaneously and conducted repeatedly during different stages of the neap-spring tidal cycle.

c) Temperature as a controlling factor of photosynthetic parameters: As the aim of this study was to investigate photo-adaptation of benthic microalgae as opposed to thermal adaptation, all production and photo-physiological experiments were conducted at constant temperature, ie. 15°C. Photosynthesis and growth of benthic microalgae is directly related to temperature (Admiraal and Peletier, 1980; Gould and Gallgher, 1990) and a number of studies have found a closer link between temperature and photo-adaptation than incident irradiance and photo-adaptation on intertidal mudflats (Blanchard et al., 1993; Barranguet et al., 1998) and in shallow subtidal sediments (MacIntyre and Cullen, 1995). Long and short-term thermal adaptations of microphytobenthos on tidal flats have been examined and were found to be limited due to strong short-term fluctuations of temperature during the tidal cycle (Blanchard et al., 1996; Blanchard et al., 1997).

d) Photoprotection of benthic diatoms: Studies on the relationship between photosynthetic quantum efficiency, non-photochemical quenching and the xanthophyll cycle should be conducted on benthic diatoms, to understand their photo-physiological adaptation to such a variable environment.
e) Photosynthetic pigments in intertidal sediments: A specific research objective of this study was a.) to use photosynthetic pigment markers in order to monitor biomass fluctuation and changes in the taxonomic composition of benthic photosynthetic assemblage and b.) to identify the factors controlling the input, storage and degradation of pigments in fine grained intertidal sediments.

The pigment diversity on Hythe intertidal mudflat is derived from in situ production of microphytobenthos, deposition of phytoplankton cells and macrophytic detritus from the adjacent salt-marsh. The correlation between accessory pigments and chlorophyll differed greatly suggesting that only some of the photosynthetic pigments could be related to active processes within the sediment. Uncertainties as to the significance of certain pigment distributions included: a.) the low correlations between all photosynthetic pigments with chlorophyll a at station 4; b.) the high concentrations of chlorophyll b, zeaxanthin-lutein and b-carotene and their weak correlation to chlorophyll a and to cyanobacteria and euglenoid abundance at all four stations; c.) the high concentration of peridinin and its absence of seasonal variation at the high shore stations and d.) the different seasonal variation of the two xanthophyll pigments diadino- and diatoxanthin and their irradiance independent distribution.

The temporal and spatial distribution patterns were clarified to a large extent when the pigment composition of motile microphytobenthos was analysed and compared to the vertical distribution of pigments in the upper 1 cm of the sediment. Figure 7.2 shows the change in proportion of pigments at the surface and within the sediment in February when chlorophyll a was found in low concentrations and in April, during the occurrence of a spring bloom.
In Fig. 7.2 the depth distribution shows that on two dates at different times of the year the upper mm of the sediment contains a very similar pigment composition to the motile fraction of microphytobenthos. On both dates below 2 mm the contribution of zeaxanthin-lutein, chlorophyll b and β-carotene increases. There are several possible reasons for this increase: these four pigments are contained in vascular plant detritus which degrades more slowly than benthic microalgae (Bianchi et al. 1993), the pigments themselves have slower degradation rates (Hurley and Armstrong, 1990; Levinton and McCartney, 1991; Repeta and Gagosian, 1987) and they are situated within the anaerobic layer of the sediment, which has been reported to enhance the preservation of pigments (Sun et al. 1993). The factors controlling the input and storage of photosynthetic pigments on Hythe tidal flat are summarised in Fig. 7.3.
A more homogeneous vertical pigment distribution was detected in winter, when low temperatures caused reduced degradation rates of pigments, pigment degradation was also reduced when the oxygenated layer was shallow such as at station 2 in the winter months and in May and at station 4 in April.

From the results of the pigment analysis in chapters 3 and 4 the following conclusions can be drawn for sediments of similar nature to those on Hythe mudflat: Fucoxanthin is a reliable taxonomic pigment marker for in situ biomass production of microphytobenthos as it correlated strongly with chlorophyll a on a vertical scale and throughout the year. When establishing sampling programs it is necessary to determine the active photic depth and sample at that resolution, as background levels of slowly degrading pigments would otherwise diffuse the trends. This resolution is sufficient to monitor the in situ growth dynamics of microphytobenthos, but it is not high enough to trace adaptational processes such as xanthophyll cycling and migration of microphytobenthos. Migration of benthic microalgae has been traced by sampling the sediment at 200μm depth resolution prior to pigment analysis and relating vertical distribution of microphytobenthos to changes in the spectral reflectance of intertidal sediment (Paterson et al., 1998). Also non-destructive tracer techniques such as PAM (photo-amplitude modulated) fluorescence which measures the variable and absolute fluorescence of the sediment surface can be used to study microphytobenthos migration (Kromkamp et al., 1998; Serodio et al., 1997).
The following improvements to the HPLC methodology used in this study can be made to increase the taxonomic and physiological information gained on pigment data of intertidal sediments:

a) The use of a solvent system which allows the separation of lutein and zeaxanthin to differentiate between input of cyanobacteria and macrophytic detritus (Barlow et al. 1997; Brotas and Plante-Cuny, 1996; Wiltshire and Schroeder, 1994) and the use of myxoxanthophyll as an exclusive taxonomic marker for cyanobacteria (Bianchi et al., 1993). 

b) Identification and quantification of neofucoxanthin which is contained in benthic diatom species at different proportions and thus gives more information on the composition of the microphytic assemblages (Brotas and Plante-Cuny, 1998).

c) Identification and quantification of fucoxanthinol to use as an indicator pigment for diatom grazing (Repeta and Gagosian, 1982).
BIBLIOGRAPHY


Barranguet, C., Herman, P.M.J. and Sinke, J.J., 1997. Microphytobenthos biomass and community composition studied by pigment biomarkers: importance and fate in the carbon


dejonge, V.N., 1980. Fluctuations in the organic carbon to chlorophyll a ratios for estuarine


MacIntyre, H.L., 1999. Physical forcing and the partitioning of shallow water primary production between the benthos and the water column, Primary productivity of planet earth, Plymouth, UK.


APPENDIX I

COMPARISON BETWEEN CHLOROPHYLL A VALUES IN THE SEDIMENT DETERMINED BY HPLC AND SPECTROPHOTOMETRY

The distribution of chlorophyll a at the four stations of Hythe was analysed with HPLC and spectrophotometry during the sampling campaign in 1996. Data obtained from both methods were compared to estimate the accuracy of both measurements. Fig. A.I.a. shows the correlation between chlorophyll a values measured with the HPLC and the spectrophotometer.

![Graph showing correlation between chlorophyll a values measured with HPLC and spectrophotometry.](image)

As data was not normally distributed, even after transformation, the correlation between the two measurements were tested with a Spearman correlation on ranks. The correlation coefficient was 0.91 (p>0.001, n=202). The 1 to 1 line drawn across the graph illustrates that the two methods gave similar values for chlorophyll a and that there was no general over or under-estimation of chlorophyll a by the spectrophotometric method.
APPENDIX II
MICROPHYTOBENTHOS SPECIES FOUND ON HYTHE MUDFLAT

D-Ni Nitzschia
D-Ni-1 Nitzschia sigma var. (Kützing) W. Smith
D-Ni-2 Nitzschia acuminata (Greg.) Grun.
D-Ni-3 Nitzschia apiculata

D-Na Navicula
D-Na-1 Navicula gregaria Donkin
D-Na-3 Navicula cryptocephala Hust.
D-Na-4 Navicula radiosa Hust.
D-Na-5 Navicula trivialis Lange-Bertalot

D-F Fallacia
D-F Fallacia pygmaea (Greg.) Stickle-Mann

D-G Gyrosigma
D-G-1 Gyrosigma fasciola W. Smith
D-G2 Gyrosigma attenuatum (Kützing) Rabenh.

D-P Pleurosigma
D-P1 Pleurosigma angulatum (Queckett) W. Smith
D-P2 Pleurosigma australii (Bréb. ex Kützing) Wm. Smith

D-C Cylindrotheca
D-C-1 Cylindrotheca closterium (Ehrenb.) Reimann & Lewin
D-C-2 Cylindrotheca gracilis (Bréb. in Kützing) Grun. in V.H.
D-C-3 Cylindrotheca fusiformis Reimann & Lewin

D-Ap Amphiprora
D-Ap-1 Amphiprora paludosa Sm.
D-Ap-1 Amphiprora large sp.
D-Ap-2 Amphiprora small sp.

D-D Diploneis
D-D-1 Diploneis sp. (Kützing) Cleve
D-D-2 Diploneis interrupta (Ehrenberg) Cleve

D-S Suriella
D-S-1 Suriella ovata Kützing

D-A Amphora
D-A-1 Amphora lineolata Ehrenberg
D-A-2 Amphora ovalis Kützing

C Cyanobacteraeia
C-O Oscillatoria sp.
C-A Anabaena sp.

Euglena
Ciliate
AUTHOR: DRANSFIELD, LEONIE

TITLE: THE ENVIRONMENTAL AND PHOTO-PHYSIOLOGICAL CONTROL OF MICROPHYTOBENTHOS PRIMARY PRODUCTION ON AN INTERTIDAL MIDFLAT

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