Microphytobenthos: Ecophysiology and Community Dynamics in Estuarine Sediments

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The ecologist capable of creating an ecosystem that passes test earns high marks: the one who fails is sure to gain new insights into ecosystem structure and function (Ewel, 1987).

Contents

Chapter 2: General Methods

Chapter 3: Can natural estuarine microphytobenthic assemblages be used as model systems in laboratory studies?

Chapter 4: The influence of light and temperature interactions on a natural estuarine microphytobenthic assemblage

Chapter 5: The influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes

Chapter 8: Can the stability of intertidal sediments be predicted from proxy parameters? An *in situ* investigation.

Chapter 9: Site-specific features influence sediment stability of intertidal mudflats

Chapter 10: General Discussion

Abstract

Deposition of fine-sediments in the intertidal zone of estuaries forms highly productive mudflats. Diatoms are often the main primary producers in these systems, with primary production estimates ranging between 29 and 234 g C m^{-2} $year^{-1}$ (Underwood & Kromkamp, 1999), and provide an important food resource for benthic infauna. The presence of biofilms has generic effects such as the control of nutrient fluxes over the sediment-water interface (Sundbäck *et al.*, 1991) and the mediation of sediment properties (Paterson, 1995). Estuaries are exposed to a wide variety of human and natural stressors. The added burden of climate change may further degrade these valuable ecosystems, threatening their ecological sustainability and the flow of goods and services they provide to humans. Thus, there is a need to understand how estuarine benthic diatoms will be affected under predicted global change scenarios, such as enhanced atmospheric CO_2 concentrations and increased temperature. How these effects will be transferred to other ecosystem processes such as primary productivity and sediment stability also needs to be assessed.

Techniques such as PAM fluorescence, high performance liquid chromatography and microscopy were used to assess the influence of light, temperature, nutrients, CO_2 and grazers were assessed in terms of the structure (i.e. assemblage composition) and functioning (i.e. health and productivity) of the biofilm. The importance of diatoms and biofilms in terms of sediment stability of estuarine mudflats was also investigated.

This thesis established the use of estuarine benthic diatom biofilms as laboratory model systems for the study of environmental variables and change. Measurements of primary productivity may be over-estimated due to diatom migration. The influence of light on a microphytobenthic assemblage was found to be temperature-dependent. Grazing from *Hydrobia ulvae* had no significant effect on diatom assemblage composition, whilst *Corophium volutator* significantly influenced assemblage composition, overriding any potential effects of temperature and nutrient conditions. Natural estuarine diatom biofilms were not carbon limited. Sediment stability cannot be predicted from proxy parameters. Models need to be site-specific due to the occurrence of features, such as *Enteromorpha* mats, that are typical to that estuary.

Abbreviations

α^{rETR}	Maximum light utilisation coefficient
ATP	Adenosine triphosphate
CSM	Cohesive Strength Meter
DA	Previously dark-adapted cells
DD	Diadinoxanthin
DMF	Dimethyformamide
DT	Diatoxanthin
E_K^{rETR}	Light saturation parameter
EPS	Extracellular Polymeric Substances
(r)ETR	(Relative) Electron Transport Rate
F'	Fluorescence yield in the light adapted state prior to the saturating beam
F_m	Maximum fluorescence yield in the dark-adapted state
F_m'	Maximum fluorescence yield in the dark-adapted state during light saturation
F_m^{15}	Maximum fluorescence yield after 15 mins dark-adaptation
FMS2	Fluorescence Monitoring System 2 (Hansatech™)
F_o'	Minimum light-adapted fluorescence yield
F_o^{15}	Fluorescence yield prior to the saturating beam after 15 mins dark-adaptation
F_q'	Fluorescence yield of PSII quenched by photochemistry ($F_m' - F'$)
F_q'/F_m'	Light adapted fluorescence measurement of photochemical capacity at PSII
F_v	Variable fluorescence ($F_m - F_o$ or $F_m^{15} - F_o^{15}$)
F_v/F_m	Theoretical maximum photochemical efficiency
h	Hours
H_2SO_4	Concentrated sulphuric acid
HPLC	High Performance Liquid Chromatography
LA	Previously light-adapted cells
LHCII	Light harvesting complex II
LN_2	Liquid nitrogen
LTSEM	Low Temperature Scanning Electron Microscopy
Min(s)	Minute(s)
ml	Millilitre
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogenase
NPQ	Non-photochemical quenching (F_v'/F_m' or $(F_m-F_m')/F_m'$)
PAM	Pulse Amplitude Modulated
PAR	Photosynthetically Active Radiation
P-E curve	Photosynthesis-Irradiance Curve
PPFD	Photosynthetic Photon Flux Density
PSI	Photosystem I
PSII	Photosystem II
Q_A	The primary quinone acceptor
q_E	Energy dependent quenching
q_I	Photoinhibitory quenching
qP	Photochemical quenching (F_q'/F_v')
q_T	Quenching related to state transitions
$rETR_{max}$	Maximum theoretical electron transport rate
s	Seconds
SE	Standard Error
µg	Micrograms

Chapter 1

Chapter 1: General Introduction

1.1 Why estuaries and mudflats are important

An estuary is a partially enclosed body of water formed where freshwater from rivers and streams flows into the ocean, mixing with seawater. Estuaries are situated at the interface between marine and terrestrial environments, and rank among the most important ecosystems in terms of their economic and ecological importance (Kennish, 2000; Underwood & Kromkamp, 1999; Costanza *et al.*, 1997). Estuaries are critical for the survival of many species. For example, many species of fish and shellfish rely on the sheltered waters at some point during their development (often as protected places to spawn; UKBAP, 2002). Estuaries are important in terms of recreation, tourism, fisheries, and other commercial activities that use the estuaries' natural resources to provide tangible and direct economic benefits. The protected coastal waters of estuaries also support key public infrastructure, serving as harbours and ports vital for shipping, transportation, aquaculture, fisheries and industry.

As a river widens and reaches the estuary, sediments settle out of the water column, leaving only the smaller particles in suspension. The mixing of fresh and salt water, accompanied by reduced flow, causes flocculation and the settling of fine particles, forming the characteristic mudflats. These fine sediment particles have a large surface area to volume ratio and are attracted to each other by Van der Waals and electrostatic charge interaction forces (Dyer, 1973). This cohesive nature helps to define the physical nature of the environment for sediment-inhabiting organisms (Paterson & Underwood, 1990). Moving from the head of an estuary towards its mouth, mudflat sediments change from being mainly silts and clays (< 63 μm) with a high organic content, to sediments with increasing proportions of sands as wave energy becomes greater. These sediments are important because they trap nutrients: although they also accumulate, filtrate and transform potentially harmful waste products and toxic pollutants (Kennish, 2000).

The UK has approximately 15% of the northwest European estuarine habitat, and the total estuarine resource has been estimated as c 588,000 ha, of which 55% is intertidal area (UKBAP, 2002). Mudflats, like other intertidal

areas, form a natural barrier to the sea, dissipating the energy of tides and waves. Mudflats thus reduce saltmarsh and coastline erosion, reduce damage to coastal defences, and reduce the flooding of low-lying land (King & Lester, 1995). They also provide an ideal place for migratory birds to rest and refuel (Burton *et al.*, 2000).

1.2 The mudflat microphytobenthos

Conditions on a mudflat can be extreme and can change rapidly. For example, wave action can mix the sediment creating a mobile and abrasive environment. The regular exposure at low tide can cause an increase in temperature and consequently an increase in salinity due to evaporating water. Bacterial decay can create an anoxic sediment layer. Mudflats are characterised by high biological productivity and abundance of organisms, but low diversity with few rare species (McLusky, 1989). The major primary producers in these systems include diatoms, euglenoids, flagellates, and cyanobacteria. Generically they are described as the 'microphytobenthos' (Paterson *et al.*, 1998; MacIntyre & Cullen, 1996; Yallop *et al.*, 1994), although in most temperate mudflats it is the diatoms (Bacillariophyceae), which are the dominant microphytes (Admiraal, 1984). Diatoms are defined as being single-celled phototrophic eukaryotic algae that posses a siliceous cell wall called the frustule. They are broadly divided, based on shape, into the pennates (Order Pennales), which are symmetrically lanceolate, and the centrics (Order Centrales), which are radially symmetrical (Admiraal, 1984). Diatoms first became abundant 144-65 Million years ago in the Cretaceous period, with centric diatoms evolving before the pennates (Gersonde & Harwood, 1990; Harwood, 1988).

Several groups of unicellular algae have become adapted to living on and moving through sediments that make up intertidal flats. Diatoms growing on sands are termed 'epipsammic' and those that thrive in mud have been termed 'epipelic' (Round, 1981). These definitions are often used to distinguish between motile (epipelic) and non-motile (epipsammic) species. True epipsammic forms belonging to the genera *Achnanthes*, *Amphora*, *Catenula*, *Cocconeis*, *Fragilaria*, and *Opephora* are generally associated with non-cohesive sediments, whilst epipelic diatoms such as *Navicula* and *Nitzschia* are associated with cohesive sediments. However, both types can occur in cohesive sediment, particularly in

regions consisting of mixed or sandy sediments, and this can have significant effects on the local diversity (Sabbe, 1993).

Whilst bilateral symmetry is a consistent feature of pennate diatoms, variations in symmetry along the major axes (longitudinal, apical, and transverse) are used for distinguishing diatom taxa. Most species have a relatively fixed form, and different species range in size from 10 μm to 500 μm, with length/breadth being related to the reproductive stage. However, a range of morphologies for the same species can exist under different conditions. A unique morphological characteristic of diatoms is the arrangement and ornamentation of pore openings within the frustule. The spatial arrangement of features, such as the raphe, striae, and punctae, is species specific and a major diagnostic tool used for species identification. A drawback to using these features is that organic matter (i.e. the protoplasm) must be removed in order for the frustule to be examined using light or scanning electron microscopy. This makes it difficult to discern viable from non-viable cells. It is important to recognize that identification and nomenclature of diatom species is hotly debated and changes frequently. Although a large field has grown around the taxonomy of diatoms, we still know little about the behaviour and physiology of individual species (Cohn & Weitzell, 1996).

1.3 Sediment and the microphytobenthos

The spatial distribution of benthic diatom patches is largely accounted for by the granulometry of the sediment, and thus by the factors influencing particle size and density (Paterson & Hagerthey, 2001; Saburova *et al.*, 1995). The EU PROMAT study (1997) reported that the structure and composition of microphytobenthic assemblages of the Wadden Sea was largely controlled by sediment properties, although conversely, the presence of microphytobenthos can also influence the sediment properties (Van de Koppel *et al.*, 2001). The overlap of the spatial conditions (i.e. grain size distribution) by temporal parameters such as temperature changes in the course of the year strongly controlled the abundance and dominance of primary producers (PROMAT, 1997). The composition and diversity of benthic diatom assemblages is also directly related to sediment grain size (Paterson & Hagerthey, 2001; Cahoon *et al.*, 1999; Sabbe & Vyverman, 1991). For example, moderate crevice size between sediment

particles increases diversity compared to small and large crevices, as they can provide refuge for taxa of varying size (Bergey, 1999).

1.4 Diatom motility

Diatom cells accumulate at the sediment/air interface in discrete layers (often referred to as 'biofilms') often of sufficient density to impart visible brown coloration to the substratum (Paterson & Underwood, 1990). These motile diatoms are able to position themselves in the upper millimetres of the sediments by distinctive chemotactic, geotactic and phototactic responses (Cohn & Weitzell, 1996; Cohn & Disparti 1994; Round *et al.*, 1990; Harper, 1977). The ability of diatoms to migrate within the sediment can be thought of as the basis of epipelic diatom ecology, and as a survival strategy that has allowed these species to proliferate in such a harsh environment.

1.4.1 Mechanisms of motility

Ehrenberg first reported motility in biraphid diatoms in 1838, yet today we are still unsure of the mechanisms that control and regulate this behaviour (Cohn *et al.*, 1999). Early suggestions regarding the mechanisms of motility included amoeboid type and ciliary movement, as well as 'jet-propulsion', although such ideas were quickly discredited (Cohn *et al.*, 1999; Cohn & Disparti, 1994; Edgar & Pickett-Heaps 1984). Although the actual mechanisms regulating diatom motility are debated, it is clear that diatom secretions of extracellular polymeric substances (EPS) through the raphe are inextricably linked with locomotion (Drum & Hopkins, 1966). Circumstantial evidence for this association includes:

1. Similarly observed rates of EPS flowing through the raphe and diatom cell velocity (Edgar & Pickett-Heaps, 1984).
2. The presence of a hydrophobic lipid coating over the raphe silica, which is hypothesised to increase the efficiency of EPS secretion (Edgar & Pickett Heaps, 1983).
3. The presence of a mucilage trail left behind a moving diatom (Edgar & Pickett-Heaps, 1984).

Various models regarding diatom motility exist, such as the Capillary model of Gordon & Drum (1970) who proposed that the force required for

movement is produced from the hydration of EPS once exuded. However, it is the Adhesion-Traction model proposed by Edgar and Picket-Heaps (1984) that has probably received the most attention. The Adhesion-Traction model suggests that vesicles located within the cytoplasm secrete mucilage into the raphe at a central pore. This mucilage forms strands of EPS that attach to the substratum. The actin bundles interact with myosin attached via a transmembrane connector to the extracellular mucilage strands, generating the force for movement. This force would cause the displacement of transmembrane components within the plasma membrane, and result sin cell movement in the opposite direction to which the force was applied (Poulsen *et al.*, 1999; Cohn & Weitzell, 1996; Round *et al.*, 1990; Drum & Hopkins, 1966).

1.4.2 Motility velocity

The rate at which diatoms move within the sediment fabric is partly a function of the physical and chemical properties of the sediment (e.g., grain size, water content, and EPS), although other factors such as temperature and pollutants can also affect motility velocity. Diatom velocity measurements have been made for several taxa using both natural and artificial substrata, although velocities measured in natural substrata are typically an order of magnitude lower than velocities measured in artificial substrata. In general, species-specific velocities range between 1 $\mu m\ s^{-1}$ and 25 $\mu m\ s^{-1}$ (Hay *et al.*, 1993; Edgar & Pickett-Heaps, 1984; Harper, 1977). Velocity is independent of the individual length of a diatom cell (Cohn & Disparti, 1994; Drum & Hopkins, 1966), although the path of diatom motion is determined by the shape of the raphe and is therefore species specific (Hay *et al.*, 1993; Round *et al.*, 1990).

The speed of diatom movement is important since it determines the rate and extent of reaction to environmental influences (Hay *et al.*, 1993). High species-specific velocities are typically associated with high light intensities and mid emersion periods (Happey-Wood & Jones 1988; Round & Palmer, 1966). Early work by Hopkins (1963) found diatom motility to be greatest between 10°C and 17.5°C and lowest or non-existent below 5°C, although temperature effects are likely to be species specific. During the emersion period, motility may be reduced by the loss of water from the sediment surface (Paterson, 1986). The

drying of sediments and high EPS content may serve as a physical barrier to diatom mobility and may account for the lack of migration often observed during the later periods of emersion.

1.4.3 Patterns of migration

The most common pattern associated with diatom motility is vertical migration. The timing and extent of migration is dependent on environmental cycles (lunar and solar), tidal cycles (Serôdio *et al.*, 1997), irradiance (Perkins *et al.*, 2002; Underwood & Kromkamp, 1999) and sediment behaviour (i.e. erosion and accretion; de Jong & van Beusekom, 1995; Underwood & Paterson, 1993). During daylight emersion periods, cells migrate to the sediment surface where they are able to maximise their photosynthesis, and on days when the sediments are immersed in the middle of the day, vertical migration may occur during both the morning and afternoon emersion periods (Pinckney & Zingmark, 1991). The close coupling between microphytobenthos and tidal phase means that migration into the sediments occurs on the low shore before the high shore, giving high shore diatoms a longer photoperiod.

Round & Palmer (1966) reported species-specific emergence from a mixed diatom assemblage in intertidal sediments. They observed that *Navicula salinarium* and *Pleurosigma angulatum* emerged within the first hour of emersion whereas *Cylindrotheca signatum* emerged later. Paterson (1986) found *Nitzschia* and *Navicula* species were the first to migrate to the surface of sediment collected from the Severn Estuary, followed by the appearance of large numbers of *Scoliopsis tumida* (*Scoliopleura tumida*) after 4 h. Consalvey (2002) followed diatom migration using low-temperature scanning electron microscopy (LTSEM) and found species-specific emergence and cell cycling to occur within biofilms. This migratory strategy of estuarine epipelic diatoms, and the ability of cells to cycle within a biofilm according to the light level at the sediment surface, serves as an effective means of avoiding photo-damage (Perkins *et al.*, 2002; Kromkamp *et al.*, 1998; Defew *et al.*, in review). Factors such as heavy rainfall may interrupt the tidal-induced migratory cycle, and consequently the precise timing and extent of migration may be dependent on local hydrology and prevailing weather conditions (Smith, 1999).

1.4.4 The advantages of migratory behaviour

There are many hypotheses as to why migratory behaviour is beneficial to estuarine benthic diatoms. For example, migration may minimize photoinhibition, pigment bleaching, and photo-induced cell damage by allowing individual cells to position themselves at optimal irradiance levels (Kingston, 1999a; Cohn *et al.*, 1999; Nultsch & Häder, 1985; Round & Palmer, 1966). Hence, photoinhibition is not commonly observed for microphytobenthos (Kingston, 1999b; Colijn & van Buurt, 1975). For example, surface cell densities of *Cylindrotheca signata* and *Nitzschia triblionella* were observed to decline at midday (Round & Palmer, 1966). Kingston (1999a) suggests that under *in situ* conditions, irradiance levels constantly fluctuate and that behavioural adaptations such as migration allow microphytobenthos to move to regions where the physiology of the cells is less stressed.

Just prior to the sediments becoming re-immersed by the incoming tide, it is postulated that diatoms migrate back down into the sediments, in order to avoid resuspension (Kromkamp *et al.*, 1998; Serôdio *et al.*, 1997; Jönsson *et al.*, 1994; Hay *et al.*, 1993; Decho, 1990; Pinckney & Zingmark, 1991; Grant *et al.*, 1986; Perkins, 1960).

It is unclear if migration provides a defence against predation or concentrates food resources for predators. The feeding efficiency and feeding rates of grazers would be enhanced if migration resulted in a concentrated resource at the sediment surface (Buffan-Dubau & Carmen, 2000; MacIntyre *et al.*, 1996). Conversely, dispersion of diatoms into the sediment would lower feeding efficiencies, decreasing the risk of grazing from deposit feeders (Pinckney *et al.*, 1994).

Microphytobenthic biomass can often be high, despite surface waters low in nutrients (Happey-Wood & Priddle, 1984). This may be because migration allows cells to maximize growth by obtaining light on the surface, whilst increasing nutrient sequestering from below the surface (Decho, 1990; Darley *et al.*, 1982). In addition, "inoculations" of diatoms from deeper sediment may promote the development of new populations on new surface sediments (Delgado *et al.*, 1991).

1.5 Extracellular Polymeric Substances

Motile epipelic diatoms produce highly hydrated carbohydrate-rich exopolymers that are extruded from the raphe during locomotion (Edgar & Pickett-Heaps, 1984). These exopolymers are collectively described as extracellular polymeric substances (EPS), although they have also been referred to as slime, mucus, mucilage and glycocalyx (Underwood *et al.*, 1995). Epipelic diatoms are the predominant source of extracellular carbohydrates on intertidal mudflats (Underwood *et al.*, 1995).

Production of EPS is fundamental to the biological success of epipelic diatoms for several reasons (Taylor, 1998). EPS provides protection from desiccation and other adverse environmental conditions such as pH change, salinity, heavy metals, low organic carbon supply, UVB radiation and sediment abrasion (Freeman & Lock, 1995; Yallop *et al.*, 1994; Hoagland *et al.*, 1993; Decho, 1990). EPS can reduce sediment resuspension, leading to increased sediment stabilization and thus stabilized light intensity within the cohesive sediments (Holland *et al.*, 1974). EPS may help maintain symbiotic relationships, and help to unite diatom cells for sexual reproduction, and it is also a vital component of motility, and thus allows diatoms to position themselves favourably within the sediment (Smith, 1999).

1.5.1 EPS characterisation

The characterisation, production, and utilisation of EPS produced by epipelic diatoms have not been fully investigated (Smith, 1999). Algal polysaccharides have principally been studied using extractions from planktonic monocultures (Hoagland *et al.*, 1993). For epipelic diatoms, carbohydrates can be separated into fractions, according to their solubility. Colloidal carbohydrates refer to extracted fractions in the liquid phase, and bound carbohydrates refer to carbohydrates bound to particles. Colloidal carbohydrates remain in solution after centrifugation and are often used as an index of EPS (Underwood *et al.*, 1995). EPS have high molecular weights (Decho, 1990) and are highly hydrated (>98 % H_2O). EPS is generally carbohydrate rich, with approximately 20-25 % of colloidal carbohydrates being polymeric (Taylor, 1998).

1.5.2 EPS production

The composition of EPS varies among and within diatom species (Decho, 1990), and depends on the physiological state of the cell and the growth phase (Underwood & Smith, 1998; Claus, 1988 as cited in Taylor, 1999). EPS composition may also be influenced by environmental factors (Taylor *et al.*, 1999) such as salinity (Allan *et al.*, 1972), temperature and light intensity (Smith, 1999; Smith & Underwood, 1998; Hoagland *et al.*, 1993; Madsen *et al.*, 1993; Decho, 1990). Concentrations of nutrients have also been found to affect EPS production (Buzzelli *et al.*, 1997; Myklestad & Haug, 1972). Staats *et al.* (2000) found that a lack of nitrogen or phosphorus not only caused cessation of diatom growth, but also stimulated exopolysaccharide accumulation in *Cylindrotheca closterium*. *Navicula pelliculosa* was also reported to have high rates of exopolysaccharide production under phosphorus limitation (Lewin, 1955). The stimulation of exopolysaccharide production under low nutrient concentration may benefit the diatom. The EPS can act as a polyelectrolyte that binds organic and inorganic nutrients, which can then be taken up again by the diatom itself (Staats *et al.*, 2000; Decho, 1990).

The extrusion of EPS by diatoms is an active metabolic process (Cooksey & Cooksey, 1986 as cited in Smith & Underwood, 1998). Smith (1999) found low light, darkness, and tidal migration all stimulated EPS production, although the highest percentage of photoassimilated carbon was incorporated into EPS immediately prior to emersion. However, Staats *et al.* (2000) found that high rates of exopolysaccharide accumulation were observed during daytime emersion on the mudflat, but not in darkened or DCMU-treated sediments, indicating that the secretion of exopolysaccharide was dependent on oxygenic photosynthesis. Such short-term variability in EPS production has implications for understanding estuarine carbon budgets, the biostabilization of sediments, and the measurement of sediment stability (Krumbein *et al.*, 1994).

1.5.3 EPS and sediment biostabilization

Microphytobenthic EPS can increase the mechanical stability of estuarine sediments (Austen *et al.*, 1999; de Winder *et al.*, 1999; Paterson, 1997; Yallop *et al.*, 1994). For the purposes of this thesis, sediment stability is defined as the

resistance of sediments to erosion. This definition incorporates aspects of both the erosion threshold (τ_{crit}) and erosion rate (E).

Frostick & McCave (1979) provided early circumstantial evidence for the *in situ* stabilisation of estuarine sediments by benthic algae. They investigated estuarine sediment dynamics over a 20-month period in the Deben Estuary, measuring bed level and suspended sediment concentrations. Their results suggested that high wind and wave activity in the winter lead to erosion of the unprotected sediments, while during the calmer summer conditions sediment was trapped by benthic algal biofilms, resulting in reduced suspended sediment concentrations.

Manzenrieder (1983) found natural sandy sediments were more stable than organic free sediment, and more stable than would be predicted from the Sheilds diagram (which relates the initiation of sediment motion to particle size and to shear developed along the bed). The higher stability of natural sediments was attributed to biological activity including the stabilising effect of diatoms. In several studies, a significant correlation between EPS and sediment stability, measured as the critical shear velocity (U^*_{crit}) or critical shear stress (τ_{crit}), has been found (Paterson *et al.*, 2000; Sutherland, 1996; Dade *et al.*, 1990). The ability of epipelic diatoms to mediate sediment stability has been termed 'epipelic stabilisation' (Paterson & Black, 1999). However, under certain conditions diatoms can destabilise the sediment; for example, oxygen bubbles can cause blisters within the biofilm, which lower the erosion threshold and decrease sediment stability (Sutherland *et al.*, 1998; Wiltshire *et al.*, 1998).

Once produced, the EPS remains in the sediment where it forms an extensive matrix and stabilises the sediment by:

1. The promotion of inter-particle binding by coating the sediment with organic material.
2. Altering the physico-chemical properties of cohesive sediments by the formation of polymer bridges (EPS strands) between sediment grains (Chenu, 1993; Chenu & Jaunet, 1992; Chenu & Guérif, 1991).
3. Smoothing the sediment surface through the filling of inter-particle voids, thereby increasing stability through the reduction of shear stress and turbulence (Delgado *et al.*, 1991).

Over the emersion period, stability is increased because the sediments and matrix dry out, and also due to a positive feedback mechanism whereby drying may stimulate the production of more EPS (Peterson, 1987). Paterson (1995) illustrated the presence of EPS matrixes using low-temperature scanning electron microscopy (LTSEM). Diatom-produced mucilages may only have a short half-life in the presence of water (Edgar & Pickett-Heaps, 1984) and the mucilage-induced surface stability usually decays after the return of the tide. However, Paterson (1989) noted that when dense diatom populations ($>10^6$ diatoms cm^{-2}) were present some residual stability remained after a full tidal inundation. Later work by Delgado *et al.* (1991) agreed with these findings providing the sediment contained low detrital content.

In various studies it has been shown that the colloidal fraction of EPS correlates well with biovolume or biomass of the epipelon (Taylor & Paterson, 1998; Underwood & Smith, 1998; Underwood & Paterson, 1993), and field investigations by Austen *et al.* (1999) have confirmed that a high cell density of epipelic benthic diatoms greatly stabilizes the sediment. Spatial variation in diatom biomass and species abundance caused a marked cross-shore variation in erosion threshold, with an increase in stability towards the saltmarsh line, where diatom growth is favoured due to increased exposure periods. It was generally shown that areas of net accumulation (trapping in diatom mats) were more difficult to erode.

Despite the circumstantial evidence that EPS produced by diatoms stabilizes sediments, attempts to correlate sediment stability with biological variables such as carbohydrate (EPS) or chlorophyll *a* content have met with mixed results (Consalvey, 2002; de Brouwer *et al.*, 2000; Paterson *et al.*, 2000; Austen *et al.*, 1999; Madsen *et al.*, 1993; Defew *et al.*, in press). Riethmüller *et al.* (1998) found a correlation between the erosion threshold and the biomass of the algae (as chlorophyll *a*). *In situ*, at low biogenic influences, erosion shear stress was ~0.5 Nm^{-2} and decreased gradually with increasing fine-grain fraction. Increasing surface concentration of diatoms significantly increased the critical erosion shear stresses, with maximal sediment stabilization occurring on muddy sediments.

1.5.4 EPS and the estuarine ecosystem

Top down regulation of benthic diatoms by infauna can influence sediment dynamics. Experiments using biocides to remove infauna typically show an increase in sediment stability, and it is hypothesised that stability increased due the formation of dense diatom biofilms (in the absence of grazing pressure) and the reduction in bioturbation thereby allowing for the compaction of the sediment (de Deckere *et al.*, 2001; Paterson & Underwood, 1993). Coles (1979) used a selective biocide to remove the diatom-grazing amphipod *Corophium volutator*. The resulting diatom bloom trapped fine cohesive sediment and turned the treated part of the sandflat into a mudflat. There are several examples of natural declines in grazer populations influencing sediment stability. Austen *et al.* (1999) found erosion threshold was negatively correlated to the number of *Hydrobia ulvae*. Daborn *et al.* (1993) documented an ecological trophic cascade effect, whereby migratory birds reduced the grazing pressure of *C. volutator* leading to a dense diatom biofilm and a significant increase in stability. Kornman & de Deckere (1998) showed that a decline in *C. volutator* densities caused by cold weather led to the formation of extensive diatom biofilms in the spring and was accompanied by significant deposition and a decrease in suspended sediment concentration. The recovery of the *C. volutator* population led to the removal of the biofilm and the suspended sediment concentration in the water column of the estuary increased.

The biostabilisation of sediments may have further positive ecological implications for the estuarine ecosystem. For example, EPS that is not dissolved or eroded during the immersion may be incorporated into the sediment carbon cycling or serve as a carbon source in the estuarine system (Decho, 2000; Middelburg *et al.*, 2000; van Duyl *et al.*, 2000; Yallop *et al.*, 2000; Underwood & Smith, 1998; Underwood *et al.*, 1995).

1.6 Photoautotrophy

The majority of estuarine benthic diatoms are photoautotrophs, and therefore synthesise organic materials from inorganic components using energy derived from the sun. This process is called photosynthesis, and is the mechanism by which light energy is converted into chemical energy by the reduction of carbon dioxide:

$$6CO_2 + 12H_20 \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O \qquad \text{Equation 1.1}$$

Oxygenic photosynthesis consists of light reactions (see section 1.6.3), and dark reactions (see section 1.6.4).

1.6.1 Light absorbing pigments

Algae use an extensive set of light-harvesting pigments, and the profiles of these pigment sets allows differentiation between taxonomic groups (Flameling, 1998). Chlorophyll *a* is present in all algae, and is frequently used as a proxy for microalgal biomass in marine systems (Blanchard *et al.*, 2000). In diatoms, chlorophyll *a*, chlorophyll *c*, and fucoxanthin are the main light harvesting pigments. Cyanobacteria contain the marker pigment zeaxanthin, and euglena contains chlorophyll *b* and lutein (although these are also present in the common green-macrophyte *Enteromorpha*) (Jeffrey & Vesk, 1997; Millie *et al.*, 1993).

The photosynthetic apparatus reflects the response of algae to changing environmental and endogenous variables. Regulating pigment concentrations maximizes light absorption. For example, in cultured planktonic diatoms acclimated to low-light levels (50 μmol m^{-2} s^{-1}), chlorophyll *a* and fucoxanthin concentrations per cell are lower than in cultures acclimated to high-light (1200 μmol m^{-2} s^{-1}) (Anning *et al.*, 2000). Alterations in pigment 'signatures', measured by high performance liquid chromatography (HPLC), provide a means by which the physiological state of an algal assemblage can be monitored (Millie *et al.*, 1993). Pigments may be utilised to indicate the health of a population, and the presence of breakdown products may be indicative of cell death through processes such as grazing (Cariou-Le Gall & Blanchard, 1995).

Pigments, found in the chloroplasts of plant and algal cells, absorb the energy of sunlight. The majority of pigments serve as an antenna, collecting light and transferring energy to the reaction centre chlorophyll *a*. The mechanism by which excitation energy is transferred from the chlorophyll that absorbs the light to the reaction centre is thought to be the nonradiative process of resonance transfer (i.e. excitation energy is transferred from a donor molecule to an acceptor molecule without the involvement/emission of a photon). Energy

absorbed in antenna pigments is funnelled towards the reaction centre by a sequence of pigments with absorption maxima that are progressively shifted toward longer red wavelengths (lower energy). The difference in energy between the two excited chlorophylls is lost as heat. This energy transfer is purely a physical phenomenon, and allows approximately 95 to 99% of the photons absorbed by the antenna pigments to have their energy transferred to the reaction centre where it can be used for photochemistry (Taiz & Zeiger, 1998).

1.6.2 Photosystems

Oxygen-evolving organisms have two photosystems that operate in series. Photosystem I (PSI) absorbs preferentially far-red light of wavelengths greater than 680 nm, whilst Photosystem II (PSII) absorbs red light of 680 nm well and is driven poorly by far-red light. Another difference between the two photosystems is that PSI produces a strong reductant, capable of reducing $NADP^+$, and a weak oxidant. PSII produces a strong oxidant capable of oxidising water, and a weaker reductant that re-reduces the oxidant produced by PSI. An electron transport chain links the two photosystems.

1.6.3 The Light Reactions

The light reactions of photosynthesis convert light energy into ATP and NADPH (Fig. 1.1). The photosynthetic pigments absorb photons (light energy), and the energy excites one of the electrons of a pigment molecule. The electron is boosted to the next orbital, but because the molecule is now highly unstable, the electron is passed to a phaeophytin molecule. This creates an electron deficiency in PSII, which is filled by the electron donor, Y_Z (a tyrosine on the reaction centre protein D_1). This enzyme is stimulated by the loss of electrons in PSII, and used to split two molecules of water. The electron from this reaction is then released to the waiting electron-deficient PSII. This step releases protons (H^+) into the thylakoid space helping to create a proton gradient, and also releases O_2. Phaeophytin transfers electrons to the plastoquinone (PQ) acceptors Q_A and Q_B. Q_A then passes the electron to a cytochrome complex called 'b6-f complex', which has a higher affinity for electrons than the plastoquinone. This complex passes protons from the stroma into the thylakoid space increasing the proton gradient. Plastocyanin transfers electrons from the b6-f complex to an

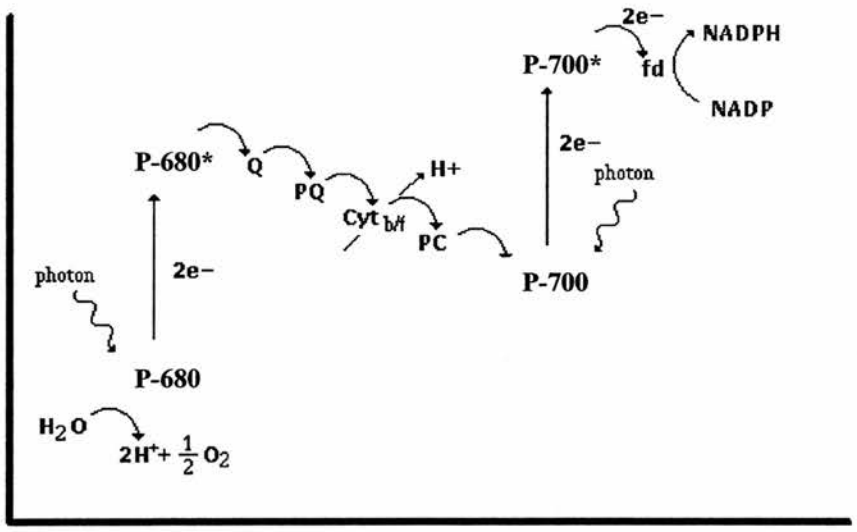


Fig. 1.1 Light reactions of oxygenic photosynthesis. P-680 = PSII; P-700 = PSI; * = excited state; Q = Phaeophytin; PQ = Plastoquinone; $Cyt_{b/f}$ = Cytochrome complex; PC = Plastocyanin; fd = Ferrodoxin. Adapted from MIT Biology Hypertextbook.

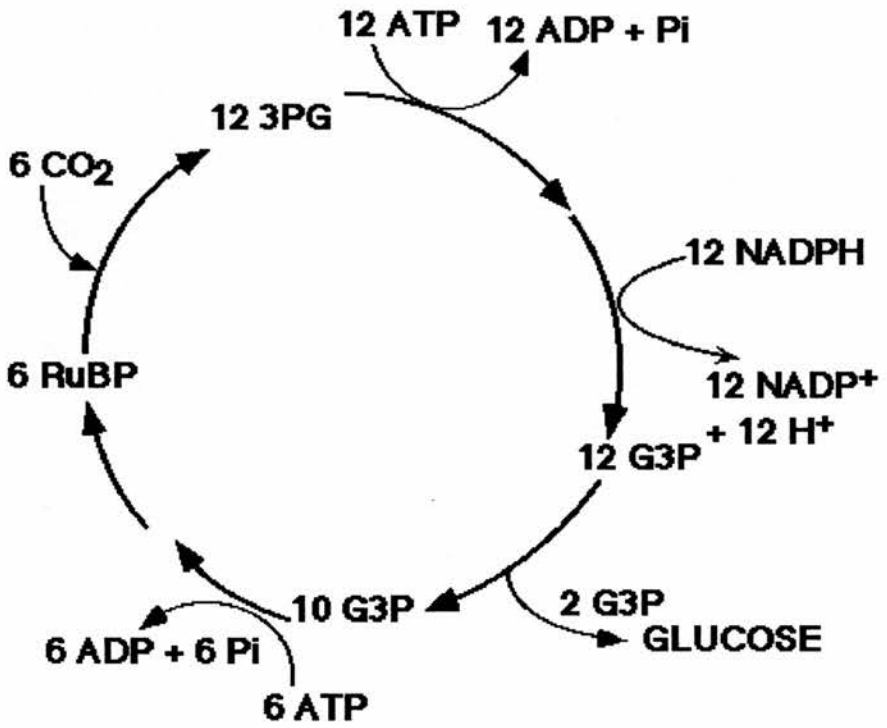


Fig. 1.2 Dark reactions (Calvin-Benson Cycle) of photosynthesis. RuBP = ribulose 1, 5 bisphosphate; 3PG = 3-phosphoglycerate; G3P = glyceraldehyde 3-phosphate. Adapted from Purves *et al.* (2002).

electron-deficient PSI (P700) and is therefore the electron donor to P700. Protons in the lumen diffuse through the enzyme ATPsynthase, and down an electrochemical gradient into the stroma, releasing energy for the synthesis of ATP (which is used in the carbon reduction reactions). PSI accepts energy from light in a similar manner to PSII, and then an electron from P700. This electron is passed to a membrane-bound iron-sulphur protein (an electron acceptor called FeS), which then passes it to soluble Ferrodoxin, which then donates its electron to $NADP^+$ reductase. $NADP^+$reductase donates the electron to a molecule of $NADP^+$ and stabilizes it by adding a proton to form NADPH (the second reducing agent for carbon fixation). This NADPH is then released into the stroma where it becomes part of the dark reactions of biosynthesis (Taiz & Zeiger, 1998).

1.6.4 The Dark Reactions

The dark reactions of photosynthesis reduce CO_2 and H_2O to carbohydrate (Fig. 1.2). During the dark reactions (also known as the Calvin-Benson cycle), the reduction of CO_2 to carbohydrate is coupled with the consumption of NADPH and ATP (generated during the light reactions). The three phases of the dark reaction cycle occur in the stroma of the chloroplast. In Phase 1 (carbon fixation), atmospheric CO_2 is incorporated into ribulose 1, 5 bisphosphate (RuBP; a five-carbon sugar). The product of the reaction (which is catalysed by Rubisco) is a six-carbon intermediate, which immediately splits to form two molecules of 3-phosphoglycerate. In Phase 2 (carbon reduction) ATP and NADPH (from the light reactions) are used to phosphorylate 3-phosphoglycerate to 1,3 diphosphoglycerate, which is then reduced to glyceraldehyde 3-phosphate (G3P). Two of these three-carbon carbohydrate precursors condense to form fructose diphosphate, which can be converted to glucose and other sugars. In Phase 3 (regeneration), more ATP is used to convert G3P back to RuBP (thereby completing the cycle). For every three molecules of CO_2 that enter the cycle, the net output is one molecule of G3P. For each G3P synthesized, the cycle spends nine molecules of ATP and six molecules of NADPH. The light reactions sustain the Calvin cycle by regenerating the ATP and NADPH (Purves *et al.*, 2002).

1.7 CO_2 uptake and assimilation

Photosynthesis is dependent on the uptake and assimilation of CO_2. Estuarine benthic diatoms take up CO_2 from the atmosphere and bicarbonate from pore water during emersion, and from the water column during immersion. The species of inorganic carbon (CO_2, HCO_3^-, or $CO_3^{=}$) in aquatic environments is a function of pH, temperature, and salinity. The majority of aquatic plants actively transport CO_2, although there is evidence that some unicellular algae obtain CO_2 by diffusion (Falkowski & Raven, 1997). It is not clear which uptake mechanisms estuarine benthic diatoms use (see Chapter 6 for further discussion).

1.7.1 Rubisco

Carbon fixed during photosynthesis is primarily assimilated by the Calvin Cycle enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). This highly conserved enzyme of low catalytic activity accounts for 5-50% of the soluble protein in algal cells (Falkowski & Raven, 1997). Isolated Rubisco can only use CO_2 as a substrate and half-saturation constants between 30-60 μM have been estimated for diatoms (Reinfelder *et al.*, 2000). Multiple factors, including light, and the supply of CO_2 and other nutrients, regulate Rubisco activity (Falkowski & Raven, 1997; MacIntyre *et al.*, 1996b). Rubisco affinities for CO_2 differ among taxa (Burkhardt *et al.*, 2001; Falkowski & Raven, 1997). The understanding of the contribution of specific taxa to primary production in estuarine systems may be greatly improved if taxon-specific Rubisco affinities are incorporated into *in situ* production models.

1.7.2 Photorespiration

Photorespiration is a metabolic pathway that occurs in the presence of light, in which Rubisco accepts oxygen, in place of carbon dioxide, resulting in the formation of a two-carbon compound, glycolate, which is subsequently metabolised to carbon dioxide. Unlike respiration there is no production of ATP.

C_3 metabolism is when the first stable compound formed from CO_2 is a 3-carbon compound at the beginning of the Calvin cycle, whilst the first organic compound incorporating CO_2 in C_4 metabolism is a 4-carbon compound. Recently, C_4 metabolism has been identified in a planktonic diatom (Morel *et al.*, 2002; Reinfelder *et al.*, 2000). In C_3 plants and diatoms, photorespiration has the

effect of reducing the rate of photosynthesis, as atmospheric oxygen can combine with Rubisco. Since oxygen is a competitive inhibitor of Rubisco, photorespiration will increase as oxygen concentration increases or as CO_2 concentration decreases. In C_4 plants and diatoms, the effect of photorespiration is negligible as the affinity of phosphoenolpyruvate carboxylase for CO_2 is extremely high.

1.8 Non-photochemical quenching and photoprotection

Non-photochemical quenching (NPQ) is a form of thermal dissipation of excess excitation energy for the avoidance of photodamage at high irradiance. In migratory diatoms, NPQ can be considered as an alternative or complementary mechanism to light-induced vertical migration. The main photoprotective pigments in diatoms are diadinoxanthin, diatoxanthin and β-carotene (Olaizola *et al.*, 1994). The extent to which different estuarine benthic diatom species use NPQ is relatively understudied (see Chapter 7 for further discussion).

Although the exact mechanisms involved in NPQ have yet to be fully resolved, it is generally accepted that the establishment of a transthylakoid proton gradient is involved, whereby an increase in the pH gradient (due to an increase in proton concentration from the splitting of water and Q_B transfer) activates enzymes which alter the epoxidation state of the xanthophyll pigments associated with the light-harvesting complex (Ting & Owens, 1993; Ruban & Horton, 1992). Cation exchange processes (involving protons and magnesium ions) may bring about changes in the ultrastructure of thylakoid membranes that switch excitation energy dissipation from fluorescence into thermal channels so that energy is lost as heat rather than light (Walker, 1990). This process acts to protect PSII from over-excitation, and prevent damage and degradation of the reaction centre D_1-polypeptide (Barber & Andersson, 1992).

If excitation transfer or photochemistry does not rapidly quench the excited state of chlorophyll, it can react with molecular oxygen to form 'singlet oxygen'. This singlet oxygen can react with and damage many cellular components (Brown *et al.*, 2000; Frank & Cogdell, 1996). The toxicity of some herbicides occurs in a similar manner, via the formation of superoxide (O_2^-),

which is very damaging to chloroplast components, especially lipids (Taiz & Zeiger, 1998).

In high light, diadinoxanthin is de-epoxidized and converted to diatoxanthin (which transfers excess absorbed energy to chlorophyll *a* less efficiently than diadinoxanthin; Robinson *et al.*, 1997), and in low light and darkness, diatoxanthin is converted to diadinoxanthin (PROMAT, 1997; Olaizola & Yamomoto, 1994). This is known as the xanthophyll cycle, and is similar to the violaxanthin (zeaxanthin) cycle in green algae and higher plants. Diatoms have two pools of diadinoxanthin, one of which does not undergo de-epoxidation and is not used in photoprotection (Arsalanne *et al.*, 1994; Olaizola & Yamomoto, 1994; Willemoes & Monas, 1991). β-carotene also dissipates excess energy away from the reaction centres via radiationless decay (i.e. heat) (Falkowski & Raven, 1997). The carotenoid accessory pigments are intimately associated with both the antenna and reaction chlorophylls, and increases in these pigments may indicate adaptation to high irradiance. Whilst they still transfer energy to chlorophyll, the efficiency of energy transfer from carotenoid to chlorophyll is less efficient than chlorophyll-to-chlorophyll.

1.9 Photoacclimation

Light penetration through cohesive sediments is very low, and so restricts microalgal production. For example, Serôdio *et al.* (1997) estimated a 0.27 mm photic zone for the Tagus estuary, whilst Paterson *et al.* (1998) used fibre optic light microsensors to estimate a 1.8 mm photic zone for the Humber estuary. Photic depths in estuarine sediments will depend on sediment type and incident light (Consalvey, 2002).

Estuarine benthic diatoms must be able to cope with variations in light availability over daily and seasonal time scales as well as changes in light intensity and spectral quality with depth. Acclimation to irradiance, and subsequent adaptation of the photosynthetic apparatus, is attributed to changes in the total amount of pigment per cell or in the ratio of different pigments, or both (Falkowski & Raven, 1997). For example, *Thalassiosira weissflogii* increases cellular chlorophyll concentrations when shifted from high to low irradiances (Post *et al.*, 1984). In a shift from lower to higher growth irradiance, carbon is

increasingly allocated away from proteins, and the cell quota of pigments and light-harvesting chlorophyll protein complexes (LHCP) decreases (Falkowski & LaRoche, 1991). Physiological acclimation serves to minimize variations in the growth rate when environmental growth-controlling factors vary.

1.10 Pulse Amplitude Modulated Fluorometry

Long-established methods of measuring photosynthesis for the estimation of primary production rates include measurement of oxygen evolution, using either Bell jars or oxygen microsensors (Barranguet, 1997; Pinckney & Zingmark, 1993; Revsback & Jørgensen, 1983), and the use of radio labelled carbon in the form of $^{14}CO_2$ or $H^{14}CO_3$ on intact sediment cores or resuspended slurries (Perkins *et al.*, 2001; Blanchard *et al.*, 1996; Blanchard & Cariou-Le Gall, 1994). The technique of Pulse Amplitude Modulated (PAM) fluorometry (as described by Schreiber *et al.*, 1986) is used for measuring minimum fluorescence (F_o; a proxy for biomass), theoretical maximum photochemical efficiency (F_v/F_m; giving an indication of the health of the biofilm), and relative electron transport rates (rETR) of microphytobenthic biofilms (Honeywill *et al.*, 2002; Perkins *et al.*, 2002; Hartig *et al.*, 1998; Kromkamp *et al.*, 1998; Serôdio *et al.*, 1997). This method has advantages over older methods since it is rapid, non-intrusive and can be deployed *in situ*, although using this method on migratory biofilms also poses problems (see Chapter 7 for further discussion).

In order to measure fluorescence under ambient light conditions, a fluorometer must be sensitive enough to detect fluorescence emission signal at specific wavelengths, but it also needs to discriminate this signal from the actinic light that drives photosynthesis (Schreiber *et al.*, 1986; Ogren & Baker, 1985). A PAM fluorometer permits the detection of very low fluorescence signals, through the use of a modulated monochromatic beam, and the measurement of the induced fluorescence at the same frequency. This technique measures the efficiency of the Photosystem II (PSII) reaction centre. *In vivo* fluorescence is emitted from the whole antenna complex, not just the reaction centre chlorophylls. At room temperature most of the fluorescence measured comes from PSII (PSI only making a small contribution – 7% at F_o) (Pfündel & Bilger, 1994; Krause & Weis, 1991).

By using the fluorometer, various parameters can be measured (Fig. 1.3) and inferences made. For dark-adapted cells, whose Q_A (the primary electron acceptor of PSII) is completely oxidised so that all reaction centres are open, all fluorescence originates from the pigment bed, and is the minimum fluorescence level (F_o; Krause & Weis, 1991). The use of F_o to trace chlorophyll *a* fluctuations is advantageous because the dark-level fluorescence yield is generally altered only under extreme conditions such as high temperatures or photo-inhibitory irradiances (Serôdio *et al.*, 2001; 1997; Krause & Weis, 1991). However, the relationship between biomass and chlorophyll *a* is a complex one. For example, measurements need to take into account the biovolume factor, where one large diatom may contain the same amount of chlorophyll as several small diatoms. Although linear relationships exist between chlorophyll *a* and dark level minimum fluorescence (Honeywill *et al.*, 2002; Honeywill, 2001; Serôdio *et al.*, 2001; 1997) the slope and intercept of the line are variable (Serôdio *et al.*, 1997). Therefore it is essential to appreciate that whilst a change in minimum fluorescence may be related to a proportional change in chlorophyll *a*, the chlorophyll *a* content *per se* cannot be determined using this method (Consalvey, 2002).

Upon application of a saturating beam of light, maximum fluorescence (F_m) is reached. This full reduction of Q_A, means that all the reaction centres are closed. The difference between the dark-adapted maximum (F_m) and minimum (F_o) fluorescence is the variable fluorescence (F_v). The ratio F_v/F_m (the efficiency of excitation capture by open PSII centres; Genty *et al.*, 1989) has become an important parameter used to assess the physiological state of the photosynthetic apparatus. A high value (~0.75) is indicative of a healthy algal biofilm (Honeywill, 2001). The values of F_v/F_m found in microalgae are often much lower than those from higher plants (Kromkamp *et al.*, 1998; Ting & Owens, 1992). Environmental stresses that affect PSII efficiency lead to a characteristic decrease in F_v/F_m (Kromkamp & Peene, 1999; Underwood & Kromkamp, 1999; Flameling, 1998; Kolber *et al.*, 1998; Geider *et al.*, 1993). For example, decreases in F_v/F_m attributed to nutrient limitation have been measured in subtidal *Gyrosigma balticum* mats, the effect being reversed by nutrient addition (Underwood *et al.*, 1999). However, care must be taken with regard to absolute values as F_v/F_m has been shown to differ between taxa (Perkins pers. comm.) and

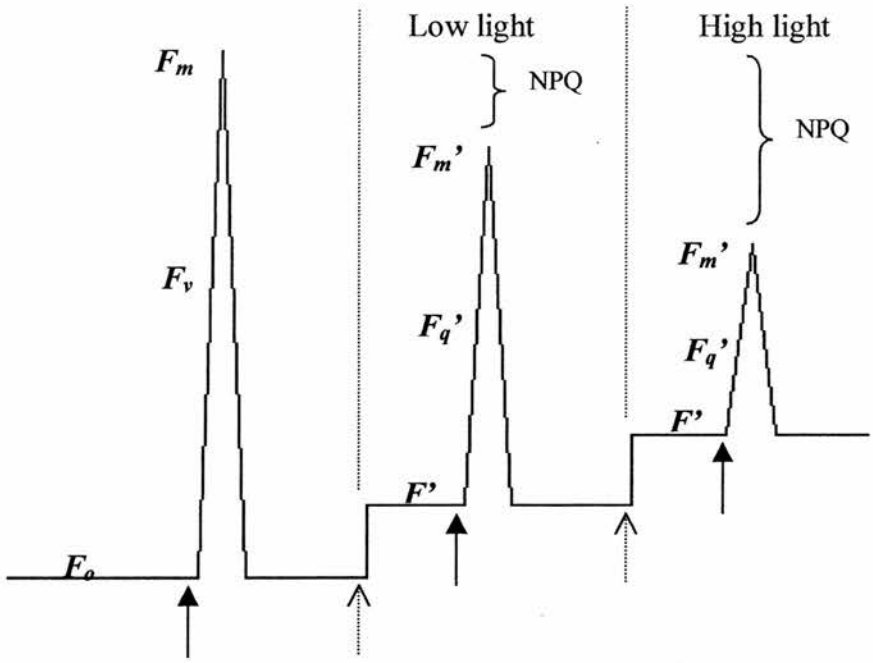


Fig 1.3 Theoretical trace of fluorescence output. F_o = minimum fluorescence where all reaction centres are open; F_m = maximal fluorescence where all reaction centres are closed; F_v = variable fluorescence (F_m–F_o); F' = fluorescence yield in the light adapted state prior to the saturating beam; F_m' = maximum fluorescence yield in light adapted state during light saturation; F_q' = fluorescence yield of PSII quenched by photochemistry (F_m'–F'); NPQ = non-photochemical quenching; Solid arrow = saturating pulse of light; dashed arrow = light on. See text (section 1.10) for further information.

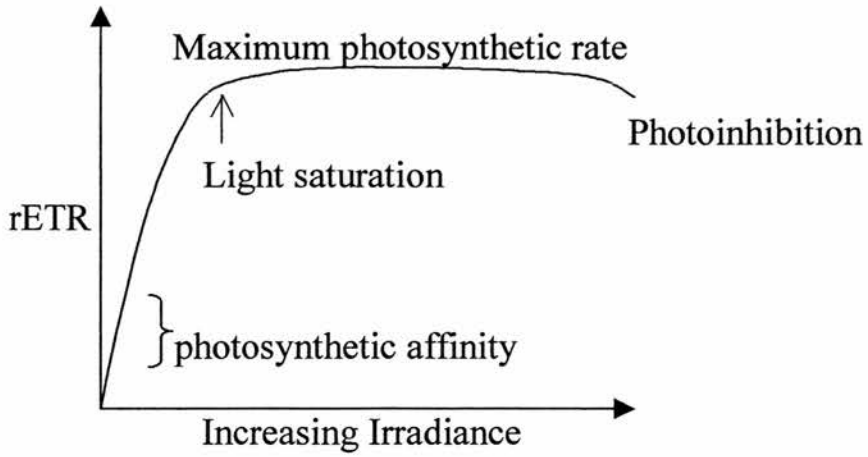


Fig 1.4 The relationship between light intensity and photosynthesis is examined using Photosynthesis-Irradiance (P-E) curves. rETR = relative electron transport rate. See text (section 1.10.1) for further information.

recent research by Parkhill *et al.* (2001) highlights that variable fluorescence can only be used as a diagnostic for nutrient-starved unbalanced growth conditions in oceanic waters.

1.10.1 P-E curves

Relative Electron Transport Rate (rETR), measured using PAM fluorescence, is increasingly being used as a proxy measurement of primary productivity (Perkins *et al.*, 2001; Kromkamp *et al.*, 1998; Sakshaug *et al.*, 1997). It is calculated as the product of the light-adapted fluorescence measurement of the photochemical capacity at PSII and photosynthetic photon flux density (i.e. $F_q'/F_m' *$ PPFD; see Chapter 2 for further details of equations).

By plotting the relative electron flow against incident irradiance, P-E curves can be constructed (P-E curves are synonymous with P-I curves. E is the correct symbol for irradiance, whilst I is the symbol for radiant intensity). Light response curves can typically be divided into three major regions (Fig. 1.4; Sakshaug *et al.*, 1997). At the lowest irradiances, photosynthetic rates are virtually linearly proportional to irradiance. This gradient/slope reflects the maximum light utilisation coefficient (α) or photosynthetic affinity, α^* (Platt & Jassby, 1976), and represents the region of the light curve in which absorption of photons is slower than the capacity of steady-state electron transport rate from water to CO_2. As irradiance increases, photosynthetic rates become increasingly non-linear and rise to a saturation level, at which the rate of photon absorption exceeds the rate of steady-state electron transport from water to CO_2. This is the maximum electron transport rate (ETR_{max}). E_k (μmol PPFD m^{-2} s^{-1}) is the light saturation index (i.e. the ratio ETR_{max}/α). This parameter denotes the irradiance at which photosynthesis is no longer limited by light absorption (and photochemical energy conversion) (Kromkamp *et al.*, 1998; Sakshaug *et al.*, 1997). With further increases in irradiance, a reduction in the photosynthetic rate relative to the saturation level may take place (down-regulation), dependent upon both the irradiance and the duration of exposure.

Henley (1993) asked whether or not the parameters ETR_{max} and α provide an adequate universal description of P-I data. For example, it is possible for some P-I data to exhibit variability in the convexity, i.e. the sharpness of transition

from PPFD limitation to saturation. Even with constant ETR_{max} and α, convexity can significantly affect photosynthetic performance at intermediate PPFDs. Whilst various models have been proposed that incorporate a convexity parameter, they are often very complex and require very little noise in the P-I data to provide robust parameter estimates. Since P-I data from estuarine microphytobenthic biofilms can be highly variable, this parameter was not investigated in this thesis.

1.11 Photoinhibition and photodamage

Photoinhibition (β) is the light-dependent reversible decrease in photosynthesis (Falkowski & Raven, 1997). Photoinhibition is a function of PPFD (above some threshold), duration of exposure, and spectral quality. It also depends on genotype as well as past and present environmental conditions (see Henley, 1993 and references therein). Photoinhibition generally causes temporary (dynamic) damage to photosynthetic units (PSU's) that results in a reduction in photochemical efficiency of the PSII reaction centre. Photoinhibition is reversible within minutes to hours, and exposure to a short dark period can aid recovery (Falkowski & Raven, 1997). Photodamage can cause temporary or permanent damage to PSU's so that a longer period of recovery is needed for regeneration of the D_1 protein and damaged PSII reaction centres.

Whereas photoinhibition is observed for cultured assemblages, Admiraal (1984) reported that photoinhibition is not often observed for natural microphytobenthic assemblages, and more recent studies support this conclusion (Perkins *et al.*, 2002; 2001; Barranguet *et al.*, 1998; Blanchard & Cariou-Le Gall, 1994; Kromkamp *et al.*, 1998; Defew *et al.*, in review). Epipelic diatoms may avoid saturating light intensities by migrating below the sediment surface. For example, *Gyrosigma* sp. and *Pleurosigma* sp. have been shown to migrate one or two cell lengths to reduce irradiance levels (Underwood & Kromkamp, 1999). This strategy may allow the biofilm to sustain high rates of productivity (Kromkamp *et al.*, 1998; Defew *et al.*, in review).

1.12 Photosynthesis and biomass

Barranguet *et al.* (1998) found a correlation between chlorophyll *a* and productivity for a sandy site but no correlation for a muddy site, whilst Wolfstein *et al.* (1998) found that *P-E* curve parameters were not correlated with biomass for Wadden Sea intertidal sediment. In the Tagus Estuary (Portugal) F_o was correlated with α and P_{max}, although the slope of the relationship varied with time of year (Serôdio pers. comm.). This suggests that other variables could be affecting the relationship between chlorophyll *a* and productivity (e.g. species composition and the migratory rhythms of the diatoms).

The short-term variability in primary production is also directly related to microalgal vertical migration. Increases in cell abundance at the sediment surface can cause significant and rapid (hourly) variations in primary productivity. For example, Pinckney & Zingmark (1991) showed a two-fold variability in production over a 6h period, and attributed these variations to vertical migration. This phenomenon should be considered when extrapolating hourly rates to monthly and annual production estimates, and primary productivity models should ideally incorporate a migration aspect (Guarini *et al.*, 2000; Pinckney & Zingmark, 1993). The 2-compartment conceptual dynamic model of Guarini *et al.* (2000) splits the microphytobenthos into the surface compartment (S), which is when the microalgae are at the sediment surface receiving the full amount of incident light, and the aphotic compartment (B) which is below the photic zone and contains potentially photosynthetically active biomass. Therefore the only difference between the two compartments is that the former actually photosynthesises because of light availability, whereas the latter does not. As a result any exchange of cells between the layers will not affect their photosynthetic characteristics. Guarini *et al.* (2000) consider this model as a basis for describing the dynamics of intertidal microphytobenthos.

Blanchard *et al.* (2001) found that in laboratory experiments, where the effects of grazing and tidal resuspension were minimal, benthic algal biomass followed a logistic-type growth curve. In the first phase of the growth curve, net production increases with the biomass level to reach its maximum value for a biomass equal to half the biotic capacity (K/2). Beyond this value, biomass increases and converged towards a maximum value at which production is

theoretically equal to zero. So when biomass is at its maximum level (K), the algal community is no longer productive. Theoretically, the biomass must be decreased down to a certain level (K/2) to maximise the net production. In the field, the kinetics of microalgal biomass exhibited biomass increases during daytime exposures and biomass decreases during other periods. This resulted in a series of oscillations (Blanchard *et al.*, 2002). Blanchard *et al.* (2000) suggested that in the field, the effect of grazing and resuspension prevents biomass from holding steady at its maximum level. Moreover, a significant negative relationship was found, which states that the higher the biomass level at the beginning of daytime exposures, the lower the net production during that daytime exposure. The high productivity of estuarine microphytobenthos was hypothesised to be due to the tight coupling between physical and biological processes.

1.13 Factors regulating microphytobenthic processes and assemblage composition

Nutrients, light, temperature, salinity and grazers regulate microphytobenthic processes, although the interactions among these factors are complex and difficult to discern. These factors will be investigated within this thesis. Stressors such as herbicides and heavy metals may also regulate microphytobenthic processes, but were not investigated in this thesis.

1.13.1 Competition

Competition among plants is primarily related to consumer-resource interactions, and Grover (1997) describes competition as the negative interactions among two or more individuals or populations for a resource. The theoretical framework and experimental evidence for resource competition is well established for phytoplankton (Grover, 1997; Tilman, 1982) and for freshwater benthic algal assemblages (McCormick, 1996). The net effect of competition is to reduce the density of one or more populations. This is a direct result of consumption of a resource by one species, below the levels required by other species, thus reducing their performance. Therefore, resources must be substances or factors that can be consumed, and that result in increased growth rates when more resources become available (Tilman, 1982). Resource

competition also assumes that species differ in their resource or nutritional requirements. Resources that are potentially important for estuarine inhabiting diatoms include nutrients, light, and space. In addition, assemblage processes are regulated by resource supply rates and the relative amount of a resource in relation to other resources (e.g., N:P).

The 'resource-ratio' hypothesis (Tilman, 1985) suggests that the supply of resources (such as light, CO_2 and mineral nutrients) might be balanced in a way that different species are limited by different resources, and thereby, coexist in perfect equilibrium at constant population densities. This was first shown by the coexistance of 2 diatom species (*Asterionella Formosa* and *Cyclotella meneghiniana*) with different optimal ratios of silicate and phosphate (Tilman, 1977). Obviously, the resource-ratio hypothesis could not account for the full species richness of primary producers, because only a few resources (usually fewer than 5) can become limiting in natural ecosystems (Sommer, 1999). Huisman & Weissing (1999) show, by numerical modelling, that the competition dynamics of systems with more than two limiting resources are fundamentally different from those with only two limiting resources. Within a wide range of parameter values, sustained oscillations or chaotic dynamics of resource concentrations and of species' abundances are possible even under constant resource supply and physical conditions. The oscillations and chaos create the environmental variability needed for the persistence of more species than the limiting resource would allow.

The 'resource-heterogeneity' model has often been proposed to explain the maintenance of plant species diversity and patterns of species diversity along productivity gradients. Resource heterogeneity should maintain biodiversity by preventing competitive exclusion (Kassen *et al.*, 2000; Crawley, 1997; Tilman, 1982; Ricklefs, 1977) because different species are superior competitors in different parts of a heterogeneous environment. In natural systems, however, resource heterogeneity co-varies with average resource supply rate, making the effect of heterogeneity difficult to isolate. Stevens & Carson (2002) found that the average supply rate of the most limiting resource controlled plant species richness, whereas heterogeneity of the resource had virtually no effect.

1.13.2 Nutrients

Cohesive sediments are generally enriched with nutrients (Underwood *et al.*, 1998; Admiraal, 1984) since tidal inundation replenishes nutrients. However, there is contradictory evidence as to whether or not estuarine benthic diatom species are nutrient limited. Nitrogen and phosphorus enrichment of intertidal and shallow water coastal systems in laboratory and field studies increased biomass (Hillebrand & Sommer, 1997; Peletier, 1996; Pickney *et al.*, 1995; Nilsson & Sundbäck, 1991; Granéli & Sundbäck, 1985; Cadée, 1984) and primary productivity (Underwood *et al.*, 1998; Nilsson *et al.*, 1991; Sundbäck & Gráneli, 1988). Nutrient enrichment frequently affects the overall structure of the diatom community, tending to increase biomass and lower species diversity and richness (Hillebrand & Sommer, 1997; Sundbäck & Snoeijs, 1991; Carrick *et al.*, 1988; Sullivan, 1976).

The capability of benthic estuarine diatoms to compete for nutrients increases as the surface to volume ratio (S:V) of the species increases (Hudon & Legendre, 1987). Species with a high S:V, such as small naviculoids, acquire nutrients more rapidly than large naviculoids and sigmoid cells (Baille, 1987; Hudon & Legendre, 1987). This may suggest that smaller species are better competitors than larger species in a limiting environment (Geider *et al.*, 1986).

Nutrient concentrations within European estuaries are likely to change due to the implementation of the Water Framework Directive (by 22 December 2003), which requires all urban sewage to be treated prior to discharge. (http://www.rec.org/REC/Programs/LocalInitiatives/Training/PDF/Water.pdf).

1.13.3 Carbon

Rothschild (1994) determined that the addition of CO_2 to *in situ* microbial mats profoundly enhanced carbon fixation rates during daylight hours. Maximum carbon fixation rates occurred in the early morning and late afternoon, coincident with times when carbon fixation would most likely be light limited. As yet there is little evidence of CO_2 limiting photosynthesis in benthic estuarine diatoms (Underwood & Kromkamp, 1999; Defew *et al.*, submitted; see Chapter 6 for further discussion), except perhaps in thick biofilms (Barranguet *et al.*, 1998; Kromkamp *et al.*, 1998; Barranguet & Peene, 1996) especially under conditions of high pH and oxygen supersaturation. For dense diatom biofilms, CO_2 may be

a limiting resource when light intensities exceed 450 μmole m^{-2} s^{-1} (Lorenzen, 1996 as cited in de Winder *et al.*, 1999). Maximum photosynthetic efficiency (F_v/F_m), measured in undisturbed cores at the beginning and near the end of the emersion period, did not differ suggesting that microphytobenthos was not limited by CO_2 (Kromkamp *et al.*, 1998). Migration into the sediment was postulated as the mechanism responsible for alleviating CO_2 limitation. Microphytobenthic algae obtain CO_2 during the immersion period; hence, early in the emersion period, microphytobenthic algae might not be limited by CO_2, whilst in the latter stages of emersion, high local respiration rates (i.e., CO_2 production) may offset CO_2 consumption associated with photosynthesis (Glud *et al.*, 1999; Kristensen *et al.*, 1997; Glud *et al.*, 1992).

There are principally two different ways in which algae may reduce carbon limitation. One is to improve the uptake of inorganic carbon into the cell, and the other is to improve the efficiency of transport within the cell (Axelsson and Beer, 2001). Limitation of energy supply by light availability decreases the rate of inorganic carbon transport, and cells grown under light-limited conditions have reduced capacity for CO_2 accumulation (Beardall *et al.*, 1998). It is possible that CO_2 limitation in benthic diatoms may be avoided by mechanisms such as CO_2 concentrating mechanisms (CCM's), HCO_3^- usage, and carbonic anhydrase activity (Burkhardt *et al.*, 2001; Riebesell 2000; Moroney & Somanchi, 1999; Beardall *et al.*, 1998; Raven, 1997).

Internal carbonic anhydrase activity has been shown to be crucial for high-affinity photosynthesis in a number of algae including marine diatoms (Matsuda *et al.*, 2002), whilst cultures of *Cylindrotheca closterium* have been shown to utilise carbonic anhydrase to increase photosynthetic rates (Defew *et al.*, submitted). Acetazolamide (Az) and ethoxyzolamide (Ez) were potent inhibitors of carbonic anhydrase in cell extracts of the marine diatom *Phaeodactylum tricornutum*. The concentration of inorganic carbon required for 50% of the maximal rate of photosynthetic oxygen evolution was increased from 53 to 542 μmol l^{-1} in the presence of Ez, whilst the membrane impermeable inhibitor Az had a lesser effect, with the concentration of inorganic carbon increasing to 130 μmol l^{-1} (Dixon & Merrett, 1988).

A CCM is one that allows the cell to create a higher CO_2 concentration at the site of Rubisco than can be explained by diffusion of inorganic carbon from

the external medium. Freshwater benthic diatoms and planktonic diatoms actively take up CO_2 and HCO_3^- (Korb *et al.*, 1997; Rotatore & Colman, 1992), whilst the marine diatom, *Phaeodactylum tricornutum*, has been shown to alter the mechanism of inorganic carbon acquisition in response to changes in the external carbon concentrations (Johnston & Raven, 1996).

It has been suggested that CO_2 limitation may occur in very thick biofilms due to rapid assimilation and a decrease in CO_2 diffusion into the sediment as depth increases (Kaplan & Reinhold, 1999; Barranguet *et al.*, 1998). Diatom mats can use different biomass-dependent biochemical pathways. Low biomass diatom mats utilise a C_3 metabolism mechanism, whilst dense diatom mats, which are more likely to limited by inorganic carbon, fix carbon using a C_4 metabolism mechanism that concentrates CO_2 around Rubisco when levels of CO_2 are low (Schwinghammer, 1983).

1.13.4 Phosphorus and Nitrogen

Annual phosphorus loading to 56 British Estuaries ranged between 0.06 and 100 mole m^{-2} yr^{-1} (0.02-3.1 kg m^{-2} yr^{-1}) (Nedwell *et al.*, 1999). Phosphorus is typically found in high concentrations in the sediments and overlying water column of estuaries. Phosphorus is unlikely to limit benthic diatom growth (Admiraal, 1977b), and maximum growth rates are attainable at very low concentrations (Admiraal, 1977b).

Microphytobenthic biomass is generally not correlated with nitrate, ammonium, or total inorganic nitrogen, indicating that nitrogen does not limit microphytobenthic growth in nutrient-rich estuaries (Underwood *et al.*, 1998). However, variability in nitrogen has been shown to influence the species composition of microphytobenthic assemblages. Several studies suggest that ammonium in estuarine sediments regulates the distribution of benthic diatoms (Underwood & Provot, 2000; Sullivan, 1999; Underwood *et al.*, 1998; Peletier, 1996; Admiraal 1977c). For example, *Navicula phyllepta*, *Navicula flanatica*, *Navicula arenaria*, *Nitzschia* cf. *dissipata*, *Nitzschia dubiformis* and *Pleurosigma angulatum* are associated with 'low' ammonium, whilst *Navicula salinarum*, *Diploneis didyma*, *Stauroneis constricta*, *Navicula cryptocepala* and *Cylindrotheca closterium* are associated with 'high' ammonium. Ammonium

toxicity may not be common in estuaries because ammonia concentrations do not generally exceed 5 mmol liter^{-1}.

1.13.5 Silicate

Silicate concentrations in estuaries may show seasonal variation, with maximum concentrations occurring during the winter and minima during the summer, after the spring bloom (Balls *et al.*, 1995; Fichez *et al.*, 1992). Silica is an essential resource for diatom growth (Keldermann *et al.*, 1988). Statistical analysis indicated that the majority of diatom taxa were not correlated with silicate pore water concentrations: *Gyrosigma fasciola* and *Nitzschia sigma* were negatively correlated, whilst *D. didyma* was positively correlated with pore water silica concentrations (Underwood *et al.*, 1998).

1.13.6 Salinity

Salinity has been suggested as an important factor regulating the distribution and species composition of diatom assemblages (Underwood & Provot, 2000; Underwood *et al.*, 1998; Peletier, 1996; Underwood, 1994; Oppenheim, 1991; Admiraal & Peletier, 1980). Estuarine diatoms can grow at salinities between 4 and 60 (Admiraal, 1984). This tolerance is especially important on exposed mudflats where rainfall can cause a sudden decrease in salinity, and evaporation can increase salinity to >40 within 1 h of exposure (Rasmussen *et al.*, 1983). Perkins (pers. comm.) found no inhibitory effects of salinity, and light curves did not differ between diatom species exposed for 7 d to salinities of 20, 35, 50 and 100.

1.13.7 Irradiance and Temperature

Early studies examined the influence of irradiance and temperature on microphytobenthic growth rates and production, often using experiments with unialgal cultures (Admiraal, 1984). However, more recent *in situ* studies have shown that irradiance and temperature effects are complex and coupled (Blanchard & Guarini, 1996; Davison, 1991). Mud surface temperature is related to incident irradiance and both factors directly influence photosynthesis (Davison 1991), making it difficult to distinguish between irradiance and temperature effects *in situ*. Moreover, photosynthetic responses will differ dependent upon

whether the irradiance is at sub-saturating or saturating levels. Whilst fundamental principles can be drawn from phytoplankton and higher plant studies (Davison, 1991), it is important to realise that extrapolation to estuarine sediments may be difficult, since estuarine microphytobenthos exhibit different dynamics, with shorter time scales of variation and the added complexity of migration, allowing diatoms to move away from adverse levels of irradiance.

The sensitivity of intertidal epipelic diatoms to light intensity and the strong correlation between light intensity and integrated microphytobenthic photosynthesis is well described (Guarini *et al.*, 2000; Underwood & Kromkamp, 1999). Microphytobenthos has previously been shown to reach photosynthetic capacity (P^B_{max}) at light intensities between 100 and 800 μmols m^{-2} s^{-1} (Hartig *et al.*, 1998; MacIntyre *et al.*, 1996a), although Perkins *et al.* (2001) have reported an inflexion point and lack of saturation occurring on light response curves taken from an *in situ* biofilm (see Chapter 7 for further details).

Monoculture studies of epipelic diatoms illustrate that differences in photosynthetic parameters between species do occur (Admiraal, 1984). Microphytobenthic diatoms tend to be shade-adapted (Pinckney & Zingmark, 1993). Diatoms in an environment characterised by low ambient light generally have increased maximum light utilisation coefficients (α) (i.e. a higher alpha) to maximise efficiency of light harvesting, lower maximum photosynthetic rates (ETR_{max}) at saturating irradiance that are limited by intrinsic processes, and a decreased saturation parameter (i.e. a lower E_K). Conversely, diatoms adapted to high ambient light conditions do not need to be as efficient at light harvesting since light is unlikely to be limiting (Hill, 1996). Therefore they are likely to have a lower α, but higher ETR_{max}.

Microphytobenthic adaptation to seasonal irradiances has been assessed using ^{14}C slurries (Blanchard & Cariou-Le Gall, 1994). E_k paralleled annual irradiance patterns, increasing between March and July and declining thereafter. Microphytobenthos may also acclimate to short-term (days) changes in irradiance. Maximum ETR and E_k for intact sediment cores were lower on days of low irradiance than days of high irradiance (Kromkamp *et al.*, 1998).

The temperature regimes within estuaries are more variable than adjacent coastal waters due to shallow water depths, different temperatures of

hydrological sources, and exposure of the sediment to the atmosphere (McLusky, 1990). Temperature is assumed to be homogenous in the horizontal plane (Guarini *et al.*, 1997) with temperature changes occurring most rapidly in the top mm of sediment (Blanchard *et al.*, 1996). For example, Vouve *et al.* (2000) reported diel surface sediment temperature variations of 10–30°C on the Marennes-Oleron Bay, France, during emersion. Such fluctuations in temperature over short periods of time can be considered a physiological stress to diatoms, particularly as temperature affects all metabolic processes. At the cellular level, PSII is believed to be the most thermolabile component of the photosynthetic apparatus, although temperature may also affect rate-limiting enzymes such as Rubisco, and temperature-sensitive steps such as diffusion and active transport across membranes (Davison, 1991).

Temperature has a significant seasonal (Admiraal & Peletier, 1980) and hourly scale effect (Blanchard & Guarini, 1996; Colijn & van Buurt, 1975) on microphytobenthic primary production and growth rates (see mini-review by Davison, 1991). The short-term effect varies among species (Admiraal, 1977a), probably due to migration (Consalvey, 2002), although many studies overlook the prospect that any variation in photosynthetic maxima might be due to differences in species composition. Blanchard *et al.* (1996) found microphytobenthic P_{max} increased along a temperature gradient, up to a 25°C maximum (T_{opt}), before declining. Photosynthesis then ceased at temperatures greater than 38°C, suggestive of a thermal threshold. T_{opt} for microphytobenthos did not differ between April, September or December suggesting that T_{opt} was a physiological constant. However, P_{max} at T_{opt} did vary with season as a function of environmental variables (Blanchard *et al.*, 1997; 1996). Guarini *et al.* (1997) modelled the spatio-temporal dynamics of mud surface temperatures and biomass specific photosynthetic capacity (P^B_{max}). Mud surface temperature dynamics were a function of three temporal scales: seasonal, lunar, and solar/daily cycles, and P^B_{max} was dependent on mud surface temperatures. Over solar and tidal cycles, P^B_{max} varied exponentially with mud surface temperatures when mud surface temperatures were below T_{opt}. When mud surface temperatures partially exceeded T_{opt}, P^B_{max} was inhibited. Along with increased grazing pressure, thermo-inhibition, which results when the range of mud surface

temperatures partially exceeds T_{opt}, may be responsible for the microphytobenthic summer biomass depression (Guarini *et al.*, 1997).

1.13.8 Predation

Both macrofauna and meiofauna consume diatoms. In addition, infauna can affect the distribution of microphytobenthic biomass by physically redistributing sediment in a process known as bioturbation. However, the overall effects of macrofauna on the species composition and biomass of intertidal benthic diatom assemblages are ambiguous.

Studies have shown macrofaunal grazing to both decrease and increase microphytobenthic biomass, depending on circumstances. For example, faecal pellets may serve as nutrient enriched microenvironments for unassimilated diatoms. The number of diatoms doubled after *Corophium volutator* were removed by the application of a pesticide (Gerdol & Hughes, 1994b), and diatom abundances have been shown to decrease with increasing densities of *Batillaria attramentaria*, *Cerithidea californica* (Byers, 2000), and *Hydrobia ulvae* (Morrisey, 1988a). Studies of the grazer effects on species composition are rare. Smith *et al.* (1996) concluded that grazers reduced the density of most diatom species, although densities of *Diploneis elliptica* and *Gyrosigma balticum* increased in the presence of macrofauna (probably due to their large and robust frustule which may protect them from consumption). In the absence of grazers (which were removed by the application of a biocide), *Navicula menissculus* and *Nitzschia epithemioides* densities increased (Underwood & Paterson, 1993b).

Attempts are being made to improve estimates of estuarine benthic primary production by incorporating microalgal-grazer effects into production models. Pheophorbide, a chlorophyll *a* breakdown product, has been used as a measure of grazing pressure; higher pheophorbide concentrations are associated with higher grazing rates (Lucas & Holligan, 1999; Brotas & Plante Cluny, 1998). However, several studies did not find a significant relationship between grazing rates and pigment breakdown products (Abele-Oescheger & Thede, 1991; Hurley & Armstrong, 1990), and Ford & Honeywill (2001) suggest that pigment breakdown products cannot be used to estimate grazing pressure in estuaries, as grazing has been seen to have different effects on chlorophyll *a* depending on the species studied.

Meiofauna have also been identified as important grazers of microphytobenthos and serve as a vital link in the transfer of energy from primary producers to higher trophic levels (Buffan-Dubau & Carmen, 2000). However, difficulties with quantifying meiofauna mean that their importance in intertidal sediment ecosystems is poorly understood. Epstein (1997a; 1997b; 1992) concluded that grazing by marine sediment flagellates, ciliates, and meiobenthos can, in some instances, control diatom production and dynamics. However, there are studies that suggest that microphytobenthos are not a significant dietary component of most meiofauna. For example, the forminifera species *Haynesina* and *Elphidium* did not show a rapid response to added phytodetritus (Moodley *et al.*, 2000) yet both these species are known to feed on diatoms (Murray, 1991). Hamels *et al.* (1998) conclude that, except for episodic blooms of herbivorous taxa, ciliate grazing does not appear to impact epipelic diatom populations.

1.14 Global environmental change

Average global surface temperature has increased by 0.6°C (or 0.06°C per decade) during the 20th Century. Mean temperature in the 1990's was 8.4°C, which is a rise of 1°C from the 1860's mean of 7.4°C. By 2100, Scottish temperature is predicted to increase by 2-3°C, with relatively more warming in winter than summer, leading to a reduced annual temperature range. Trends in coastal water temperatures around Scotland have shown a rise of 1°C since 1970 (Scottish Executive, 2002). Computer models which simulate the effects on climate of increasing concentrations of greenhouse gases project that global average surface temperature will rise by a further 3°C by the end of the 21st Century (or 0.3°C per decade). It is currently believed that most ecosystems can withstand at most a 0.1°C global temperature change per decade, before experiencing severe ecological stress. Temperature, in particular, influences organism biology, affects dissolved oxygen concentrations in water, and plays a direct role in sea level rise due to thermal expansion and melting of polar ice-caps (Braithwaite & Raper, 2002; Chen *et al.*, 2002; Pew Centre, 2002).

Since 1750, atmospheric concentrations of greenhouse gases have risen with the greatest concern being a 31% increase in CO_2. Of the increase in CO_2

emissions, three-quarters are attributed to the burning of fossil fuels (Freund, 2003), and over the last two decades, CO_2 has increased at a rate of 1.5 ppm yr^{-1}. Increases in the concentration of these greenhouse gases will reduce the efficiency with which the Earth cools to space, and will tend to warm the lower atmosphere and surface. Whilst the correlation between carbon dioxide and temperature is high, carbon dioxide often lags behind major temperature changes. Evidence from modelling studies indicates that the sensitivity of global mean surface temperature to doubling CO_2 is unlikely to lie outside the range 1.5 to 4.5°C (Bryant, 1997).

Forecasts of a rising sea level are based on climate model results, which indicate that the Earths average surface temperature may increase between 1.4 and 5.8°C during the 21st Century. Global warming is expected to cause a further rise of between 9 and 88 cm by 2100, with a best estimate of 50 cm, if emissions of greenhouse gases remain uncontrolled. This expected rate of change (an average of 5 cm per decade) is significantly faster than that experienced over the last 100 years. However, forecasting sea-level rise involves many uncertainties, and other complications include local coastline variation, changes in major ocean currents, regional land subsidence and emergence and differences in tidal patterns and seawater density (IPCC, 2001). Higher sea level and increased storm frequency may affect the sedimentation patterns of mudflats and estuaries.

In coastal zones, climate change has been predicted to have seven key impacts (IPCC, 2001):

1) Lowland inundation and wetland displacement.
2) Accelerated shoreline erosion and reduced wetlands area.
3) More severe storm-surge flooding.
4) Saltwater intrusion into estuaries.
5) Altered tidal range.
6) Changes in sedimentation patterns.
7) Decreased light penetration to benthic organisms.

The scientific community has some reasonable knowledge of how increases in temperature will affect plant and animal physiology, abundances and distributions; aquatic oxygen concentrations; and sea level. However, they know less about temperature's influence among organisms, and we know almost nothing about the effects of high temperature on the microscopic organisms

(such as diatoms) that play significant roles in the 'microbial loop' of food webs. Thus we cannot predict how increased temperature might affect webs or systems as a whole (Pew Centre, 2002).

Estuaries are already exposed to a wide variety of human and natural stressors. The added burden of climate change may further degrade these valuable ecosystems, threatening their ecological sustainability and the flow of goods and services they provide to humans. Thus, there is a strong need to understand how estuarine benthic diatoms will be affected under the IPCC's predictions and how these effects will be transferred to other ecosystem processes.

1.14.1 The potential effects of increased temperature

Predicted increases in atmospheric temperature due to climate change could result in either enhanced thermal stresses within the sediments, or optimised growth conditions for benthic diatoms. Harvey (2000) suggests that warmer temperatures may suppress the down regulation of the photosynthetic response as higher temperatures increase the cellular demand for photosynthetic products. However, in terms of estuarine microphytobenthos, this would suggest a higher ETR in warmer habitats, which has not been observed (Perkins *et al.*, 2001).

Higher global temperatures may influence microphytobenthic competition dynamics. For example, Watermann *et al.* (1999) determined that temperature was a key factor regulating the competition dynamics between cyanobacteria and epipelic diatoms. At 10°C cyanobacteria growth rates were low compared to diatoms. However, at 25°C, the cyanobacterium, *Microcoleus chthonplastes*, dominated. The dominance of cyanobacteria at high temperatures and diatoms at low temperatures has also been observed in natural microphytobenthic assemblages (PROMAT, 1997). It is likely that any taxonomic shifts observed at higher temperatures would be mediated by the physiological constraints imposed by higher temperatures on diatom processes (Blanchard *et al.*, 1996, Davison, 1991). For example, although diatoms can actively photosynthesise at temperatures greater than 25°C, they require lower temperatures at night to maintain high rates of cell division (Admiraal, 1977a). If

the trend of increasing nocturnal temperatures continues at the present rate, diatom cell division could be adversely affected.

The composition of diatom EPS varies inter- and intraspecifically (Holland *et al.*, 1974). Diatoms can stabilise the sediment, but also different epipelic diatom species may do so to differing degrees. An increase in global temperature may result in a shift in species composition, which in turn could have important consequences for sediment stability, sediment transport and coastal erosion. One scenario is that matrix strength and thus sediment stability could be compromised if the resultant assemblage extrudes less colloidal carbohydrate. Alternatively, an assemblage that produced significantly larger amounts of EPS could lead to an increase in critical erosion shear stress (Underwood, 1994). The formation and decomposition rates of EPS are also temperature dependent (Wolfstein & Stal, 2002). To date, the possible feedback of temperature and sediment stability through the effects of temperature on EPS dynamics has received little attention. Sediment erosion dynamics may also be affected, due to temperature effects on assemblage composition and vertical migration.

Higher temperatures may affect the timing and duration of benthic algal blooms. Short-term and seasonal variations in P^B_{max} suggest that the microphytobenthic communities take advantage of spring environmental conditions, allowing the onset of blooms (Blanchard *et al.*, 1997). If a region warms due to predicted climate change, seasonal boundaries could be affected, so that optimal temperatures and consequently blooms occur earlier in the year. This in turn could potentially lead to a negative cascade effect, which could be traced up through the estuarine food chain (Daborn *et al.*, 1993).

1.14.2 The effects of increased atmospheric carbon dioxide

The effects of increased atmospheric CO_2 on benthic diatom processes have not been adequately addressed and therefore limit our predictive capabilities. However, we can draw information from the plant literature. The initial direct effect of higher atmospheric CO_2 would be to stimulate photosynthetic rates. However, the long-term effect of higher CO_2 on photosynthesis and carbon storage in ecosystems is complicated by feedback

mechanisms, such as changes in biochemistry, nutrient cycling and relative species abundance (Harvey, 2000).

It is widely agreed that the terrestrial biosphere sink is limited in its capacity to absorb excess CO_2 (IPCC, 2001). At present, carbon cycling models may be missing a significant sink term that may further reduce CO_2 concentration projections. This sink could solely be explained by the CO_2 fertilisation effect, which is based upon the idea that plants in a CO_2-rich atmosphere grow faster, and so absorb more CO_2. Taking into account missing sinks could lead to different CO_2 projections regarding the carbon budget and radiative forcing (Kaldy *et al.*, 2002; Plattner *et al.*, 2002; Wigley & Raper, 1992). The influence of increased atmospheric CO_2 concentration on estuarine benthic diatom species has yet to be addressed. Photosynthesis may be enhanced under increased CO_2 concentration, but estuarine benthic diatoms are unlikely to provide a significant sink for carbon since they are themselves consumed by estuarine macro- and meiofauna. A sink has to trap and remove the carbon from the carbon cycle (Blanchard, pers. comm.).

If CO_2 limits microphytobenthic growth, then elevated atmospheric CO_2 concentrations could influence benthic diatom processes in a manner similar to nitrogen or phosphorus enrichment. For example, as a result of phosphate inputs many previously unproductive lakes have become highly productive with diatoms, whilst green algae have been replaced by cyanobacteria (Tilman, 1993). In general, the addition of a limiting nutrient is thought to cause a shift in the composition and diversity of that community. Shifts in carbon supply rates or in the ratio of carbon to other essential resources (i.e. nitrogen or phosphorus) could alter assemblage composition, so that taxa with a high affinity for carbon dominate. If CO_2 was previously limiting, then increased atmospheric CO_2 concentrations will cause species to be limited by a different resource (competitive release). The best competitor for that newly limiting resource could potentially out-compete other species (Tilman, 1993).

Increased atmospheric CO_2 concentrations could potentially alter physiological processes. For example, under high CO_2 concentration the probability of a CO_2 molecule binding with the Rubisco enzyme is expected to increase, thereby reducing photorespiration and increasing net photosynthesis. However, nutrient availability, irradiance, or pollutants/toxins might confound

primary production by down-regulating the photosynthetic response, partitioning production, or changing the relative species abundances (Harvey, 2000). When CO_2 and light are not limiting, nutrient limitation often results in unbalanced growth (Lorenzen, 1996 cited in de Winder *et al.*, 1999) indicating that the fixed CO_2 is used for the production of EPS rather than for structural cell material.

There is the possibility that the effects of climate change will vary among estuaries with different trophic states. It is hypothesised that nutrient limited ecosystems are to some extent buffered against global changes, so that net primary production and total carbon storage of nutrient-limited systems are likely to be less affected by climate change than enriched systems. If climatic and atmospheric conditions become more favourable for growth then the frequency of nutrient limitation may increase; alternatively, adverse climatic conditions might increase nutrient supply rates (Shaver *et al.*, 1992).

1.14.3 Increases in storm frequency and sea level

It is expected that increases in storm intensity and frequency will negatively affect intertidal ecosystems. Storms can disturb and mobilise as much as the top 10cm of intertidal sediments (Yallop & Paterson, 1994), increase the probability of nutrient release (Malcolm & Sivyer, 1997), reduce light availability, and disturb mature biofilms (Colijn & Dijkema, 1981). The impact of rain has also been shown to lower the stability of intertidal sediments and increase sediment erosion (Tolhurst, unpublished data).

Recovery from storms is strongly influenced by the microbiology of the sediment. Although estuarine diatom populations are able to recover rapidly from episodic disturbance events (Consalvey, 2002; Underwood & Paterson, 1993b), it is unclear what the long-term effect of more frequent and intense storms will be on the development and longevity of benthic diatom biofilms. If storm frequency increases as predicted under the IPCC 'business as usual' scenario, this is likely to have important consequences for coastal ecology and the associated communities, as well as the processes of land reclamation and coastline erosion.

Predicted sediment deposition rates are thought to be high enough to compensate for the area loss associated with sea level rise (Temmerman *et al.*, 2003; Wigley & Raper, 1992). Thus, in theory there should be no net loss of sand and mud deposits and impacts on benthic diatoms should be minimal. However,

as low water moves landward, and sea defences prevent a compensating landward migration of the high water mark, intertidal flats are likely to be squeezed out. It has been estimated that by 2013, sea level rise could result in a loss of 8,000 to 10,000 ha of intertidal flats in England. Much of this loss is expected in southern and southeast England, although research suggests that the major firths in Scotland will also be affected (UKBAP, 2002). Within estuaries, mudflats deposited in the past may erode due to changed estuarine dynamics, and remobilised sediment may be redeposited elsewhere.

1.15 Biodiversity

Although poorly defined, the term 'biodiversity' or 'biological diversity' has become widely used by environmentalists, conservationists and ecologists. Gaston (1998) presents 10 different definitions of biodiversity and discusses whether it is a measurable entity. For the purpose of this thesis, biodiversity is examined in terms of the taxonomic variety in estuarine benthic diatom assemblages, and how this changes in response to different environmental variables. The reasons why biodiversity should be conserved include: 1) it provides sources of marketable commodities; 2) it provides non-market goods and services; 3) it has intrinsic value and therefore human kind has moral and ethical responsibilities towards it (Gaston, 1998).

In ecological studies, samples will frequently consist of information on the number and relative abundance of the species present, with the diversity of the sample being dependent on the two distinct components of species richness and species evenness or equability (Waite, 2000). Diversity is used as an index of ecosystem well being, and thus diversity measures have a potential application as a monitor of environmental change. The assumption that the adverse effects of 'change' will be reflected in a reduction in diversity or by a change in the shape of the species abundance distribution is a central theme (Magurran, 1988). Species diversity has functional consequences because the number and kinds of species present determine the organismal traits that influence ecosystem processes (Chapin *et al.*, 2000). For example, a change in biodiversity of estuarine benthic diatom assemblages could affect ecosystem functions such as carbon turnover, grazing rates, and primary production.

1.16 System monitoring

It is important to study the effect of environmental variables on natural microphytobenthic diatom populations. However, studies on such small spatial and temporal scales make the prediction and extrapolation of carbon fixation over large areas dubious and the investigation of environmental change difficult. Recent developments in a number of techniques will allow scientists and interested parties to determine 'system status' and monitor changes due to natural factors and anthropogenic pressures. In conjunction with ground-truthing measurements and process modelling, remote-sensing techniques such as spectral reflectance and fluorescence can be used to make inferences about the distribution, abundance and productivity of benthic autotrophs without destructively sampling the sediment (Kromkamp *et al.*, 1998; Paterson *et al.*, 1998).

1.17 Thesis Aims

Using a variety of techniques including fluorescence, high performance liquid chromatography and microscopy, the responses of microphytobenthic biofilms to aspects of global change were investigated. The environmental factors of light, temperature, nutrients, carbon dioxide and grazers were assessed in terms of the structure (i.e. assemblage composition) and functioning (i.e. health and productivity) of the biofilm. The importance of diatoms and biofilms in terms of sediment stability of estuarine mudflats was also investigated.

The principle aims are:

- Determine if estuarine benthic diatom biofilms can be used as laboratory model systems to investigate the influence of environmental factors.
- Investigate how temperature influences the structure and functioning of estuarine benthic diatom assemblages grown at sub-saturating irradiance.
- Study the influence of grazing on diatom assemblage structure, and determine the importance of grazing under different nutrient and temperature regimes.
- Investigate how enhanced carbon dioxide conditions might influence the structure and functioning of natural benthic diatom assemblages.

- Determine the effect of diatom migration on photophysiological parameters measured by chlorophyll fluorescence.
- Investigate how estuarine microphytobenthos influences sediment stability, and verify whether sediment erosion thresholds can be determined from biological and/or physical proxy parameters.

Chapter 2

Chapter 2: General Methods

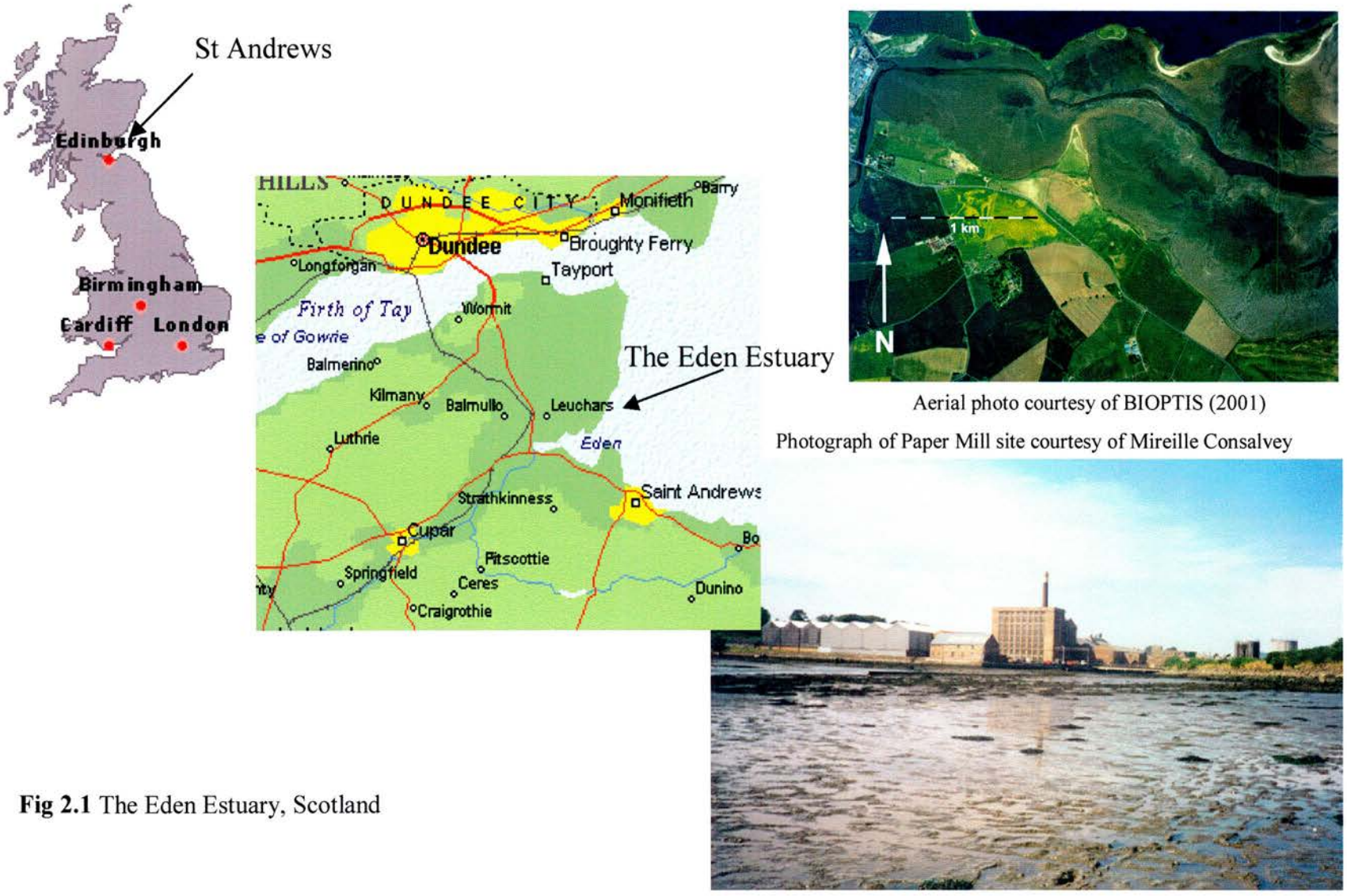
2.1 Study sites

The main sampling sites were the Eden Estuary, Scotland, and the Westerschelde and Oosterschelde estuaries in the Netherlands. All sites are extremely well classified since being extensively surveyed during the 1999 and 2000 BIOPTIS field campaigns (BIOPTIS EU MAS3-CT97-0158). This surveying included the ground-truthing of a number of variables including sediment grain size, sediment infaunal species composition, photosynthetic parameters, biomass (chlorophyll *a* and minimum fluorescence), pigment fingerprinting, sediment surface reflectance, and sediment erosion characteristics. Remote-sensing and ground-truthing measurements were made simultaneously.

2.1.1 The Eden Estuary

The Eden Estuary is a relatively small estuary situated on the east coast of Scotland between St Andrews and the Firth of Tay (56°22'N, 2°50'W) (Fig 2.1). This mesotidal estuary has a total area of 10.41 km^2, of which 9.37 km^2 are intertidal mudflats exposed at low tide (Davidson & Buck, 1997). Grain size of the Eden Estuary is heterogeneous, with grain size increasing towards the mouth of the estuary. The sediment bed is generally flat with minor drainage channels running down to the central channel of the River Eden, above which water height does not generally exceed 5 m at high tide. The hydrodynamics of the system are dominated by tidal currents rather than by wave action due to the presence of a sand bar at the mouth of the estuary.

The Eden Estuary has recently been appointed as a Special Area of Conservation (SAC; JNCC, 2003) The Estuary is adjacent to an extensive dune system nature reserve, has two Sites of Special Scientific Interest, and is an important area for migrating birds and waterfowl populations. The high nitrogen concentration associated with the Eden estuary is likely to be from the prime agricultural land that makes up more than three quarters of the surrounding catchment area (Clelland, 1994; Mathieson & Atkins, 1995), but will also have nutrient inputs from the golf courses and RAF base.



Aerial photo courtesy of BIOPTIS (2001)

Photograph of Paper Mill site courtesy of Mireille Consalvey

Fig 2.1 The Eden Estuary, Scotland

The polychaete worms, *Nereis diversicolor* and *Arenicola marina*, the amphipod *Corophium volutator*, the gastropod *Hydrobia ulvae*, and the mussel *Mytilus edulis* are the dominant macrofaunal species found in the Eden Estuary. Other macrofaunal species include *Cerastoderma edule*, *Eteone longa*, *Macoma balthica, Scrobicularia plana*, *Spio filicornis* and the Oligochaete and Tubificoides spp. Much less is known about the meiofaunal populations of the Eden estuary, although the seasonal abundance of foraminifera populations and their link with diatom blooms is currently under investigation (Austin. pers. comm.). Enteromorpha spp. is the dominant macrophyte and forms extensive blooms from spring to autumn, whist the seagrass Zostera spp., *Fucus spiralis*, Porphyra spp., and Ulva spp., form small localised populations at various sites along the estuary. Diatoms are the dominant microphytobenthos, and often form biofilms on the sediment surface, although cyanobacteria are occasionally present.

The most frequently used site was situated near Guardbridge paper mill. Sediments from this site were classified as sand and sandy-mud sediments, whose fine grain fraction (>0.063 μm) was between 10-25% (classification after Figge *et al.*, 1980 cited in Riethmüller *et al.*, 1998). *F. spiralis* is found on boulders at the top shore, but diatom assemblages dominate the sediments. Sampling dates are specified for individual experiments in each chapter.

2.1.2 The Westerschelde and Oosterschelde Estuaries.

The Westerschelde and Oosterschelde Estuaries are situated in the southwest of the Netherlands, near the town of Yerseke and the NIOO laboratory (Fig. 2.2). These two estuaries differ naturally in their nutrient status (Table 2.1). The sampling sites were Biezelingse Ham, the Molenplaat, and Zandkreek.

Table 2.1 Nutrient levels in the Westerschelde and Oosterschelde Estuaries (Data courtesy of Dr J. Kromkamp and the NIOO laboratory).

	Westerschelde	Oosterschelde
NH_4 μmol N l^{-1}	0.8	3.7
NO_2 μmol N l^{-1}	3.7	0.9
NO_3 μmol N l^{-1}	248	6.0
PO_4 μmol P l^{-1}	4.25	0.6
Molar N:P	59	18
Salinity	22	31

Fig 2.2 The Westerschelde and Oosterschelde Estuaries

A: Zandkreek site; B: Biezelingse Ham site; C: Molenplaat

Photographs courtesy of Dr Richard Ford

The Biezelingse-Ham mudflat is a protected embayment located on the north shore of the turbid, eutrophic and well-mixed Westerschelde Estuary (51° 26' N, 3° 55' E). It has an area of about 1.5 km^2 and a mean tidal range of approximately 4 m. The mudflat surface is mostly level with some minor drainage channels, and major bedforms are generally absent (de Brouwer *et al.*, 2000). The dominant macrofaunal species at this site was *C. volutator*, although other species were present including *C. edule*, *E. longa*, *Heteromastus filiformis*, *H. ulvae*, *M. balthica*, Oligochaete spp., *S. plana*, and *S. filicornis*. One of the local features of this site was a thick, confluent, migratory microphytobenthic biofilm found on the top-shore at various times of the year.

The Molenplaat (51° 26' N, 3° 57' E) is a small intertidal flat situated in the Westerschelde estuary, and is only accessible by boat. Most of the tidal flat is located between –1 and +1 m relative to mean tidal level. The mean tidal range is approximately 5 m with the average emersion period ranging between 4.5 – 8 hours (Herman *et al.*, 2001). Diatoms dominate the microphytobenthic assemblage for most of the year, with the occasional occurrence of cyanobacteria and euglenoids (Barranguet *et al.*, 1997; Sabbe, 1993; Sabbe & Vyverman, 1991). At the time measurements were taken, the dominant macrofaunal species were *Bathyporeia* spp., *C. edule*, *C. volutator*, *H. filiformis*, *H. ulvae*, *M. balthica*, *Pygospio elegans* and *T. marioni*. This mudflat is known for the seasonal appearance and disappearance of a centralised area where silt and diatoms interact and accumulate (Herman *et al.*, 2001; Lucas & Holligan, 1999).

The Zandkreek mudflat is a relatively sheltered subsytem with ca. 3 km^2 tidal flats, and is situated on the south shore of the oligotrophic Oosterschelde Estuary (51° 32' N, 3° 54' E). The tidal amplitude of this site is approximately 3.5m (Vermaat *et al.*, 1987). This is a silty-mud, nutrient-poor site characterised by low biomass and productivity. Dominant macrofauna were *A. marina*, *C. edule*, *C. volutator*, *E. longa*, *H. filiformis*, *H. ulvae*, *M. balthica*, Oligochaete spp., *Scoloplos armiger*, *S. plana*, *S. filicornis* and *Tharyx marioni*.

2.2 Sediment collection

Sediment from experimental cores was sampled using one of two methods. Surface sediment was obtained using either a contact core (Chapters 3, 4, 7 and 8) or a mini-core (Chapters 5 & 6). Both methods required the sediment

to be frozen in liquid nitrogen (LN_2) immediately upon collection, until returning to the laboratory where samples were stored at -80°C until required for further analysis.

2.2.1 The contact core

The contact core consists of an aluminium dish fitted with a plastic collar (Honeywill *et al.*, 2002). The dish was lightly pushed downwards into the sediment, until the base of the dish touched the sediment surface (a depth of ~ 2mm). LN_2 was then poured into the dish and left for between 15 and 30 s (depending on the sediment water content) before the contact core was removed from the sediment. Any sediment not level with the edge of the collar was quickly removed by scraping a knife across the base of the contact core. The collar was then removed and the frozen disc of sediment wrapped in labelled foil and stored in LN_2. The contact core had an area of 2463 mm^2, and sediment depths varied between 2 and 5 mm.

2.2.2 The mini-core

The base (i.e. the tapering section) of a small syringe (1.8 cm diameter) was removed. The syringe was then placed into the sediment to a depth of ~5 mm, rotated 360° within the sediment and removed. Any sediment protruding from the syringe was removed by scraping a knife across the base of the syringe. The sediment was extracted from the syringe using the syringe-plunger, and wrapped in labelled foil before being frozen and stored in LN_2.

2.3 Sediment grain size and organic content

Granulometry samples were collected with a 70 mm (diameter) core to a depth of 0.1 m. Samples were oven-dried for 48 h at 100°C. 25 g dried samples were treated with 10% sodium hexametaphosphate solution (a deflocculating agent) and left to stand overnight. Sediment was stirred for 15 min and wet sieved through a 63-μm sieve. The residue was re-dried at 100°C for a further 48 h before sediments were dry sieved with a stacked shaker (ranging from 2.0 mm to 0.063 mm) (method modified from Buchanan & Kain, 1971). Surface organic matter was calculated using the ash-free dry weight method. Samples were dried

to a constant weight in an oven at 100°C. A sub-sample of known weight was ashed at 550°C for 6 h, and then re-weighed after being allowed to cool at room temperature in a desiccator. Any data presented in this thesis regarding sediment grain size and organic content are thanks to the Aquatic Services Unit, Cork, Ireland (BIOPTIS, 2001).

2.4 Water content

Sediment water content was determined as the percentage water of frozen wet sediment, where Wt_{wet} was the wet frozen sediment mass and Wt_{dry} was the dry frozen sediment mass:

$$\% \text{ Water Content} = ((Wt_{wet} - Wt_{dry}) / Wt_{wet}) * 100 \qquad \text{Equation 2.1}$$

Frozen sediment was placed into labelled plastic bags, and weighed before and after freeze-drying (lyophilisation). Sediments were lyophilised in an Edwards Modulyo Freeze Dryer, rather than in an oven at 110°C, in order to avoid pigment breakdown. Lyophilisation took place in the dark, at -60°C and under vacuum, until sediments were completely dry (approx. 24 h). Upon removal from the freeze-dryer, and after being weighed, samples were sealed and stored in the dark at -80°C.

2.5 The quantification of carbohydrates

Sediment carbohydrates were quantified using the spectrophotometric Dubois assay (Dubois *et al.*, 1956), which is based upon the reaction of a carbohydrate-phenol mixture to the addition of concentrated sulphuric acid (H_2SO_4). This exothermic reaction results in the production of the colour compounds furfurals and hydroxymethylfurfurals (from pentoses and hexoses). The extinction of this colouring (orange/yellow) is measured by spectrophotometry at a wavelength of 485 nm, which corresponds to peak absorbencies of the coloured compounds (Taylor, 1998). Total, colloidal fractions, and polymeric components (EPS) of the colloidal fraction are measured and quantified against glucose standards; hence results are expressed as glucose equivalents.

2.5.1 Dubois assay

0.5 ml of 5% w/v Phenol solution, followed immediately by 2.5 ml conc. H_2SO_4 onto the solution surface, were added to 1 ml of sample extract. The solution was vortexed, and left to stand for 45 min (to allow the colour to develop). The solution was decanted to a centrifuge tube and centrifuged at 2500 rpm for 15 min. After being decanted into cuvettes, absorbencies were measured on a Spectrophotometer at 485 nm (BIOPTIS protocols).

2.5.2 Carbohydrate extraction

For total carbohydrate extraction, approximately 5 mg of freeze-dried sediment was weighed into a boiling tube. Freeze-drying has been determined to increase the yield of carbohydrate (Underwood *et al.*, 1995). 1 ml of distilled water was added to the sediment samples, and a 1 ml blank was made. The sediment/water mixture was vortexed, after which the Dubois assay procedure was followed.

For colloidal and EPS in colloidal fractions, approximately 100 – 150 mg of sediment was weighed into a centrifuge tube. 5 ml of distilled water was added to the sediment and left for 15 min at room temperature. The samples were then vortexed and centrifuged (2500 rpm for 15 min). For the colloidal fraction, 1 ml of supernatant from the 5 ml extract was placed in a clean boiling tube, and assayed. For the EPS in colloidal fraction, 7 ml of cold ethanol (between 2 – 4°C and for a final concentration of 70% ethanol) was added to 3 ml of the supernatant in a centrifuge tube. The samples were left overnight in the refrigerator before being centrifuged (2500 rpm for 15 min). The supernatant was discarded and the pellet was resuspended in 1 ml of distilled water. The Dubois assay procedure was followed (without the centrifugation).

2.5.3 Glucose standards

A calibration curve of glucose dilutions was produced before each batch of samples. 3 repetitions of 0, 1, 2, 5, 10, 20, 50 and 100 $\mu g\ ml^{-1}$ glucose were made up to 1 ml from a stock solution, followed by Dubois assay. Coefficients and constants were calculated from linear regressions of the standards.

2.5.4 Calculations

Carbohydrate concentrations were calculated as:

$$\text{Total Carbohydrate } (\mu g\ g^{-1}) = \frac{((Abs - C)/M)}{Wt} \qquad \text{Equation 2.2}$$

$$\text{Colloidal Carbohydrate } (\mu g\ g^{-1}) = \frac{((Abs - C)/(M))(5)}{Wt} \qquad \text{Equation 2.3}$$

$$\text{EPS in colloidal Carbohydrate } (\mu g\ g^{-1}) = \frac{((Abs - C)/(M))(1.67)}{Wt} \qquad \text{Equation 2.4}$$

Where Abs is the absorbance at 485 nm, C is the constant (intercept of the line), M is the coefficient (gradient of the line) and Wt is the sample weight (g). The number 5 is derived from 1 ml being used out of a 5ml sample (5/1=5) and 1.67 is derived from 3 ml being used out of a 5ml sample (5/3=1.67).

2.6 Pigment analysis

Algal groups have characteristic pigments (Jeffrey *et al.*, 1997), and certain groups within microphytobenthic assemblages can be distinguished on the basis of pigment fingerprints (Wiltshire *et al.*, 1998). High Performance Liquid Chromatography (HPLC) has proven effective in rapidly separating and distinguishing pigments, their breakdown products and carotenoids within monotypic and mixed algal samples. This chromatographic technique produces a chemotaxonomic signature for the algal types (Jeffrey *et al.*, 1997), and allows for the quantitatively accurate determination of pigments in estuarine sediments. It is particularly useful for the determination of chlorophyll *a*, since this is used as an indicator of algal biomass.

2.6.1 The HPLC

The HPLC consisted of a quaternary high-pressure pump (Perkin-Elmer 410), an autosampler (Waters WISP 417) cooled to 4 – 6°C, a column oven containing a reversed phase Nucleosil C18 column (Capital HPLC Ltd), an oven

thermostat which maintained the column oven at 25°C, and a Photo-diode Array Detector (PDA; Waters 910). The Waters software programme 'Millennium' coordinated the HPLC components, and amalgamated and stored the resulting chromatograms. The column was replaced every 300-600 samples, when chromatogram peaks became diffuse.

2.6.2 Pigment extraction

Pigments were extracted using either 90% Dimethylformamide (DMF) or 100% acetone (only used in Chapter 3). The change in extraction method was used because 90% DMF was found to be more effective at extracting chlorophyll *c* from sediment samples and was particularly useful for extracting chlorophyll *a* from cyanobacteria. Sediments dominated by microphytobenthos have been found to be comparable between extraction methods (BIOPTIS final report, 2001; Honeywill, 2001).

1 ml of 90% DMF or 100% acetone was added to a known quantity (~ 0.1 g) of lyophilised sediment. Extraction took place in the dark, for a minimum of 24 h at 4°C (DMF) or at -70°C (acetone). Sediment was separated from the solvent prior to HPLC analysis, by filtration through a 0.2 μm pore syringe filter (Whatman™).

Sample extractions were injected into a tertiary solvent gradient at a flow rate of 1.0 ml min^{-1} (Wiltshire, BIOPTIS protocols). Solvent A was 80% methanol, 10% water and 10% buffer (3.75 g tetrabutylammonium acetate and 19.25 g ammonium acetate in 500 ml distilled water), solvent B was 90% methanol and 10% acetone, and solvent C was 56.5% methanol and 43.5% propanol. All solvents were HPLC grade and degassed prior to use by bubbling helium through them for 5 min. In order to sharpen the chromatogram peaks, 30 μl of distilled water was injected prior to injection of 70 μl of a sample (Wiltshire, 2000). Each sample ran for 40 min.

2.6.3 Pigment identification

Each sample produced a chromatogram at a wavelength of 430 nm. Pigments are retained on the column for different periods (called the retention time) and the amount of pigment leaving the column determines the intensity of

the signal (measured as a signal peak) produced in the detector. Pigments were identified and quantified from their retention times and peak areas. A spectrum of each peak, from 350 – 700 nm, was produced and used to identify pigments, by comparison against a known set of pigment spectra (Honeywill, 2001; Jeffrey *et al.*, 1997; Millennium library database compiled during the early stages of BIOPTIS). This spectrum was also useful in determining whether peaks consisted of an individual pigment, or a mixture of pigments that were not adequately separated.

2.6.4 Pigment quantification

Chlorophyll *a* was the only standard run with each set of samples (see below). Three concentrations were used to obtain a standard curve, from which chlorophyll *a* concentrations of sample extracts were calculated using regression analysis:

Chlorophyll *a* (mg l^{-1}) Equation 2.5
= ((Peak area–Intercept)/Gradient)*Dilution factor

Chlorophyll *a* (μg chlorophyll *a* / g dry sediment) Equation 2.6
= ((Equation 2.5*(Dilution factor/1000))/Sample weight)*1000

Chlorophyll *a* (mg m^{-2}) Equation 2.7
= ((Equation 2.6*Sample dry weight)/Surface area)*1000

Only chlorophyll *a* was expressed as a concentration, whilst all other minor pigments were expressed in terms of a ratio against chlorophyll *a*, using their chromatogram peak areas at 430 nm. Using pigment ratios is an accepted method, but comparison between ratios is not always possible, as ratios differ depending on whether they are expressed as peak area:peak area, mass:mass, or mole:mole (Wiltshire, 2000; Wiltshire *et al.*, 1998; Barranguet *et al.*, 1997; Cariou-Le Gall & Blanchard, 1995; Wiltshire & Schroeder, 1994).

2.6.5 Preparation of the chlorophyll *a* standards

Stock chlorophyll *a* standard solutions, derived from *Anacystis nidulans* (Sigma™), were made by dissolving 1 mg in the same extraction solvent used for the samples, in a 250 ml volumetric flask covered with tin foil. Since the dry weight of standards can vary by as much as 50% (BIOPTIS protocols), each standard concentration was quantified by a spectrophotometric method (Jeffrey *et al.*, 1997).

The absorbance of a blank (extracting solvent) and the chlorophyll *a* standards were measured in separate 1 cm cuvettes, at the corrected peak maxima (λ max) of chlorophyll *a*, which is 662 nm. All samples were also measured at 750 nm to correct for light scattering in the sample. The concentration was calculated as:

$$C = ((\ [abs\ 662] - [abs\ 750] - blank\ ) / E\ ) * 1000 \qquad \text{Equation 2.8}$$

Where C is the concentration of chlorophyll *a* (mg l^{-1}), abs 662 and abs 750 are the absorbance at 662 nm and 750 nm, and E is the extinction coefficient for chlorophyll *a* in a given solvent (L g^{-1} cm^{-1}). E is 88.15 for 100% acetone (Jeffrey *et al.*, 1997) and 88.74 for 100% DMF (Porra *et al.*, 1989). E in 90% DMF is currently under investigation (Consalvey, pers. comm.).

Standards were always in the range of ~5 mg l^{-1} and ~0.5 mg l^{-1}, since concentrations higher than this can overload the column, and concentrations less than this become inaccurate to quantify. In order to minimise breakdown of the standards, they were stored in the dark at 4°C for a maximum of 3 months.

2.7 Fluorometry

Measurements of chlorophyll *a* fluorescence were taken from intact sediment cores in the laboratory. Data from this technique gave an indication of microphytobenthic biomass, a quantification of stress response, and an estimate of various photosynthetic parameters (see below). The pulse-amplitude modulated (PAM) fluorescence technique was applied using a portable Hansatech™ FMS2 with a blue measuring light and an actinic light source that could saturate photochemistry (except in Chapter 5 when a Walz™ Dive Pam

with a red measuring light was used). The blue light enhanced fluorescence yields from diatoms and green algae, but less so from cyanobacteria (Yentsch & Yentsch, 1979).

2.7.1 The FMS2 settings

In all experiments, FMS2 instrument settings and probe height (4 mm above the sediment surface) followed those of Honeywill (2001) and remained constant for comparative purposes. The FMS2 settings were: gain 99, modulation frequency level 3, minimum fluorescence duration 2.8 s, saturation intensity level 60 (which gave a Photosynthetic Photon Flux Density (PPFD) of 9500 $\mu mol\ m^{-2}\ s^{-1}$ (Consalvey, 2002)) and a saturation intensity pulse width of 1.0 s. Mouget & Tremblin (2002) concluded that the FMS may be satisfactorily used to measure *in vivo* chlorophyll fluorescence on algae in the laboratory, and that the FMS2 is preferred for field-based experiments.

2.7.2 Fluorescence measurements

Dark-adapted measurements were made on surface biofilms after fifteen min. This length of time is thought to be optimal to minimise changes in the biofilm community structure resulting from vertical migration, whilst achieving a stable minimal fluorescence yield (termed F_o^{15}) after reversal of non-photochemical quenching and re-oxidation of photosystem II (PSII) reaction centres (Honeywill *et al.*, 2002; Perkins *et al.*, 2001; Barranguet & Kromkamp, 2000; Kromkamp *et al.*, 1998; Serodio *et al.*, 1997). Chapter 7 discusses this point further.

In dark-adapted cells, the 'minimum fluorescence' level was measured, and was termed F_o^{15}. Since F_o^{15} originates from the pigment bed, it can provide a proxy measure of biomass (Honeywill *et al.*, 2002). Upon application of a brief, very strong pulse of actinic light, the 'maximum fluorescence' emission from the sample was measured, and was termed F_m^{15}. When using the FMS2, a F_o^{15} value of 10 would indicate low biomass, but in practice, it is preferable not to use values lower than 100 (Honeywill, pers. comm.). Calibration has shown that the F_o^{15} value can increase up to 4000 indicating high biomass, although if the F_o^{15}

values are to be used in calculating the theoretical maximum photochemical efficiency (F_v/F_m), then a value up to 700 is preferred.

F_o^{15} and F_m^{15} were used to calculate F_v/F_m (also referred to as F_v/F_m^{15}) an estimate of the efficiency of excitation capture by open PSII reaction centres (Genty *et al.*, 1989). This ratio was used to examine the possibility that cells within the experimental assemblages were under stress (Büchel & Wilheim, 1993). A ratio of 0.75 indicated a healthy biofilm (Honeywill pers. comm.) and ratios were calculated as:

$$F_v/F_m = (F_m^{15} - F_o^{15})/F_m^{15} \qquad \text{Equation 2.9}$$

Light adapted fluorescence measurements of the photochemical capacity at PSII (F_q'/F_m') were made under incremental increases in ambient light (applied using the FMS2 actinic light source, and calibrated against a Licor cosine corrected quantum sensor):

$$F_q'/F_m' = (F_m' - F')/F_m' \qquad \text{Equation 2.10}$$

Where F' is the fluorescence yield just prior to the saturating pulse, and F_m' is the maximum light-adapted fluorescence yield during light saturation. The minimum and maximum light levels used, and the time period between each light level are specified in each chapter.

Relative Electron Transport Rate (rETR), a proxy measurement of primary productivity (Perkins *et al.*, 2001; Kromkamp *et al.*, 1998; Sakshaug *et al.*, 1997) was calculated as the product of F_q'/F_m' and PPFD:

$$\text{rETR} = F_q'/F_m' \times \text{PPFD} \qquad \text{Equation 2.11}$$

rETR vs. PPFD response curves from experimental samples, were constructed using equation 2.11. Light response curves are independent of biomass, as long as the minimum fluorescence yield is high enough (Underwood, pers. comm). Values of the maximum theoretical electron transport rate ($rETR_{max}$) and the maximum light utilisation coefficient (α^{retr}) were calculated by iteration (Long &

Hällgren, 1993). The light saturation parameter (E_K) was calculated as $rETR_{max}$ / α.

The photochemical quenching coefficient, qP and the extent to which photochemistry is down-regulated by non-photochemical quenching (NPQ) can be quantified by the following parameters:

$$qP = F_q'/F_v' = (F_m' - F') / (F_m' - F_o') \quad \text{Equation 2.12}$$

$$NPQ = F_v'/F_m' = (F_m' - F_o') / F_m' \quad \text{Equation 2.13}$$

Where F_o' is the minimum light-adapted fluorescence yield which was calculated from measurements of F_o^{15}, F_m^{15} *and* F_m', following the equation of Oxborough & Baker (1997a):

$$F_o' = F_o^{15} / (F_v/F_m + F_o^{15}/F_m') \quad \text{Equation 2.14}$$

Using equation 2.14 for assemblages that had previously been light adapted (see Chapter 7), F_o^{15} and F_v/F_m were unavailable, and thus instantaneous values from the light curve (in darkness before the actinic light was applied from the FMS2) were used instead.

Since calculations of F_o' assume complete oxidation of Q_A, NPQ was also calculated by:

$$(F_m - F_m') / F_m' \quad \text{Equation 2.15}$$

Patterns of NPQ were identical using either equation, taking into account that F_v'/F_m' (equation 2.13) is inversely proportional to NPQ and $(F_m-F_m')/F_m'$ is proportional to NPQ. This justified the calculation of F_o' by equation 2.13 in order to determine patterns of NPQ.

2.8 Diatom identification

Samples of microphytobenthos were assessed in order to identify and ascertain the number and type of species present, the population diversity, and the similarity of populations between experimental treatments. Diatoms were collected by two methods: surface scrapes and lens tissue method.

2.8.1 Surface scrape method

An aluminium stub was used to scrape off ~2 cm^2 of surface sediment. This was placed in a labelled glass vial, and then studied fresh or preserved in 4-5 drops of 1.25% gluteraldehyde for further analysis and storage.

2.8.2 Lens tissue method

This method collected the motile diatom fraction (Eaton & Moss, 1966). Two pieces of lens tissue (1.5 cm^2) were placed on the sediment surface. After one hour, the top piece of lens tissue was removed, placed in a labelled eppendorf, and preserved in 4-5 drops of 1.25% gluteraldehyde, until permanent slide preparation was possible (see below).

2.8.3 Permanent slide preparation

Permanent slide preparations were made by acid-cleaning samples in order to remove organic matter (Simonsen, 1974). Lens tissue were shaken vigorously in distilled water, and filtered through a thin mesh (in order to separate the cell suspension from lens tissue fibres) into a centrifuge tube. The samples were centrifuged (1500 rpm for 15 min), with the purpose of concentrating the diatoms into a pellet. Most of the excess water was removed, and the pellet resuspended.

1 ml of saturated potassium permanganate solution was added to the solution and left for 24 h at room temperature. This solution was then hydrolysed by the addition of 2 ml (or more if still effervescing) of concentrated hydrochloric acid, and left in an oven (70°C) until the solution had changed from a purple to a clear colour. The solution was left to cool, then centrifuged, and the supernatant removed. The solution was re-suspended in distilled water and centrifuged again. This process was repeated 8 times in order to remove all traces of acid from the diatom pellet. On the final washing, the diatom pellet was re-suspended in ~2 ml of distilled water. 1 ml of this solution was then placed on a clean coverslip and left to air-dry in a dust free environment for 24-48 h.

The coverslips were mounted onto alcohol-cleaned slides by being placed cell-side down in a few drops of the embedding material Naphrax (Northern Biological Supplies). The slide was placed onto a hotplate in order to boil off the

solvent (Toluene), and was removed when the mountant had almost finished bubbling (usually 30-60 s). The mountant set after a few minutes.

2.8.4 Slide examination

Permanent slides and fresh material were examined using a Zeiss Universal light microscope (bright field; total magnification 1250x). Where possible, 300 valves per slide were counted and identified for determination of species composition and relative abundance. Cells were identified from a SERG directory of local species and from the literature (Sabbe *et al.*, 1999; Hartley *et al.*, 1996; Sabbe & Vyverman 1995; Sabbe *et al.*, 1995; Sabbe, 1993; Sabbe & Vyverman 1991; van der Werff & Huls, 1976).

Both types of slide count have inaccuracies associated with them. A degree of error is associated with counts of the motile epipelic fraction, since the species community data presented here included empty frustules. However, according to Underwood & Paterson (1993) this error was probably small. For counts of the whole sediment assemblage, naviculoid species (with the exception of *Navicula digitoradiata* and *Navicula gregaria*) were separated into three size fractions (length of small species = <12.75µm, length of mid-sized species = 12.75-21.25µm, length of large species = >21.25µm; with each size group being considered a taxon). This would have influenced values of species richness, evenness and diversity.

2.9 Assemblage change

Diversity indices are relatively easily calculated and have the advantage of making no assumptions about the underlying distribution or processes determining the abundance of species. For the research presented in this thesis, the Shannon-Wiener index (H') will be used since this has proved to be a good robust general index of diversity, its values being influenced by species richness and evenness (Waite, 2000). Within an ecological context, it measures the amount of uncertainty associated with the identity of a randomly selected individual:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

Equation 2.16

The term p_i is the proportion of a particular species in a sample, which is multiplied by the natural logarithm of itself ($\ln p_i$). Summing the product for all species in the sample derives H. The minus sign is to make the final value of H positive (Fowler *et al.*, 2001)

Assemblage analysis was carried out using Reciprocal Averaging (RA) Ordination. Ordination techniques summarise community data by producing low-dimensional ordination space in which similar species and samples are close together and dissimilar entities far apart. Since the experiments investigated gross assemblage change, the RA ordination was down-weighted. (The effect of RA ordination without down weighting was assessed and concluded to be insignificant). Canonical Correspondence Analysis (CCA) was used in Chapter 5 to assess the relationships between the ordination of samples and species, and measured environmental variables.

The SIMI similarity index was used to compare assemblages between treatments:

$$SIMI_{1,2} = S\sum_{i=1} P_{i1} P_{i2} / \sqrt{S\sum_{i=1} P_{i1}^2 \cdot S\sum_{i=1} P_{i2}^2} \qquad \text{Equation 2.17}$$

Where P_{i1} and P_{i2} are the proportions of each taxon in samples 1 and 2 (Stander, 1970 cited in Medlin, 1983). The index has a value of 0 when samples have no taxa in common, and 1 when samples have identical taxa at the same relative abundance. This statistic gives more weight to the abundant taxa.

2.10 Low Temperature Scanning Electron Microscopy

Following the protocol of Paterson (1995), Low-temperature Scanning Electron Microscopy (LTSEM) allowed for the qualitative visualisation, with a minimal amount of distortion, of the sediment surface structure, and composition of the associated microphytobenthos. Paterson (1995) and Defarge (1997) outline the advantages of LTSEM for sediment analysis. Samples of surface sediment were collected using a strip of stiff metal foil that was inserted ~5 mm into the sediment, and pushed parallel to the surface for a length of ~2cm. The sample and foil were then removed from the sediment and plunged and stored in LN_2 until return to the laboratory, where they were stored in a -80°C freezer until

being viewed. Samples were mounted under LN_2 onto special cryo-stubs, and transferred to the cryo-apparatus (Oxford Instruments CT1500). The sediment was partially freeze-dried within the LTSEM until enough extraneous water had come off to allow a clear view of the sample. The sample was then gold sputter-coated (in order to get a better picture) while still frozen (usually ~10 nm layer of gold) and examined under SEM (Jeol JSM 35 CF). This maximised the visualisation of the sample whilst minimising energy build-up. Photographs were taken using an attached camera (MAMIYA).

2.11 Sediment Stability and the Cohesive Strength Meter

Sediment stability was measured using the Cohesive Strength Meter (CSM), which allowed for the rapid measurement of the erosion threshold of exposed intertidal sediments. The device consists of a water-filled chamber 30 mm in diameter, pushed into the sediment. A jet of water is released from the top of the chamber and directed at the sediment surface. The velocity of the jet pulse is systematically increased over time. Accuracy of the water jet pressure is extremely important and should the actual pressure exceed the desired pressure by > 0.69 kPa, the system automatically vents to reduce the pressure to the required level (Tolhurst, 1999).

Sediment stability was expressed as a threshold for sediment erosion, determined when the light transmission across the test chamber dropped below 90% (0.01 kg m^{-2}) as the bed failed (Tolhurst *et al.*, 1999). Sediments whose erosion thresholds were above 2 Nm^{-2} were considered to be relatively stable. This CSM device measures sediment stability up to 9.08 Nm^{-2}, which is quite high compared to the upper limits of most erosion devices. High stability values are unlikely to be reported in the literature since this device has only been operating for the last 5 years.

2.12 Macrofauna

Macrofauna were sampled from the top 15 cm using a 19 cm (diameter) stovepipe core (0.028 m^2). Samples were sieved over a 1 mm-grade sieve, fixed with 4% formalin and stained with Rose-Bengal. Macrofauna were identified to species level whenever possible (BIOPTIS, 2001).

Chapter 3

Chapter 3: Can natural estuarine microphytobenthic assemblages be used as model systems in laboratory studies?

Abstract

The use of complex species mixtures is becoming more common in laboratory investigations of ecological theory. Natural assemblages of microphytobenthos provide a model system of considerable species richness that can be examined and easily manipulated under laboratory conditions. However, the relative temporal stability of estuarine microphytobenthic assemblages maintained under laboratory conditions, in terms of species composition and assemblage metabolism, is not known. This information is required before the results from model systems employing assemblages of microphytobenthos can be properly interpreted. Natural assemblages of microphytobenthos were sampled, prepared and incubated in the laboratory under light levels representative of those found in the literature. Analysis of microphytobenthic assemblage composition (gross community change), biomass (chlorophyll a and minimum fluorescence), composition of pigments and photophysiological status were assessed after a 14 d period. No changes in species richness were found, whilst diversity declined from the initial field values but was similar when compared between assemblages maintained at different light levels. Field assemblages contained greater numbers of larger diatoms compared to the cultured assemblages. Photophysiological responses were similar between the two light treatments, although signs of photophysiological stress were observed. It was concluded that estuarine microphytobenthic assemblages appear to possess a certain degree of inertia when brought from the field into the reduced light regime of a laboratory. Microphytobenthic assemblages therefore provide a useful experimental model with relevance to natural conditions.

Chapter 3: Can natural estuarine microphytobenthic assemblages be used as model systems in laboratory studies?

3.1 Introduction

Natural estuarine microphytobenthic assemblages are a reservoir of genetic and species diversity. Diatoms have a rapid doubling time and respond quickly to changes in environmental conditions. As a result, microphytobenthic assemblages can potentially provide a model system that can be successfully manipulated to illustrate the potential effects of environmental change (Petchey *et al.*, 1999; McCormick & Cairns, 1994), or ecosystem stress (Medley & Clements, 1998).

Understanding the complex factors that drive ecosystem structure and function in nature, and how system change will affect organisms and assemblages, is challenging. The ultimate goal is to determine, and then predict, how assemblages will react to stress in the natural environment. The reductionist approach is to examine the response of single populations (Staats *et al.*, 2000; Kromkamp & Limbeek, 1993) or reduced diversity mixtures to specific variables under controlled conditions (Underwood & Provot, 2000; Watermann *et al.*, 1999; Olaizola &Yamamoto, 1994; Metaxas & Lewis, 1991; van Donk & Kilham, 1990). Mixtures of assemblages ranging in complexity from two species systems, such as the classic experiments of Gause (1934), to increasingly complex mixtures (Emmerson *et al.*, 2001; Huisman & Weissing, 1999) have been used previously. However, a reductionist approach has profound limitations for the understanding of natural interactions in complex systems, and the wider interpretation of these studies must always be treated with caution since natural assemblages contain the possibility of so many interactions and responses. The ultimate aim would be to conduct experiments *in situ*, although this is often impractical. Therefore a compromise is to maintain a diverse natural assemblage under laboratory conditions: 'a natural assemblage culture'. These can potentially provide a useful model system to study ecological theories such as the intermediate disturbance hypotheses (IDH, Connell, 1978) and the grazer reversal hypothesis (Proulx & Mazumder, 1998). Indeed, this approach is already being used by a number of authors (Yallop *et al.*, 2000; Serôdio *et al.*, 1997).

Many factors (e.g. nutrients, grazing pressure, tidal flooding) are altered on removal from the field, but light availability is arguably one of the most fundamental changes when an assemblage is moved from the field into a laboratory system. Generally, light levels in laboratory systems are much lower than in the field, and are constant rather than constantly fluctuating. Laboratory light levels range between 50 and 200 $\mu mol\ m^{-2}\ s^{-1}$ (Smith & Underwood, 1998; Serôdio *et al.*, 1997; Peletier *et al.*, 1996; Hay *et al.*, 1993; Madsen *et al.*, 1993; Glud *et al.*, 1992; Admiraal, 1977). Irradiance has been selected as a key factor for limiting photosynthesis, and the low light levels reported in the literature have clear implications relating to the physiological acclimation of diatom cells. In addition, irradiance can be responsible for variations in growth rate and hence competitive ability, thereby affecting the assemblage composition both inter- and intra-specifically. For example, studies have shown filamentous cyanobacteria to be positioned under the diatom layer, indicating that they have a preference for an environment of lower irradiance (Underwood & Kromkamp, 1999 and references therein), and therefore may out-compete diatoms under laboratory culture conditions.

Microphytobenthos provide a convenient model, since assemblages are species rich and samples easily manipulated. However, assemblages will begin to change immediately upon removal from their ambient conditions. Whilst the stability of natural microphytobenthic communities has been observed for shallow-water coastal sandy sediments (Nilsson *et al.*, 1991; Sundbäck & Snoeijs, 1991; Sundbäck *et al.*, 1990), it has yet to be discussed for muddy intertidal estuarine sediments. It is not known how fast changes in the species diversity or physiology of the assemblage occur under the reduced irradiance of normal laboratory conditions. If any changes occur upon entry to laboratory conditions, then the validity of such experiments would have to be questioned since control measurements will no longer be related to the natural system. Therefore, the soundness of an approach may rest on how rapidly assemblages change under laboratory conditions.

This experiment aimed to:

- Question the validity of examining cores of estuarine microphytobenthic assemblages under irradiance conditions imposed under laboratory culture.
- Establish to what extent the diatom species composition of a natural estuarine microphytobenthic assemblage would alter, when exposed to fourteen days of reduced light intensity.

3.2 Methods

In October 1999, surface sediment from the Eden Estuary was collected and sieved through a 150 μm mesh to remove macrofauna and most meiofauna. A layer of sieved, homogenously mixed sediment (approx. 3 cm deep) was placed into 23 sand-filled cores (surface area: 50 cm^2). Three cores were used for initial measurements, and 20 cores (10 unshaded and 10 shaded) were distributed between two tanks, in a randomised block design. Shading was provided using a computer-generated black and white pattern, printed onto acetate and placed appropriately onto a thin Perspex lid covering both tanks. A natural tidal regime was simulated (using filtered coastal water of 22 salinity), and the growth chamber maintained a temperature of 10°C. White fluorescent lamps provided a mean Photosynthetic Photon Flux Density (PPFD) of 180 μmol m^{-2} s^{-1} for unshaded cores, and 65 μmol m^{-2} s^{-1} for shaded cores (on a 12:12 Light:Dark cycle).

Initial measurements of chlorophyll *a*, pigment composition, carbohydrate fractions, and assemblage composition (species richness, diversity and LTSEM) were taken from 3 individual cores, 1 day after sieving the sediment. After 14 days, these same variables were measured from the 20 cores (10 unshaded and 10 shaded) maintained under experimental conditions. Sediment samples were collected using a contact core. Chlorophyll *a* was determined using HPLC and EPS concentrations determined using the Dubois assay. Fluorescence measurements were made using a FMS2 fluorometer. F_o^{15}, F_v/F_m^{15} and photosynthesis-irradiance curves (3 minute intervals after each incremental increase in light level) were measured at the end of the experimental period in order to assess differences in photophysiology between the 2 light treatments. See Chapter 2 for individual technique methodologies.

3.2.1 Statistics

The Kruskal-Wallis test was used to determine if the non-normally distributed chlorophyll *a* data differed between the initial and experimental assemblages. 1-way ANOVA was used to test for significant differences between initial and experimental assemblages in the fucoxanthin:chlorophyll *a* ratios and carbohydrate concentrations. The Mann-Whitney test was used to determine if the non-normally distributed F_v/F_m^{15} data differed between the experimental light treatments. The T-test was used to determine if measured fluorescence variables were significantly different between experimental light treatments (Zar 1999). Assemblage analysis was carried out using Reciprocal Averaging (RA) Ordination, and the SIMI similarity index (Medlin 1983) was used to compare assemblages between treatments.

3.3 Results

3.3.1 Alterations in biochemical parameters and photophysiology

No significant change in biomass was found over 14 d, either from initial levels or between light treatments. Chlorophyll *a* concentration was consistent across the treatments and against initial values, and minimum fluorescence did not differ significantly between experimental light treatments. Fucoxanthin / Chlorophyll *a* ratios did not differ significantly between light treatments nor against initial values. Sediment carbohydrate concentrations were consistent across the treatments and against initial values, indicating no significant change in EPS production over 14 d, either from initial levels or between light treatments (Table 3.1).

Mean F_v/F_m^{15} ratios of shaded experimental cores were significantly higher than unshaded cores; mean values and S.E. were 0.65 (0.01) and 0.60 (0.02) respectively. Both unshaded and shaded assemblages had mean F_v/F_m^{15} ratios of less than 0.75, indicating that the cells were under stress (Honeywill, 2001). Maximum photosynthetic rate, photosynthetic affinity and light saturation values were estimated from P-E curves and did not differ significantly between unshaded and shaded cores (Table 3.2).

Table 3.1 Mean (± SE) values for measurements of chlorophyll *a*, fucoxanthin/chlorophyll *a* ratios, and carbohydrate concentrations, from initial and experimental light treatments.

	Initial	**Unshaded** 180 µmol m^{-2} s^{-1}	**Shaded** 65 µmol m^{-2} s^{-1}	**Significance**
Chlorophyll *a* mg m^{-2}	89.5 (3.1) *n = 3*	90.5 (5.9) *n = 10*	107.2 (10.2) *n = 10*	Kruskal-Wallis p = 0.242
Fucoxanthin / Chlorophyll *a* Ratio (peak area:peak area)	0.64 (0.01) *n = 3*	0.66 (0.01) *n = 10*	0.66 (0.02) *n = 10*	1-way ANOVA p = 0.659
Total carbohydrate µg glucose equivalents g^{-1}	5.72 (0.14) *n = 3*	5.31 (0.74) *n = 10*	5.81 (0.49) *n = 10*	1-way ANOVA p = 0.831
Colloidal carbohydrate µg glucose equivalents g^{-1}	1.60 (0.02) *n = 3*	1.62 (0.06) *n = 10*	1.56 (0.03) *n = 10*	1-way ANOVA p = 0.718
EPS in colloidal carbohydrate µg glucose equivalents g^{-1}	0.53 (0.01) *n = 3*	0.50 (0.02) *n = 10*	0.50 (0.01) *n = 10*	1-way ANOVA p = 0.589

Table 3.2 Mean (± SE) values of F_v/F_m, Minimum Fluorescence (Fo^{15}), Maximum Photosynthetic Rate, Photosynthetic Affinity and Light Saturation Index measurements taken from experimental light treatments. ETR = Electron Transport Rate.

	Unshaded 180 µmol m^{-2} s^{-1}	**Shaded** 65 µmol m^{-2} s^{-1}	**Significance**
Genty factor F_v/F_m^{15} ratio	0.60 (0.02) *n = 10*	0.65 (0.01) *n = 10*	Mann-Whitney **p = 0.014**
Minimum fluorescence Fo^{15}	375 (47) *n = 10*	399 (40) *n = 10*	T-test p = 0.72
Max. Photosynthetic Rate Relative ETR	73.1 (17.3) *n = 3*	89.4 (7.5) *n = 3*	T-test p = 0.48
Photosynthetic affinity Relative ETR	0.46 (0.07) *n = 3*	0.54 (0.02) *n = 3*	T-test p = 0.65
Light saturation Index µmol m^{-2} s^{-1}	154.9 (18.2) *n = 3*	165.1 (7.2) *n = 3*	T-test p = 0.34

3.3.2 Assemblage structure

Low-temperature scanning electron micrographs demonstrated the development of a confluent biofilm over the surface of the test cores (Fig. 3.1.1 to 3.1.5). The assemblages varied slightly in composition, but were comprised of a mixed assemblage of mainly diatoms interspersed with other microphytobenthos. The size of diatom cells varied among treatments, with the initial assemblage dominated by *Gyrosigma fasciola* and *Pleurosigma angulatum* (Fig. 3.1.1). There was an apparent shift towards dominance by smaller diatom species after 14 d under laboratory conditions, with this effect being more pronounced for the unshaded experimental assemblage (Figs. 3.1.2 & 3.1.3). The cyanobacterium, *Merismopodia punctata*, was found forming rafts on the surface of some samples (Fig. 3.1.4) while other cyanobacteria were also noted as part of the assemblage (Fig. 3.1.5).

3.3.3 Assemblage diversity

A total of 63 diatom taxa were identified from lens tissue samples. In all samples, of live and motile counts, 95% of the overall percentage composition consisted of the epipelic fraction. Cyanobacteria were found in all live samples, at an overall percentage abundance of <1%. The initial assemblage was found to contain a *Gleotheca* species and *M. punctata*, whilst the experimental cores contained a *Cylindrospermum* species and an unidentified colonial species.

After 14 d under the experimental light treatments, species richness (Fig. 3.2.1) and diversity (Fig. 3.2.2) were similar between experimental light treatments, for both methods of cell collection. There was no significant difference in species richness between the initial assemblage and the experimental assemblages for the motile epipelic fraction ($F_{2,8}$=2.33; p = 0.159) and counts of the whole sediment assemblage ($F_{2,8}$=1.28; p = 0.33). Diversity of the initial assemblage, taking into account the whole sediment assemblage, was found to be significantly higher than the assemblages under the light treatments ($F_{2,8}$=9.68; p = 0.007), but was not significantly different for the motile epipelic fraction ($F_{2,8}$=2.24; p = 0.168).

The correspondence of both the motile and whole sediment assemblage samples and species ordinations can be evaluated by comparing Figs. 3.3.1 & 3.3.2. Qualitative LTSEM evidence suggesting that the initial diatom assemblage

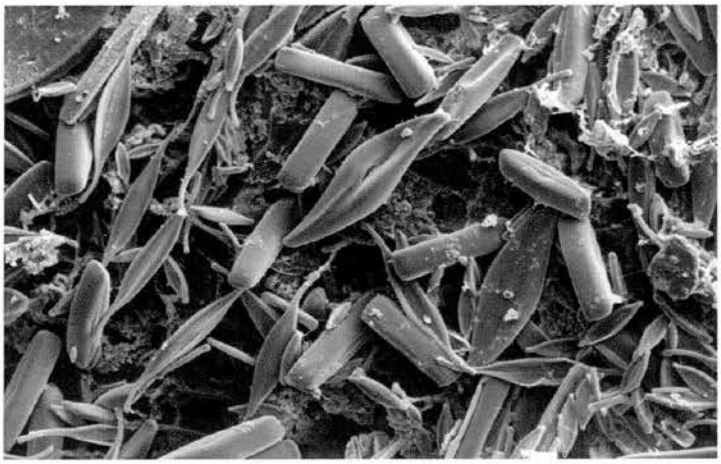


Fig. 3.1.1 Low-temperature scanning electron micrograph of surface sediment of the initial diatom assemblage. Abundant species include *Gyrosigma fasciola* and *Pleurosigma angulatum*. Bar marker = 10 μm.

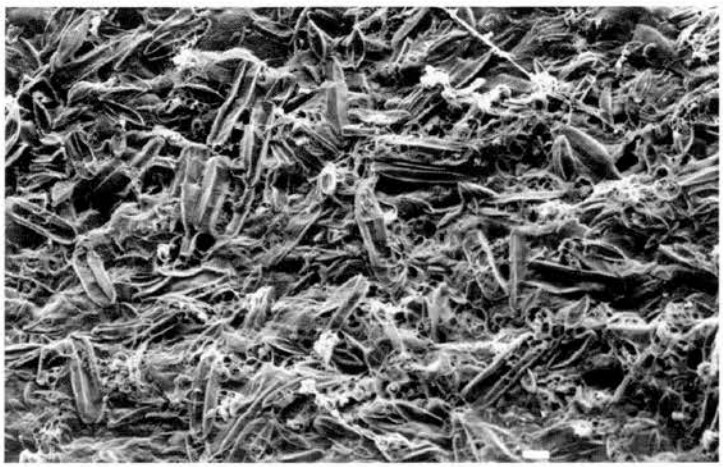


Fig. 3.1.2 Low-temperature scanning electron micrograph of surface sediment of an unshaded core after 14 d. Bar marker = 10 μm.

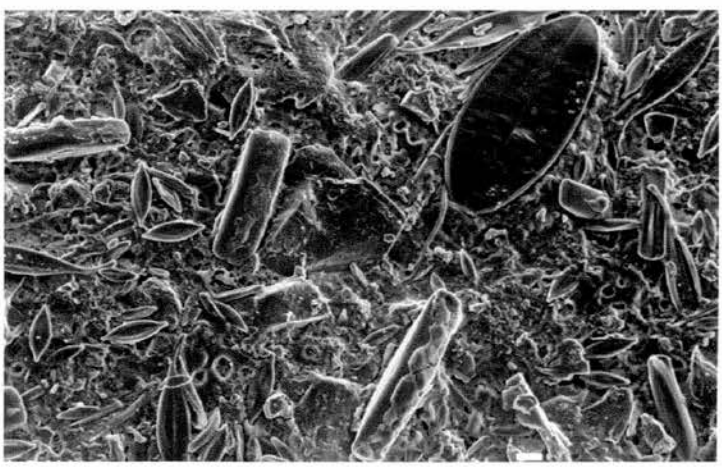


Fig. 3.1.3 Low-temperature scanning electron micrograph of surface sediment of a shaded core after 14 d. Bar marker = 10 μm.

Fig. 3.1.4 Low-temperature scanning electron micrograph of surface sediment of a shaded core. A mixed-diatom assemblage surrounds the tabular colony of *Merismopedia punctata*. Bar marker = 10 μm.

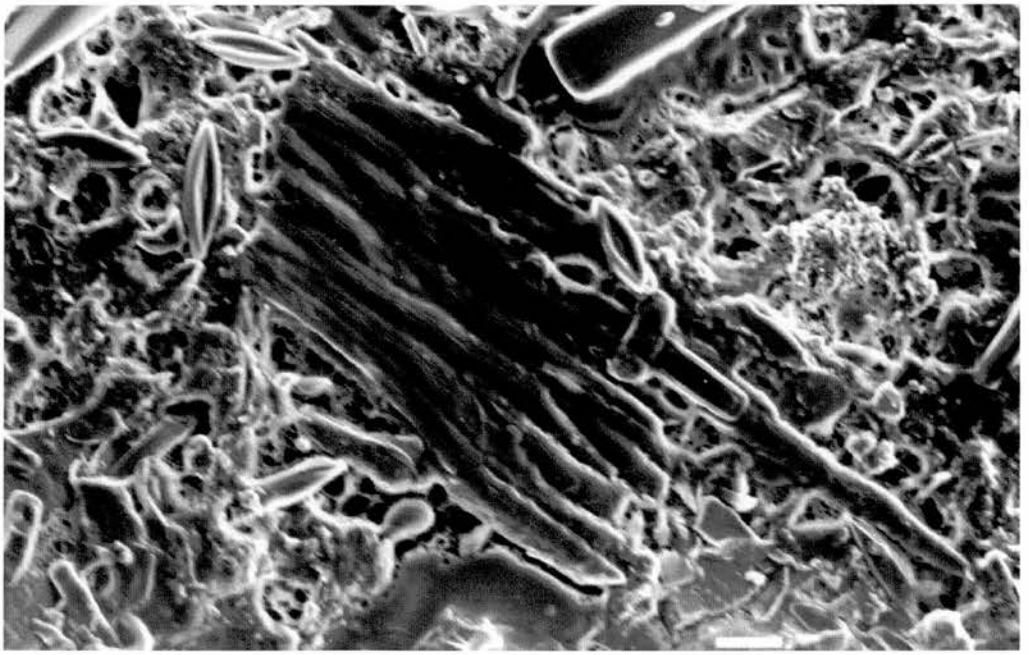


Fig. 3.1.5 Low-temperature scanning electron micrograph of surface sediment of a shaded core. A mixed-diatom assemblage surrounds a cyanobacterium. Bar marker = 10 μm.

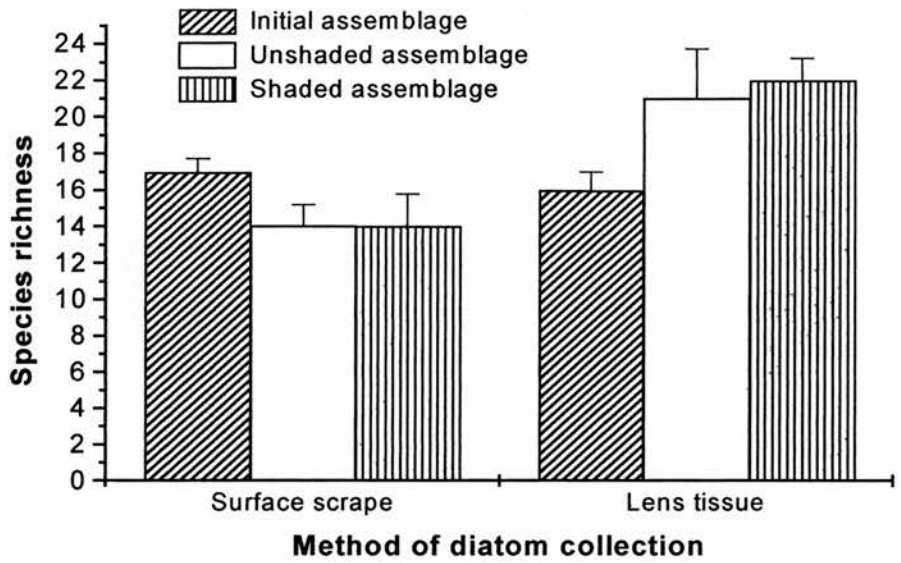


Fig. 3.2.1

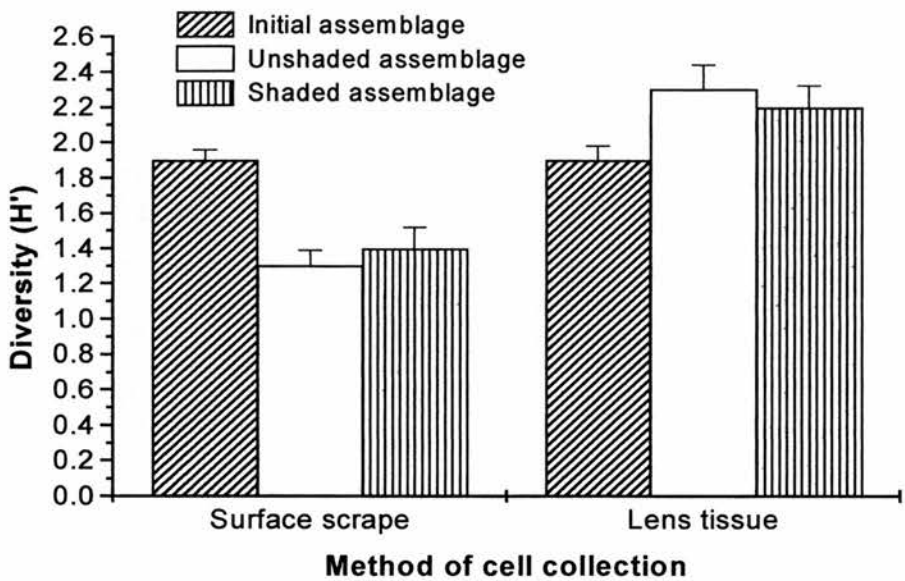


Fig. 3.2.2

Fig. 3.2. Species richness (Fig. 3.2.1) and diversity (Fig. 3.2.2) of the initial and experimental assemblages for counts of the whole sediment assemblage and motile epipelic fraction. In both graphs, error bars represent SE; for initial cores, $n = 3$ and for experimental treatments, $n = 4$.

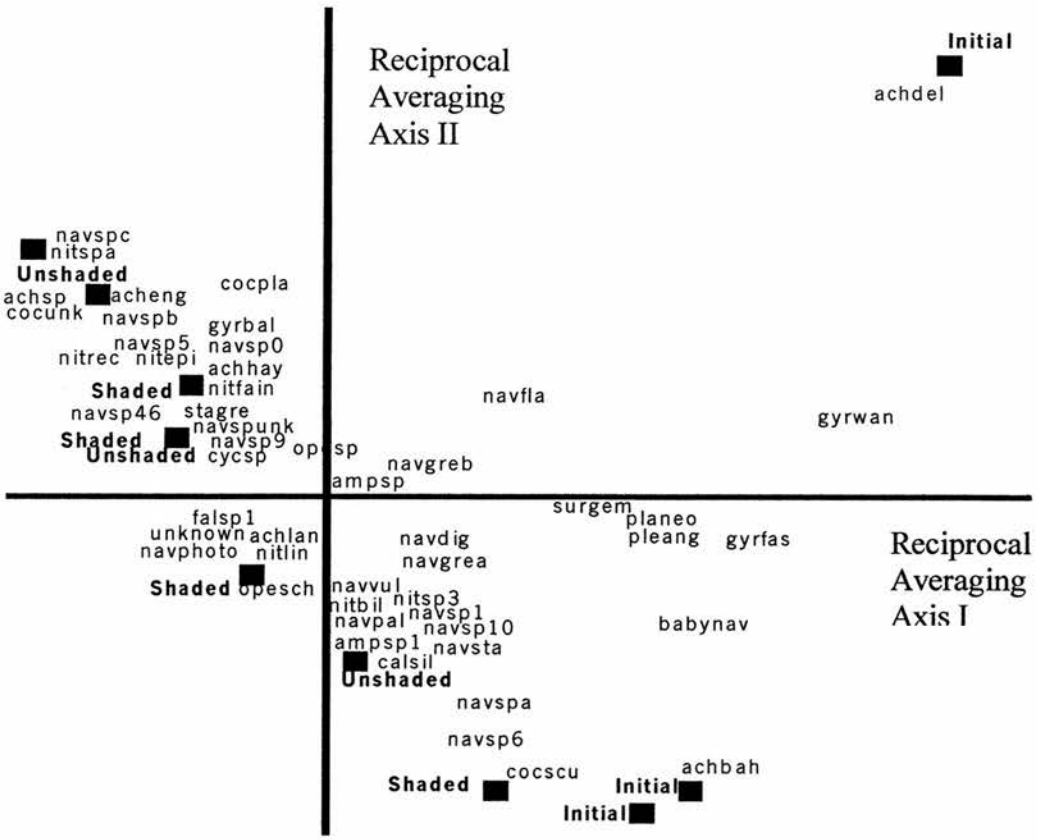


Fig. 3.3.1

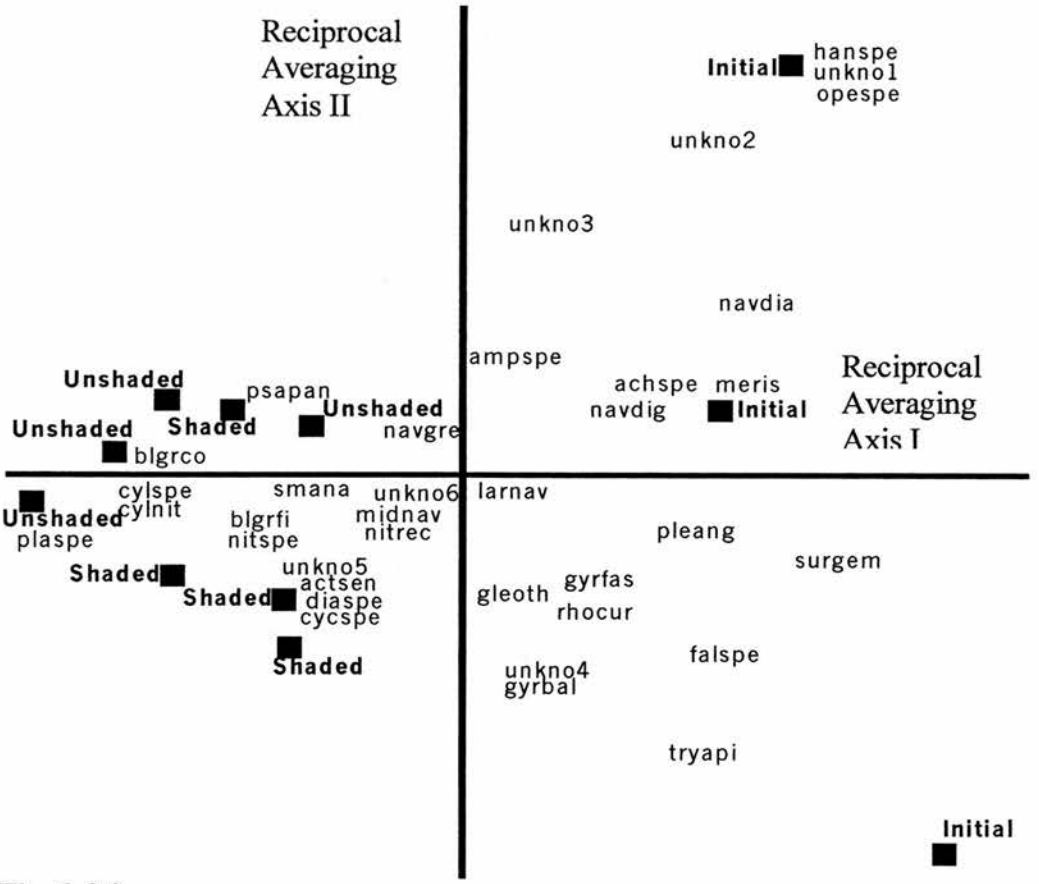


Fig. 3.3.2

Fig. 3.3. Characterisation of algal assemblages of the motile epipelon (Fig. 3.3.1) and the whole sediment assemblage (Fig. 3.3.2). For the initial cores $n = 3$, and for experimental treatments $n = 5$. Reciprocal averaging and selected species scores for algal assemblages of unshaded cores, shaded cores and initial samples. Species designations include: achdel=*Achnanthes delicatula*, acheng=*Achnanthes engelbrechtii*, achhay=*Achnanthes haynaldii*, achlan=*Achnanthes lanceolata*, actsen=*Actinoptychus senarius*, achspe=Achnanthes sp., achsub=*Achnanthes submarina*, ampspe=Amphora sp., ampsp1=Amphora sp. 1, babynav=unidentified small Naviculoid, blgrco=Blue-green colony, calsil=*Caloneis silicula*, cocpla=*Cocconeis placentula*, cocscu=*Cocconeis scutellum*, cocpel=*Cocconeis peltoides*, cocunk=Unknown Cocconeis sp., cycspe=Cyclotella sp., cylnit=unknown Cylindrotheca sp., Cylspe=Cylindrotheca sp., cylsper=Cylindrospermum sp., diaspe=Diatoma sp., diavul=Diatoma *vulgare*, falspe=Fallacia sp., falsp1=Fallacia sp. 1, gleoth=Gleotheca sp., gyrbal=*Gyrosigma balticum*, gyrfas=*Gyrosigma fasciola*, gyrwan=*Gyrosigma wansbeckii*, hanspe=Hantzschia sp., larnav=Large Navicula species, merpun=*Merismopedia punctata*, midnav=Mid-sized Navicula species, navdia=Unknown Navicula sp., navdig=*Navicula digitoradiata*, navfla=*Navicula flanatica*, navgre=*Navicula gregaria*, navgrea=*Navicula gregaria a*, navgreb=*Navicula gregaria b*, navpal=*Navicula palpebralis*, navpin=Unknown Naviculoid or Pinnularia sp., navphoto=Unknown large Naviculoid, navsp0=Unknown Navicula sp. 0, navsp1=Unknown Navicula sp. 1, navsp10=Unknown Navicula sp. 10, navsp46=Unknown Navicula sp. 46, navsp5=Unknown Navicula sp. 5, navsp53=Unknown Navicula sp. 53, navsp6=Unknown Navicula sp. 6, navsp9=Unknown Navicula sp. 9, navspa=Unknown Navicula sp. A, navspb=Unknown Navicula sp. B, navspc=Unknown Navicula sp. C, navsta=*Navicula stankovicii*, navvul=*Navicula vulpina*, nitbil=*Nitzschia bilobata*, nitdis=*Nitzschia dissipata*, nitepi=*Nitzschia epithemioides*, nitlin=*Nitzschia linearis*, nitrec=*Nitzschia recta*, nitfain=Unknown Nitzschia sp., nitspe=Nitzschia sp., nitspunk=Unknown Nitzschia sp., opespe=Unknown Opephora sp., opesch=*Opephora schwartzii*, planeo=*Plagiotropis neovitrea*, plaspe=Unknown Plagiotropis sp., pleang=*Pleurosigma angulatum*, psapan=*Psammodictyon pandiformis*, rhocur=*Rhoicosphenia curvata*, smanav=Small-sized Navicula species, stagre=*Stauroneis gregorii*, surgem=*Surirella gemma*, surspa=Unknown Surirella sp., tryapi=*Tryblionella apiculata*, unkno1-6=Unknown sp. 1-6, unknown=Unknown sp.

Table 3.3 Comparison of diatom assemblages using the SIMI similarity index.

	Motile Epipelic Fraction	Whole Sediment Assemblage
Initial assemblage vs. Unshaded assemblage	0.56	0.82
Initial assemblage vs. Shaded assemblage	0.71	0.83
Unshaded assemblage vs. Shaded assemblage	0.90	0.99

was made up of larger species was supported by the reciprocal averaging analysis. The initial motile samples were typified by the larger diatom species of *Navicula digitoradiata* (25 to 80 μm long), *Gyrosigma balticum* (200 to 400 μm long), *G. fasciola* (60 to 150 μm long), *P. angulatum* (150 to 360 μm long), and *Surirella gemma* (70 to 140 μm long). They were typically grouped to the right on Reciprocal Averaging axis I. With the addition of some smaller *Achnanthes* species and a small-unidentified *Navicula* species, the initial species composition of the whole sediment assemblage, produced similar findings. Species that typified the experimental light treatment assemblages were grouped to the left on reciprocal averaging axis I.

There was little difference in species composition between the two experimental light treatments, both being dominated by small naviculoid species. Similarity analysis (Table 3.3) indicated that the greatest change from the initial assemblage structure occurred for the unshaded cores (SIMI of 0.56 and 0.82 respectively for the motile fraction and samples of the whole sediment assemblage). The motile fraction showed greater variation with time than the samples of the whole assemblage.

3.4 Discussion

Light intensity may influence the composition of a laboratory-grown microphytobenthic assemblage, since field irradiance levels are generally much higher than those imposed in the laboratory. Seasonal factors should be taken into account however, since changes between ambient light levels and laboratory cultures are likely to be more extreme in summer than in winter. In the absence of perturbations such as waves and grazers, it was not known how rapidly a microphytobenthic assemblage would change under laboratory conditions. In theoretical terms, with a conservative doubling time of 24 h, a change in assemblage structure could be rapid. A clear drift toward smaller cells, but without any substantive change in richness or total diversity between systems was found during this experiment. It is suggested that this information is important given that systems examined in a state of change may lead to idiosyncratic responses and dubious conclusions.

3.4.1 Light climate

When an algal assemblage is placed in a given light regime, species will acclimate within the limits of their genetic potential and environmental constraints. Inter- and intraspecific competition for light may influence the assemblage structure, and vertical partitioning of the light gradient through the sediment could serve as a mechanism of coexistence (Grover, 1997).

Acclimation to lower photon fluxes is commonly reflected by increased levels of chlorophyll proteins (Falkowski & Raven, 1997), and accessory pigments such as chlorophyll *c* and fucoxanthin have been shown to be especially important in ensuring maximum photosynthetic capacity (Gallagher *et al.*, 1984). MacIntyre *et al.* (1996) found high fucoxanthin:chlorophyll *a* ratios to be indicative of low light acclimation in phytoplankton. Monoculture studies of epipelic diatoms illustrate that differences in photosynthetic parameters between species (Admiraal, 1984) and between low and high light environments (Hill, 1996) do occur. However, photosynthetic responses were not significantly different between the two experimental light treatments (Table 3.1) and no significant differences in fucoxanthin:chlorophyll *a* ratios between experimental treatments and the initial assemblage were observed in this experiment. These results gave the first indication that experimental assemblages were similar in their composition. Before transplantation into the laboratory culture conditions, irradiance levels in the field during October were quite low, so that assemblages may already have adapted to a reduced light intensity.

With the reduced light climate imposed in this experiment, there was the possibility that the cyanobacteria might have out-competed the diatoms and consequently dominated the assemblage. However, the experimental assemblage structure remained diatom-dominated even after 14 d under experimentally reduced light intensities. Whilst LTSEM and light microscopy data unequivocally demonstrated the presence of cyanobacteria, numbers were sufficiently low to prevent the cyanobacterial marker pigment, zeaxanthin, being detected by HPLC. One possible reason for the low abundance of cyanobacteria is that such populations may have been limited by other factors such as sediment size and temperature (Watermann *et al.*, 1999; Stal, 1995).

3.4.2 Physiological stress

Environmental stresses that affect PSII efficiency, lead to a characteristic decrease in F_v/F_m^{15}, with a ratio of 0.75 indicating a healthy algal cell (Honeywill pers.comm.). The F_v/F_m^{15} ratio of assemblages from both experimental light treatments indicated signs of stress, which could be attributable to the reduced light intensities of the experimental system, or perhaps some other limiting factor, such as the assemblage entering the stationary phase by the end of the experiment (Perkins pers. comm.), or due to nutrient limitation within the biofilm (Kromkamp & Peene, 1999; Flameling, 1998; Geider *et al.*, 1993).

It is difficult to state that the decrease in F_v/F_m^{15} is due to the experimental set-up without having the initial values (which were not taken due to the equipment not being available at this time). The results also suggest that it would be a good idea to monitor the health of the system on a daily basis, in order to determine the optimum amount of time an assemblage can be maintained in the laboratory, or to allow for a fresh input of nutrients.

3.4.3 Diversity

The highest assemblage diversity (H') was found in surface scrapes from the initial assemblage, and these were significantly more diverse than those from experimental assemblages, although separating the *Navicula* species into 3 size-fractions (length of small species = <12.75µm, length of mid-sized species = 12.75 to 21.25µm, length of large species = >21.25µm) would have influenced these values.

Reciprocal averaging (Figs. 3.3.1 & 3.3.2) and LTSEM images (Figs. 3.1.1 to 3.1.5) highlighted the change in the diatom assemblage from the initial assemblage. Diversity was marginally reduced at the expense of larger species, since experimental assemblages were characterised by much smaller cells and species. Cell size is known to influence many properties of algal cells, including competitive ability (Grover, 1989 and references therein), and smaller taxa may become more dominant for the following reasons. In studies of marine and freshwater phytoplankton, the majority of species have shown some increase in cell volume with increasing irradiance (Thompson *et al.*, 1991 and references therein). Larger algae often have higher light requirements due to intra- and inter-cellular self-shading, and higher minimum nutrient requirements. Larger

cells are also more resistant to grazing (Leibold, 1999) and so the removal of grazers from the assemblages may have favoured smaller cells. If the reduction in maximum PSII quantum efficiency (F_v/F_m^{15}) indicates nutrient limitation, then cells of a smaller size may have been favoured given that their surface area/volume ratio improves their ability to sequester nutrient resources from the surrounding environment (Thompson *et al.*, 1991). Small size may be an advantage in low light environments since the amount of energy available for division may be reduced.

A gradient from right to left along reciprocal averaging Axis 1, of both the motile and whole sediment fractions, indicates that light intensity could be a factor regulating diatom assemblage composition, since both experimental light intensities were much lower than average October field conditions. However, it could also describe the effect of the experimental protocol, since grazers were removed, the sediment was sieved, and nutrients may have been limiting towards the end of the 14 d.

3.5 Conclusion

Results from this study suggest that major changes occur relatively slowly under the specific experimental conditions outlined. Despite perturbations such as sediment sieving, reduced light and a fixed temperature regime, the system was relatively slow to respond. However, in a variable and unpredictable environment, such as an estuary, only a community that is dynamically robust would be expected to persist. Intertidal sediments are exposed to strong vertical gradients and regularly influenced by tidal inundation and are thus far more variable and dynamic than phytoplankton systems. Indeed, intertidal estuarine sediments are recognised as stressful, depauperate systems, due partly to sudden changes in conditions (i.e. temperature variation, salinity, erosion, deposition). The organisms that can cope with the natural environmental stress can reach a high biomass, but they must be adapted to cope with these variable and changing conditions in a poorly buffered system.

This experiment has shown that estuarine microphytobenthic assemblages appear to possess, in contrast to phytoplankton communities, a certain degree of inertia when brought from natural field conditions into the laboratory. Phytoplankton are likely to experience much more rapid changes in variables

such as nutrients and temperature, whilst diatoms use their ability to migrate in order to escape from grazing pressure, damaging irradiance, reduced nutrients and anoxia, therefore buffering themselves against these changes. Consequently, estuarine microphytobenthic assemblages can provide a useful experimental model provided the experimenters are aware that slow changes in species composition are occurring and that more extreme differences between laboratory light treatments may still produce changes in photophysiology. Whenever possible, the final test of theory should always be made in the field.

3.6 Publication

Data from this study have been published in Marine Ecology Progress Series. See Defew, E.C., Paterson, D.M. & Hagerthey, S.E. (2002). The use of natural microphytobenthic assemblages as laboratory model systems. *Mar Ecol Prog Ser* **237**: 15-25.

Chapter 4

Chapter 4: The influence of light and temperature interactions on a natural estuarine microphytobenthic assemblage

Abstract

A natural microphytobenthic assemblage from the Eden Estuary, Scotland was used to study the effect of temperature and irradiance on the sustainability and species composition of a natural transient biofilm. Three tidal tanks were maintained: tank 1 at 10°C; tank 2 at 18°C; and tank 3 at 26°C. Within each tank, 5 cores were unshaded (350 µmol m^{-2} s^{-1}), 5 cores were semi-shaded (175 µmol m^{-2} s^{-1}), and 5 cores were shaded (70 µmol m^{-2} s^{-1}). Minimum fluorescence (F_o^{15}) and biofilm health (F_v/F_m^{15}) were monitored daily. Species composition and pigment concentrations/ratios (chlorophyll a, diadinoxanthin and zeaxanthin) were determined for the initial assemblage (day 0) and for the final assemblages (day 21). Relative electron transport rate (rETR), maximum theoretical electron transport rate ($rETR_{max}$), maximum light utilisation coefficient (α^{rETR}), light saturation parameter (E_K) and photochemical (qP) and non-photochemical quenching (NPQ) coefficients of the assemblages were monitored every four days. As surface biomass increased, health of the biofilms decreased. The biofilms grown at 10°C and 18°C were sustainable in a 21 d experiment. Species composition of experimental assemblages were different from the initial assemblage after 21 d, and species richness and diversity illustrated a variety of responses to the temperature and light conditions. Diatoms at 10°C acclimated to the different light levels by varying the ratios of diadinoxanthin:chlorophyll a, whilst at 18°C the assemblages composition changed dramatically in response to shading. After 14 d, F_o^{15} and F_v/F_m^{15} values substantially decreased for biofilms grown at 26°C, probably due to nutrient limitation. Ratios of zeaxanthin/chlorophyll a increased with increasing temperature, indicating an increase in cyanobacterial biomass at 26°C, after 21 d. Chlorophyll a decreased with increasing temperature. Temperature had significant effects on $rETR_{max}$ and E_K in the short term (days). Light and temperature treatments did not affect α^{rETR}, and not all P-E curves saturated, which was attributed to vertical migration in response to light.

Chapter 4: The influence of light and temperature interactions on a natural estuarine microphytobenthic assemblage

4.1 Introduction

Early physiological studies employed monocultures to examine the influence of irradiance and temperature on microphytobenthic growth rates and production (see Admiraal, 1984). However, *in situ* studies have shown that irradiance and temperature effects are complex and coupled, since mud surface temperature is related to incident irradiance and both factors directly influence photosynthesis (Blanchard & Guarini, 1996; Davison, 1991). This makes it difficult to distinguish between irradiance and temperature effects in field-studies.

The sustainability of a laboratory-grown algal biofilm, when placed under particular light and/or temperature conditions for a significant time period, will be largely dependent on species-specific growth rates. The species within the assemblage will acclimate to the imposed conditions within the limits of their genetic potential and environmental constraints. If the environmental conditions are towards the limits of species capabilities, the thermal and light environment may act as a selective force, consequently altering assemblage composition (Geider, 1987) toward more tolerant or better-adapted species. Many experimental studies examining environmental variables, such as light and temperature, have not identified and/or quantified the biofilm species composition (Underwood & Kromkamp, 1999). Studies that include species composition would help to link the findings of monoculture laboratory experiments with observations from the field.

When algae experience changes in light regime, acclimation of the photosynthetic apparatus occurs, resulting in the optimisation of photosynthesis. This can happen on very short time scales (minutes/hours). At low light, this ensures that maximum photosynthetic rate is maintained, whilst at high light it helps prevent photodamage. This is known as photoacclimation. At the cellular level, photoacclimation can be attributed to changes in both light harvesting and reaction centre pigments, in either the total amount of pigment per cell, the ratio of different pigments, or both (Richardson *et al.*, 1983). Temperature can

influence algal photosynthesis by changing photosynthetic rates, or by inducing phenotypic or genotypic changes among algal species (Davison, 1991). For instance, microphytobenthic maximum photosynthetic rate (P_{max}) was found to increase progressively along a temperature gradient up to an optimum temperature at 25°C (T_{opt}), beyond which P_{max} declined, before photosynthesis ceased at temperatures greater than 38°C (Guarini *et al.*, 1997; Blanchard *et al.*, 1996).

Most research has examined the effect of a single variable, and in the case of temperature its effects at saturating light intensities has possibly been over-emphasised (Davison, 1991). The research presented in this chapter concentrates on the effect of temperature at the sub-saturating levels that are more typical for natural microphytobenthic populations. Light levels are reduced in nature by light attenuation through sediments, self-shading within the biofilm, the ability to migrate, and microcycling of diatom cells (i.e. the movement of cells in and out of the surface layer, as a means for motile diatoms to optimise their position in the light climate) (Consalvey, 2002; Kromkamp *et al.*, 1998). Studies looking at the effect of temperature, such as those of Watermann *et al.* (1999), are restricted due to the limited diversity of the test assemblage. Natural estuarine biofilms are species rich and may show more subtle responses. Defew *et al.* (2002) demonstrated the utility of natural microphytobenthic assemblages as a laboratory model system to study the effect of environmental change. The work presented in this chapter aimed to:

- Describe how the interactive effects of temperature and irradiance over a 21 d period affected biomass and species composition of a natural estuarine microphytobenthic assemblage.
- Investigate how the photosynthetic response of a natural estuarine microphytobenthic biofilm changed with time in response to different light and temperature conditions.

4.2 Methods

In March 2000, sand and sandy-mud surface sediments were collected from the Eden Estuary and sieved through a 150 μm mesh to remove macrofauna

and most meiofauna. A 3 cm deep layer of sieved, homogenously mixed sediment was placed into 45 sand-filled cores (surface area: 50cm^2), which were distributed equally between 3 tidal tanks. A further 3 cores were used for initial analysis. A natural tidal regime was simulated (using filtered coastal water of salinity 22), the air temperature of the environmental room was maintained at 10°C, and aquarium heaters at 3 constant temperatures maintained the water in individual tanks. Tank 1 was maintained at 10°C, tank 2 at 18°C and tank 3 at 26°C. Within each tank, 5 cores were unshaded (350 µmol PPFD m^{-2} s^{-1}), 5 cores were semi-shaded (175 µmol PPFD m^{-2} s^{-1}), and 5 cores were shaded (70 µmol PPFD m^{-2} s^{-1}). This gave a total of 9 treatments. White fluorescent lamps were suspended 60 cm above the tanks, and shading was provided using neutral density filters (Fig. 4.1).

Three replicate measurements of minimum fluorescence (F_o^{15}; Honeywill *et al.*, 2002; a proxy for microphytobenthic biomass), and the theoretical maximum photochemical efficiency (F_v/F_m^{15}; Honeywill *et al.*, 2002), an indicator of system health, were measured daily from each experimental core, at the time corresponding to low tide at the original site. In order to maintain adequate replication of light-response curves, day 1 for F_o^{15} and F_v/F_m^{15} was staggered between the 3 temperature tanks (hence due to biofilm growth, starting F_o^{15} values were higher as temperature increased). Diatom species composition was determined for the initial assemblage, and for the unshaded and shaded treatments at each temperature after 21 d. Sediments were sampled with a syringe core for analysis of pigment composition (chlorophyll *a* and zeaxanthin). Samples were taken from 3 individual cores, 1 d after sieving the sediment, and from all experimental cores after 21 d. P-E curves (3 min interval after each incremental increase in light level) were taken from each experimental treatment (n=5) every 4 d. The photosynthetic responses (relative electron transport rate (rETR), maximum theoretical electron transport rate ($rETR_{max}$), maximum light utilisation coefficient (α^{rETR}), light saturation parameter (E_K) and photochemical (qP) and non-photochemical quenching (NPQ) coefficients of the assemblages were monitored. See Chapter 2 for methodologies of the individual techniques.

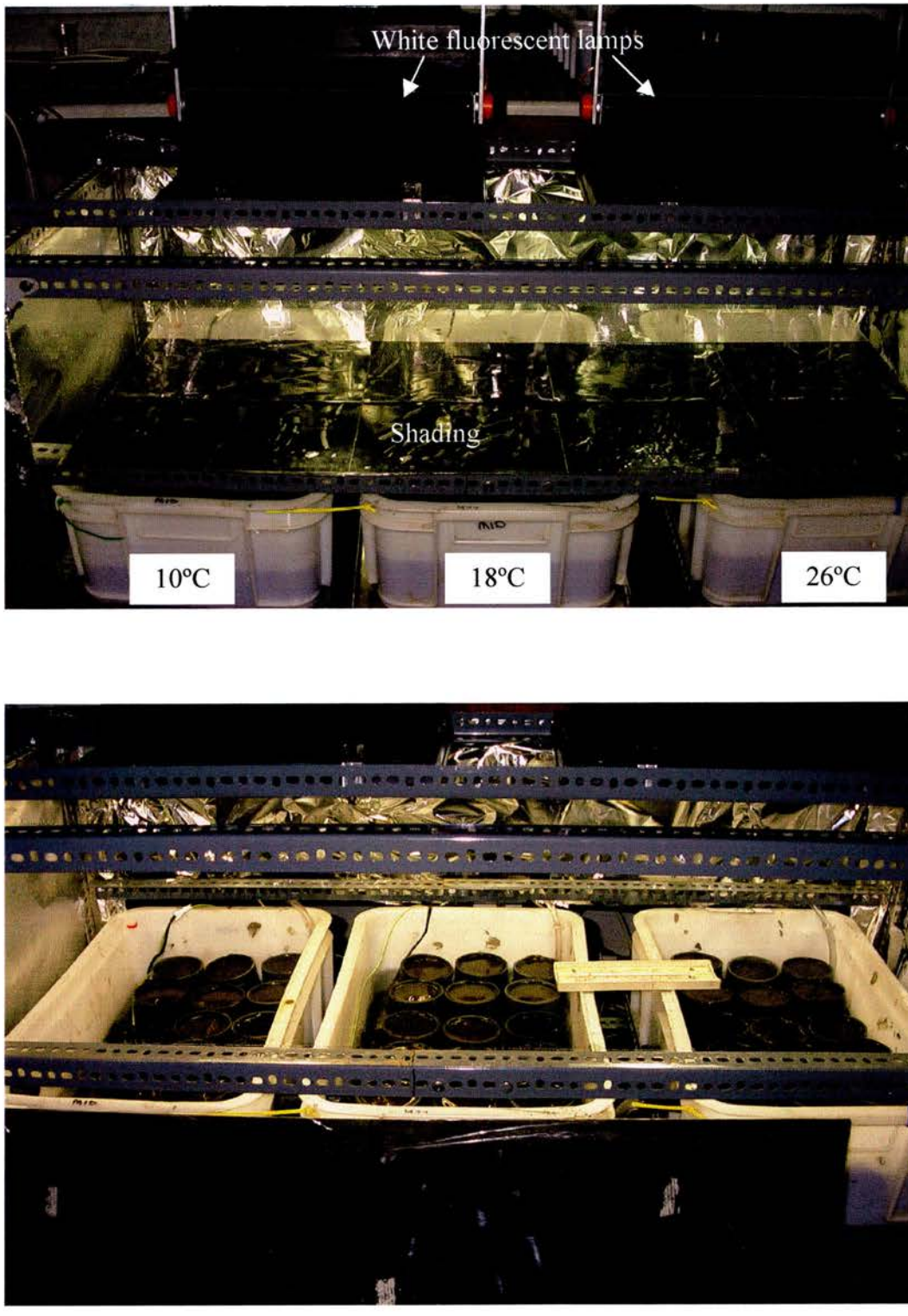


Fig. 4.1 Experimental set-up. Tank 1 was maintained at 10°C, tank 2 at 18°C and tank 3 at 26°C. In each tank, 5 cores were unshaded (350 µmol PPFD m^{-2} s^{-1}), 5 cores were semi-shaded (175 µmol PPFD m^{-2} s^{-1}), and 5 cores were shaded (70 µmol PPFD m^{-2} s^{-1}).

4.2.1 Statistics

Fully nested analysis of variance (ANOVA) and post-hoc Tukey tests were used to determine if measured parameters were significantly different between experimental light and temperature treatments. Multiple regression analysis and post-hoc Tukey tests were used to test for significant increases/decreases in F_o^{15} between temperature treatments (Zar, 1999). Assemblage analysis was carried out using Reciprocal Averaging (RA) Ordination to investigate gross assemblage change, and the SIMI similarity index to compare taxa presence and abundance of assemblages between treatments. Further details can be obtained from Chapter 2, section 2.9.

4.3 Results

4.3.1 Biomass and system health

Water content of cores from the 26°C treatment was significantly lower than the cores from the 10°C and 18°C treatments after 21 d under experimental conditions, but did not differ from the initial water content of the sediment ($F_{3,44} = 20.14$; $p < 0.001$) (data not shown). Neither light regime nor the interaction of temperature and light regime significantly affected sediment water content.

After 21 d, algal biomass (chlorophyll *a*) had increased above the initial level of 37.5 mg m^{-2} (± 4.1 mg m^{-2}) in all treatments except the shaded 18°C and unshaded 26°C treatments (Fig. 4.2). Chlorophyll *a* decreased with increasing temperature treatment ($F_{2,36} = 4.41$; $p = 0.019$). The interaction of temperature and light had a significant effect on the chlorophyll *a* concentration of the sediments ($F_{4,36} = 6.58$; $p < 0.001$). As light level decreased, chlorophyll *a* concentration significantly decreased at 10°C and 18°C, whilst chlorophyll *a* concentration was not significantly different among light levels at 26°C ($F_{9,38} = 4.62$; $p < 0.001$).

F_o^{15}/chlorophyll *a* ratios were significantly lower at 26°C ($F_{2,36} = 30.55$; $p < 0.001$), but did not differ among light treatments at any temperature ($F_{2,36} = 1.0$; $p = 0.38$) (Fig. 4.3). Thus changes in F_o^{15} were due to changes in surface biomass rather than changes in pigmentation due to photoacclimation.

F_o^{15} (a proxy for surface biomass) slowly increased for the first 6 d in all treatments. The rates of increase in surface biomass did not differ significantly among temperature regimes ($F_{0.05,2,21} = 2.24$). Between days 7 and 14, surface

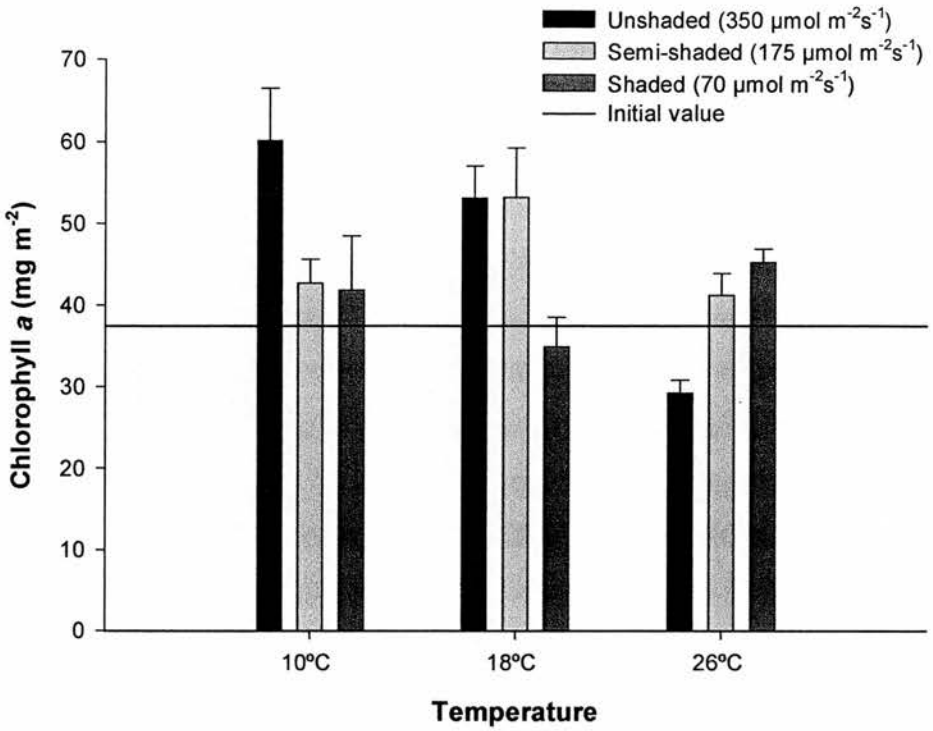


Fig. 4.2 Chlorophyll *a* (mg m^{-2}) after 21 d under the experimental conditions (Mean ± S.E.; n=5 cores in each treatment).

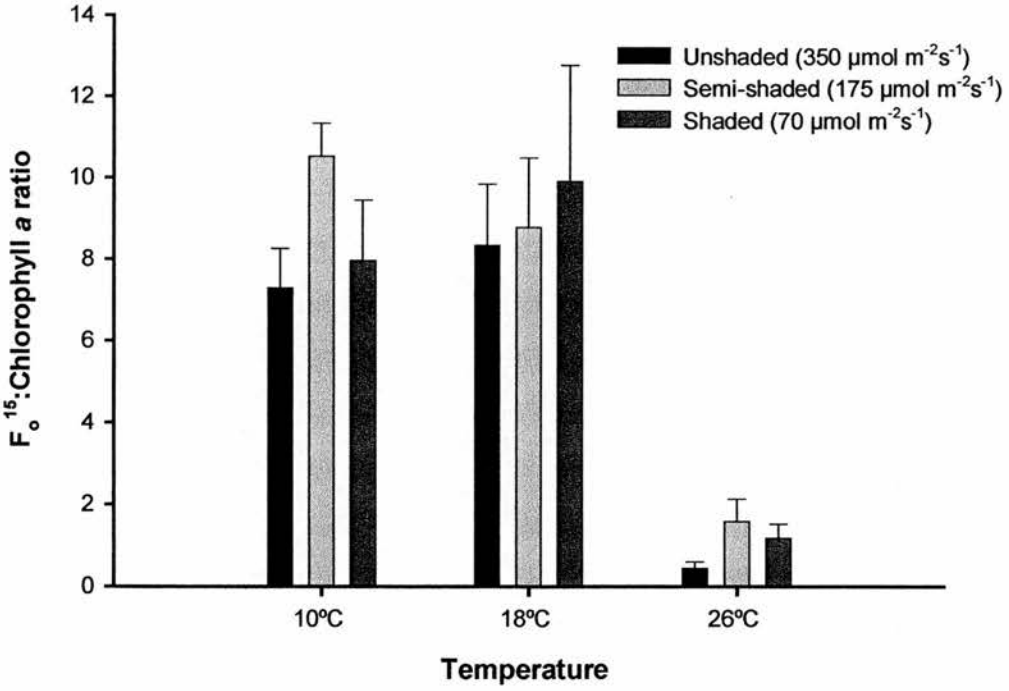


Fig. 4.3 F_o^{15}:Chlorophyll *a* ratios after 21 d under the experimental conditions (Mean ± S.E.; n=5 cores in each treatment).

biomass increased in the 10°C and 18°C treatments, before slowly declining for the remaining 7 d (Fig. 4.4A & B). At 26°C, surface biomass continued to slowly increase until day 9, before declining for the remainder of the 21 d experiment (Fig. 4.4C). In the final 7 d of the experiment, surface biomass decreased at a significantly faster rate as temperature increased ($q_{0.001,18,3} = 6.196$). At 10°C and 18°C, the highest surface biomass was achieved in the semi-shaded treatments, whilst at 26°C, highest surface biomass occurred in the shaded treatment.

Since there were no significant differences in F_v/F_m^{15} (a measurement of system health) among light levels at all temperatures, F_v/F_m^{15} values were averaged over the 3 light treatments (Fig. 4.5). Health of the 10°C biofilm slowly decreased for the duration of the experiment, whilst health of the 18°C biofilm decreased initially, before slowly increasing for the remainder of the experiment. Health of the 26°C biofilm increased until day 6, then slowly decreased between days 7 and 14, before increasing for the remainder of the experiment. Mean F_v/F_m^{15} values at the end of the experiment were 0.63, 0.69 and 0.81 for the 10°C, 18°C and 26°C treatments respectively (averaged over the 3 light regimes).

4.3.2 Microphytobenthic species composition

Cyanobacterial biomass increased with increasing temperature, indicated by an increase in the zeaxanthin/chlorophyll *a* ratios at 26°C. At 26°C, cyanobacterial biomass significantly increased with decreasing light levels ($F_{8,36} = 10.91$; $p < 0.001$) (Fig. 4.6).

A total of 114 diatom taxa were identified from all the lens tissue samples. Using the correspondence of experimental treatments and dominant species (>1%) ordinations (Fig. 4.7), the unshaded 18°C assemblages grouped out to the right of reciprocal averaging axis I. The other experimental treatments were situated to the left of reciprocal averaging axis I, and separated out along reciprocal averaging axis II. Reciprocal averaging axis II separated temperature treatments, whilst reciprocal averaging axis I separated light treatments.

The greatest change in composition from the initial diatom assemblage occurred for the 18°C unshaded and 26°C shaded assemblages (SIMI indices of 0.14 and 0.16 respectively), whilst the least change occurred for the 10°C assemblages (SIMI indices of 0.78 and 0.94 for unshaded and shaded

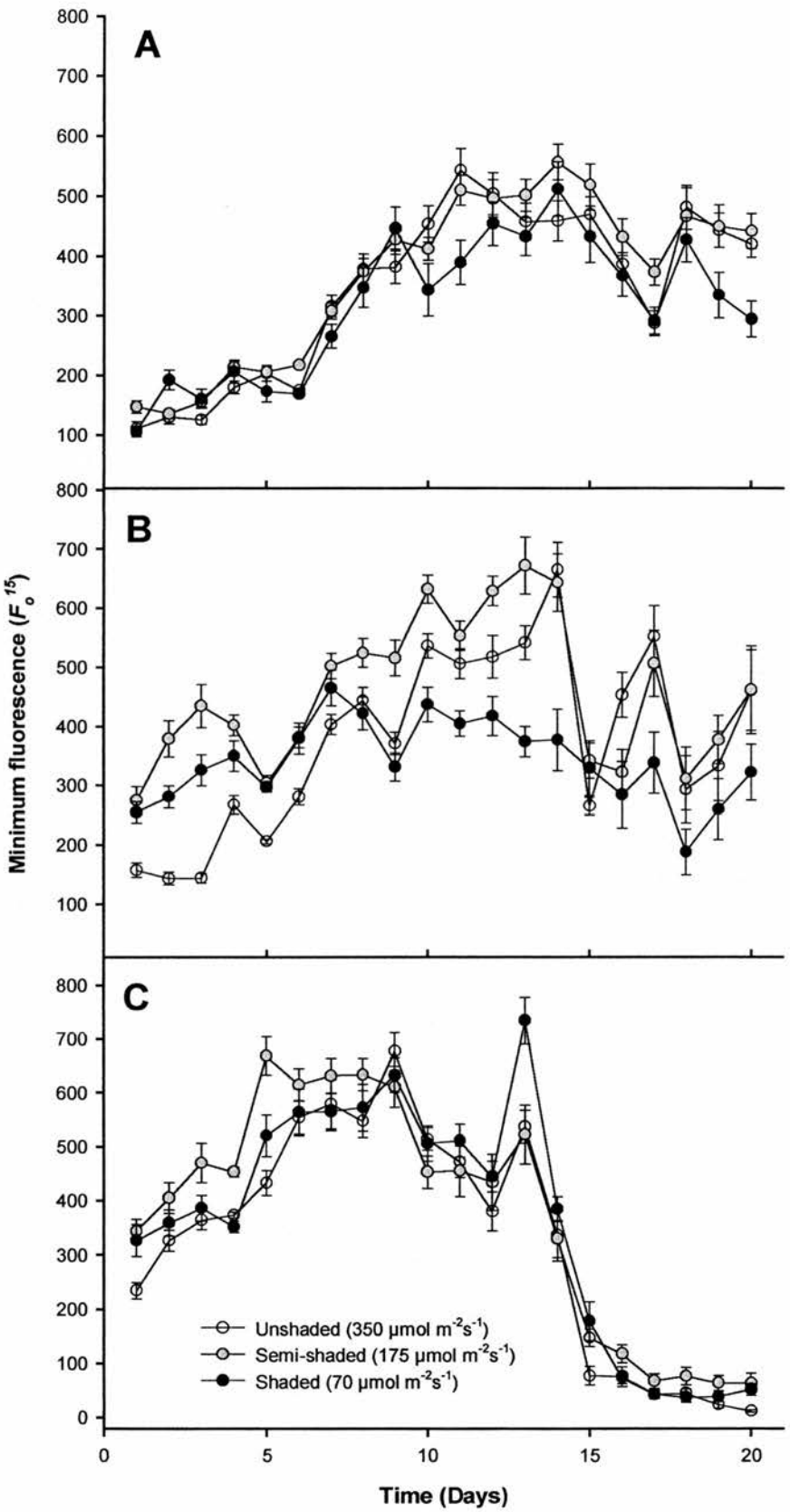


Fig. 4.4 Daily F_o^{15} values (mean) for each experimental light treatment over the 21 d experiment: (A) 10°C (B) 18°C (C) 26°C.

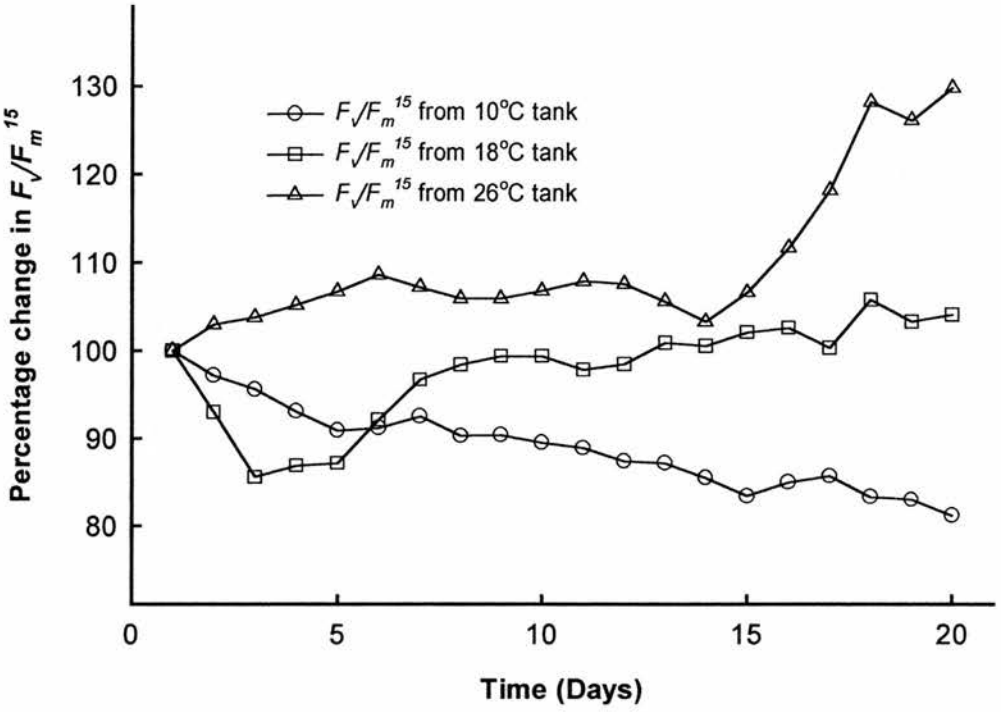


Fig. 4.5 Percentage change in health of the biofilm (F_v/F_m^{15}). Measurements were taken every day for the 21 d duration of the experiment. Values were averaged over the three light treatments at each temperature (Mean; n=15 cores at each temperature).

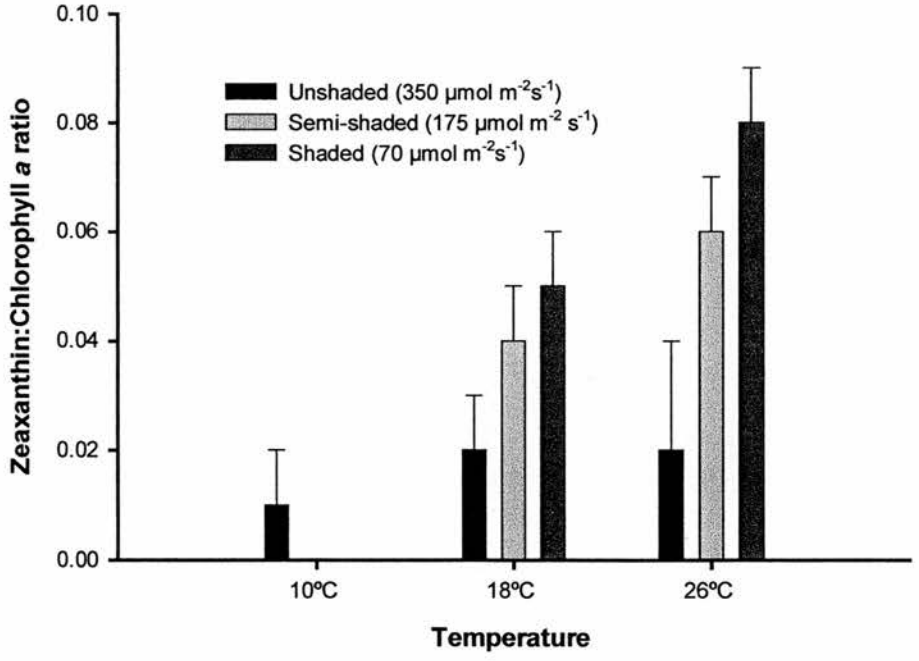


Fig. 4.6 Zeaxanthin:Chlorophyll *a* ratios after 21 d under the experimental conditions (Mean ± S.E.; n=5 cores in each treatment).

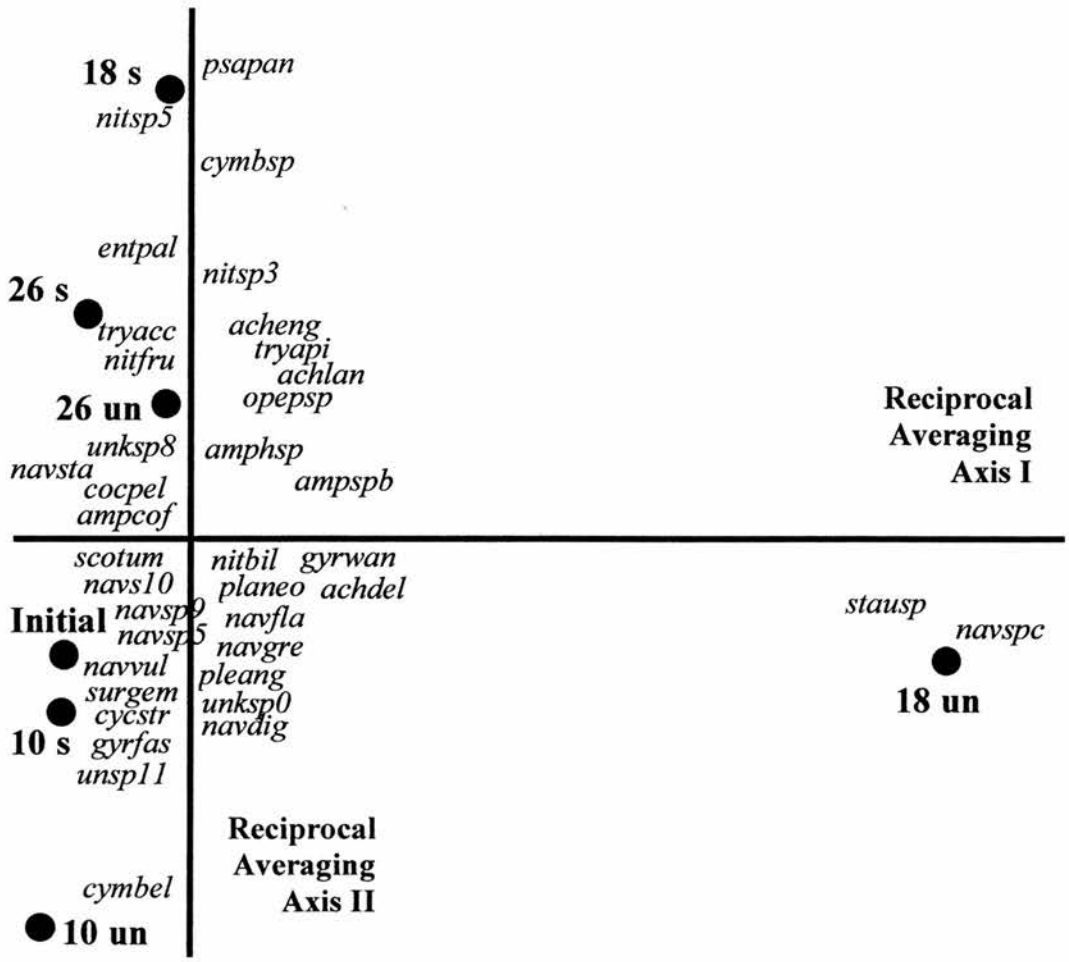


Fig. 4.7 Characterisation of diatom assemblages for selected experimental treatments. Initial = initial assemblage; 10 un = 10°C unshaded assemblage; 10 s = 10°C shaded assemblage; 18 un = 18°C unshaded assemblage; 18 s = 18°C shaded assemblage; 26 un = 26°C unshaded assemblage; 26 s = 26°C shaded assemblage. Reciprocal averaging and selected species scores (only species whose relative abundance was >1% are shown). achdel = *Achnanthes delicatula*; acheng = *Achnanthes engelbrechtii*; achlan = *Achnanthes lanceolata*; ampcof = *Amphora coffeaeformis*; amphsp= *Amphiprora* species; ampspb = *Amphora* species b; cocpel = *Cocconeis peltoides*; cycstr = *Cyclotella striata*; cymbel = *Cymatosira belgica*; cymbsp = *Cymbella* species; entpal = *Entomoneis paludosa*; gyrfas = *Gyrosigma fasciola*; gyrwan = *Gyrosigma wansbeckii*; navdig = *Navicula digitoradiata*; navfla = *Navicula flanatica*; navgre = *Navicula gregaria*; navsta = *Navicula stankovicii*; navvul = *Navicula vulpine*; navsp5 = *Navicula* species 5; navsp9 = *Navicula* species 9; navs10 = *Navicula* species 10; navspc = *Navicula* species c; nitbil = *Nitzschia bilobata*; nitfru = *Nitzschia frustulum*; nit sp 3 = *Nitzchia* species 3; nitsp5 = *Nitzchia* species 5; opepsp = *Opephora* species; planeo = *Plagiotropis neovitrea*; pleang = *Pleurosigma angulatum*; Psapan = *Psammodictyon pandiformis*; scotum = *Scoloneis tumida*; stausp = *Stauroneis* species; surgem = *Surirella gemma*; tryacc = *Tyblionella accuminata*; tryapi = *Tryblionella apiculata*; unksp0 = unknown species 0; unksp8 = unknown species 8; unsp11 = unknown species 11.

respectively) (Table 4.1). Dominant diatom species (>10%) of the experimental treatments can be compared in Table 4.2.

Compared to the initial assemblage (taken on day 0), species richness of 10°C assemblages, and unshaded 18°C assemblages, were significantly reduced by the end of the experiment (day 21) (Table 4.3). The greatest assemblage change between unshaded and shaded treatments occurred at 18°C (SIMI index of 0.18; Table 4.1), where species richness, evenness and diversity were significantly lower in the unshaded assemblages. Species richness evenness and diversity did not differ between unshaded and shaded treatments at 10°C and 26°C. Temperature did not affect species richness of unshaded assemblages, whilst species richness of shaded 10°C assemblages were significantly lower than the 18°C assemblages ($F_{6,14} = 5.54$; $p = 0.004$) (Table 4.3). Species evenness was not affected by temperature under either the unshaded or shaded light regime. Species diversity of 18°C unshaded assemblages was significantly lower than 26°C unshaded assemblages, whilst temperature did not significantly affect diversity of shaded assemblages ($F_{6,14} = 4.68$; $p = 0.008$ and $F_{6,14} = 7.38$; $p = 0.001$ for evenness and diversity respectively) (Table 4.3).

4.3.3 Photophysiological parameters

Replicate light response curves were made on each treatment every fourth day (except on the final two measurement days on the 26°C biofilms, when the signal was too low; $F_o'^{15}$ values were < 150). Saturation of the light curves (mean values) did not always occur, and thus $rETR_{max}$ is only an estimate. This was the case in the 26°C treatment on measurement day 1 (Fig. 4.8A); in the 18°C unshaded treatments on measurement days 2, 3 and 5 (Fig. 4.8B, 4.8C and 4.8E); in the 18°C shaded treatment on measurement day 3 (Fig. 4.8C); in the 18°C semi-shaded treatment on measurement day 4 (Fig. 4.8D).

Temperature and time had significant effects on $rETR_{max}$ ($F_{2,6} = 9.90$, $p < 0.01$ and $F_{30,154} = 6.04$, $p < 0.001$ respectively; Table 4.4) and E_K ($F_{2,6} = 9.93$, $p < 0.01$ and $F_{30,154} = 6.24$, $p < 0.001$ respectively; Table 4.5). Light treatment had no significant effect on $rETR_{max}$ or E_K. Temperature and light treatments did not have a significant effect on the maximum light utilisation coefficient (α^{rETR}; Table 4.6), whilst time did have a significant effect ($F_{30,154} = 5.77$, $p < 0.005$).

Table 4.1 SIMI similarity indices comparing the experimental treatments - an index of 0 meant the samples had no taxa in common, and an index of 1 meant the samples had the same taxa and relative abundance.

	Initial	10°C Unshaded	10°C Shaded	18°C Unshaded	18°C Shaded	26°C Unshaded
10°C Unshaded	0.78					
10°C Shaded	0.94	0.79				
18°C Unshaded	0.14	0.13	0.16			
18°C Shaded	0.42	0.23	0.39	0.18		
26°C Unshaded	0.66	0.54	0.67	0.27	0.71	
26°C Shaded	0.16	0.10	0.22	0.04	0.34	0.44

Table 4.2 Percentage composition of the dominant species (>10%) for unshaded and shaded assemblages at all temperatures, taken after 21 d. Values are from pooled data (Mean; n=3).

	Initial assemblage	10°C Unshaded assemblage	10°C Shaded assemblage	18°C Unshaded assemblage	18°C Shaded assemblage	26°C Unshaded assemblage	26°C Shaded assemblage
Achnanthes engelbrechtii	7%	0.3%	6%	6%	24%	15%	10%
Cymatosira belgica	0.1%	23%	0.3%	1%	0%	0%	0%
Navicula gregaria	29%	27%	28%	6%	6%	13%	2%
Navicula species 5	15%	22%	16%	6%	4%	16%	4%
Nitzschia frustulum	2%	3%	2%	0%	11%	20%	10%
Nitzschia species 3	2%	1%	1%	0.8%	12%	2%	5%
Stauroneis species	0.1%	0.6%	0.9%	66%	3%	5%	0.2%
Unknown species 8	0%	0%	2%	0%	0%	4%	40%

Table 4.3 Species richness, evenness, and diversity values for each experimental assemblage, taken after 21 d. Values are from pooled data (n=3).

		Species Richness	Species Evenness	Species Diversity (H')
Initial value		39 ± 4	0.77 ± 0.01	2.81 ± 0.09
10°C	Unshaded	22 ± 2	0.72 ± 0.02	2.20 ± 0.02
	Shaded	25 ± 2	0.82 ± 0.05	2.63 ± 0.20
18°C	Unshaded	22 ± 4	0.53 ± 0.07	1.61 ± 0.16
	Shaded	40 ± 5	0.78 ± 0.01	2.86 ± 0.14
26°C	Unshaded	33 ± 1	0.76 ± 0.01	2.68 ± 0.03
	Shaded	32 ± 3	0.65 ± 0.08	2.26 ± 0.30

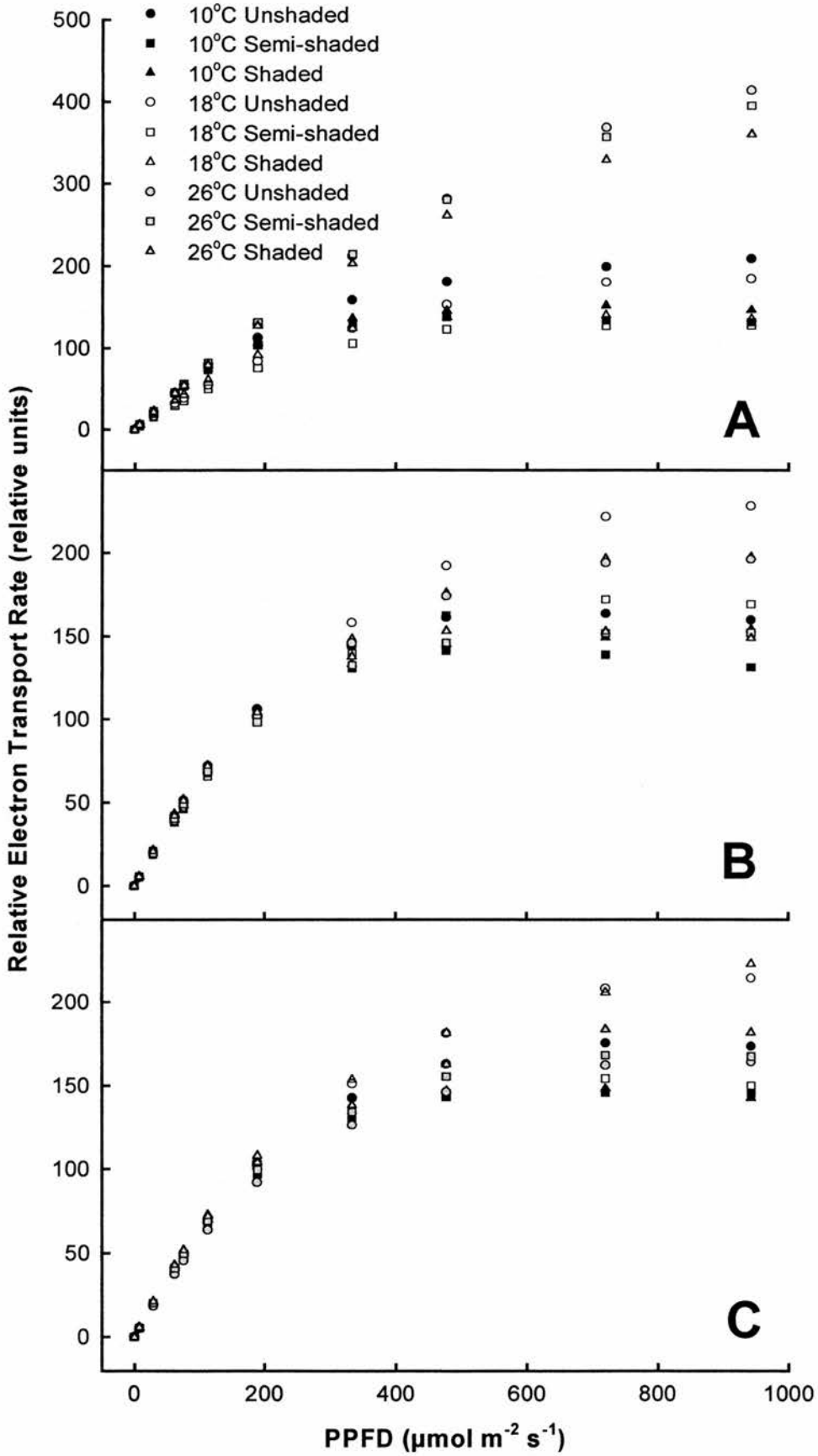


Fig. 4.8 (legend next page)

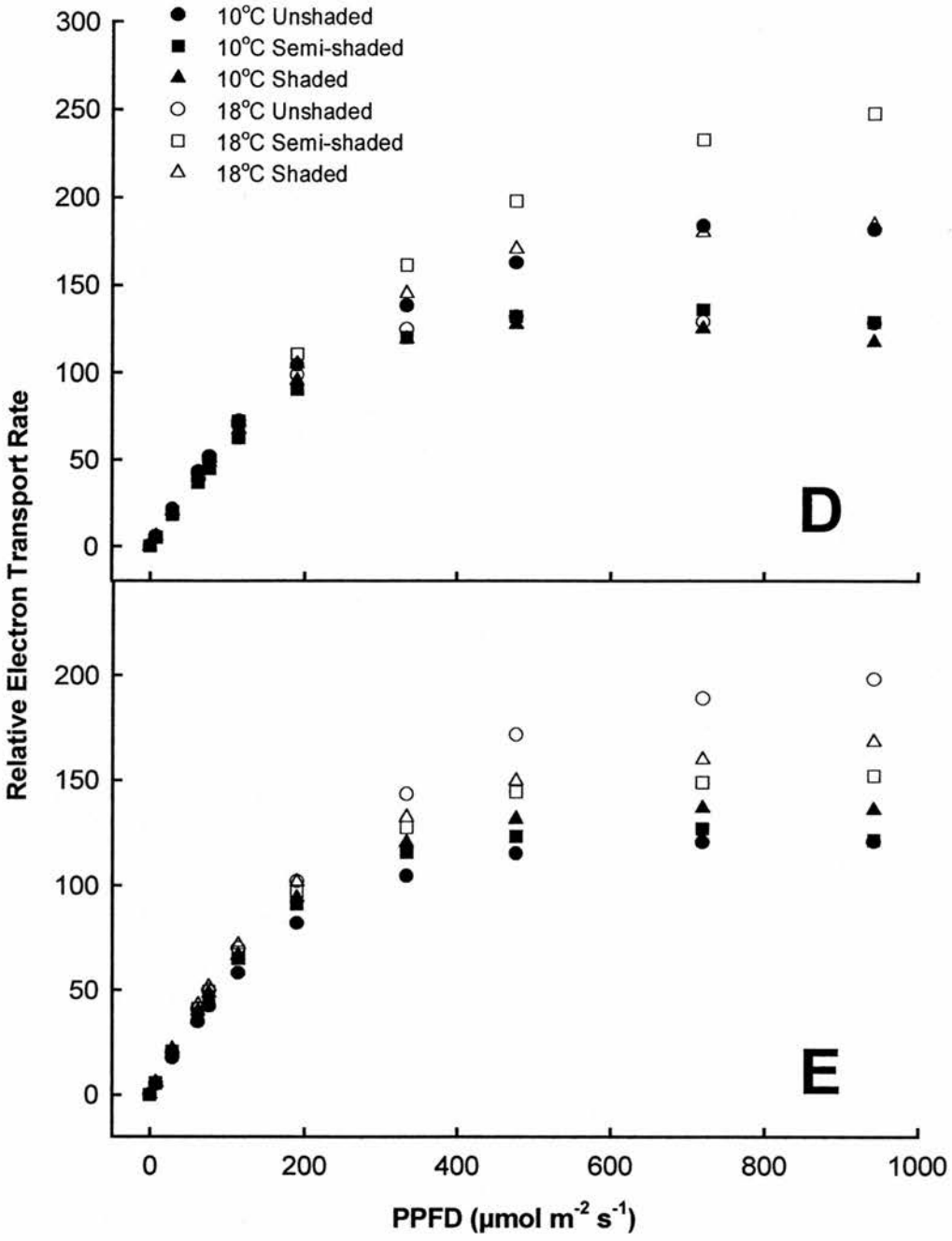


Fig. 4.8 Light response curves for all experimental treatments on:
(A) Measurement day 1;
(B) Measurement day 2;
(C) Measurement day 3;
(D) Measurement day 4;
(E) Measurement day 5.
At 26°C, biomass was too low on measurement days 4 and 5 for accurate measurements to be obtained.

Table 4.4 Maximum Relative Electron Transport Rates ($rETR_{max}$) for each experimental treatment (Mean ± S.E.; n=5). P-E curves were taken every 4 days.

Temperature and Light Treatment		Measurement Set 1	Measurement Set 2	Measurement Set 3	Measurement Set 4	Measurement Set 5
10°C	Unshaded	248.4 ± 41.9	172.8 ± 22.2	189.4 ± 15.5	141.0 ± 10.0	131.4 ± 15.7
	Semi-shaded	139.0 ± 18.0	139.6 ± 8.3	154.8 ± 15.9	125.6 ± 11.0	130.4 ± 11.7
	Shaded	161.4 ± 46.0	169.2 ± 26.7	154.4 ± 18.2	135.6 ± 14.0	146.2 ± 12.7
18°C	Unshaded	214.4 ± 38.1	267.2 ± 39.3	249.2 ± 19.0	330.6 ± 48.7	241.6 ± 48.1
	Semi-shaded	135.0 ± 10.1	180.4 ± 13.6	165.0 ± 21.7	207.4 ± 36.8	163.6 ± 31.9
	Shaded	143.0 ± 12.6	221.8 ± 33.8	305.0 ± 62.1	238.3 ± 49.1	190.2 ± 23.3
26°C	Unshaded	401.2 ± 80.2	221.6 ± 28.4	192.8 ± 41.7		
	Semi-shaded	513.6 ± 63.4	162.0 ± 16.2	191.8 ± 40.0	Signal too low	Signal too low
	Shaded	467.8 ± 28.2	157.4 ± 16.0	153.3 ± 18.4		

Table 4.5 Light saturation parameters (E_K) for each experimental treatment (Mean ± S.E.; n=5). P-E curves were taken every 4 days.

Temperature and Light Treatment		Measurement Set 1	Measurement Set 2	Measurement Set 3	Measurement Set 4	Measurement Set 5
	Unshaded	327.2 ± 39.9	262.7 ± 27.3	293.9 ± 23.0	244.8 ± 13.4	219.6 ± 16.7
10°C	Semi-shaded	204.2 ± 23.2	219.9 ± 10.3	243.5 ± 21.0	206.1 ± 16.6	212.0 ± 18.3
	Shaded	227.7 ± 52.4	233.5 ± 24.7	239.4 ± 21.1	203.4 ± 13.5	218.7 ± 11.4
	Unshaded	421.9 ± 53.6	409.1 ± 46.3	384.7 ± 23.5	472.4 ± 54.3	350.5 ± 57.6
18°C	Semi-shaded	300.9 ± 19.7	306.8 ± 18.5	263.6 ± 30.9	306.2 ± 39.0	248.1 ± 42.6
	Shaded	264.6 ± 19.1	330.8 ± 27.3	421.8 ± 82.2	320.3 ± 38.0	246.1 ± 21.5
	Unshaded	574.2 ± 117.5	352.6 ± 40.4	302.1 ± 54.8		
26°C	Semi-shaded	694.6 ± 72.0	245.0 ± 20.5	282.1 ± 44.6	Signal too low	Signal too low
	Shaded	647.0 ± 34.5	243.9 ± 23.4	240.2 ± 25.9		

Table 4.6 Maximum light utilisation coefficients (α^{rETR}) for each experimental treatment (Mean ± S.E.; n=5). P-E curves were taken every 4 days.

Temperature and Light Treatment		Measurement Set 1	Measurement Set 2	Measurement Set 3	Measurement Set 4	Measurement Set 5
10°C	Unshaded	0.74 ± 0.04	0.65 ± 0.02	0.64 ± 0.01	0.57 ± 0.01	0.59 ± 0.03
	Semi-shaded	0.68 ± 0.01	0.63 ± 0.01	0.63 ± 0.02	0.61 ± 0.01	0.62 ± 0.01
	Shaded	0.68 ± 0.03	0.71 ± 0.04	0.64 ± 0.03	0.66 ± 0.03	0.67 ± 0.04
18°C	Unshaded	0.49 ± 0.04	0.64 ± 0.03	0.65 ± 0.02	0.69 ± 0.03	0.68 ± 0.03
	Semi-shaded	0.45 ± 0.01	0.59 ± 0.01	0.62 ± 0.02	0.66 ± 0.03	0.65 ± 0.01
	Shaded	0.54 ± 0.02	0.66 ± 0.05	0.71 ± 0.04	0.72 ± 0.05	0.77 ± 0.05
26°C	Unshaded	0.71 ± 0.02	0.63 ± 0.01	0.62 ± 0.02		
	Semi-shaded	0.73 ± 0.02	0.66 ± 0.02	0.66 ± 0.03	Signal too low	Signal too low
	Shaded	0.72 ± 0.01	0.64 ± 0.01	0.64 ± 0.01		

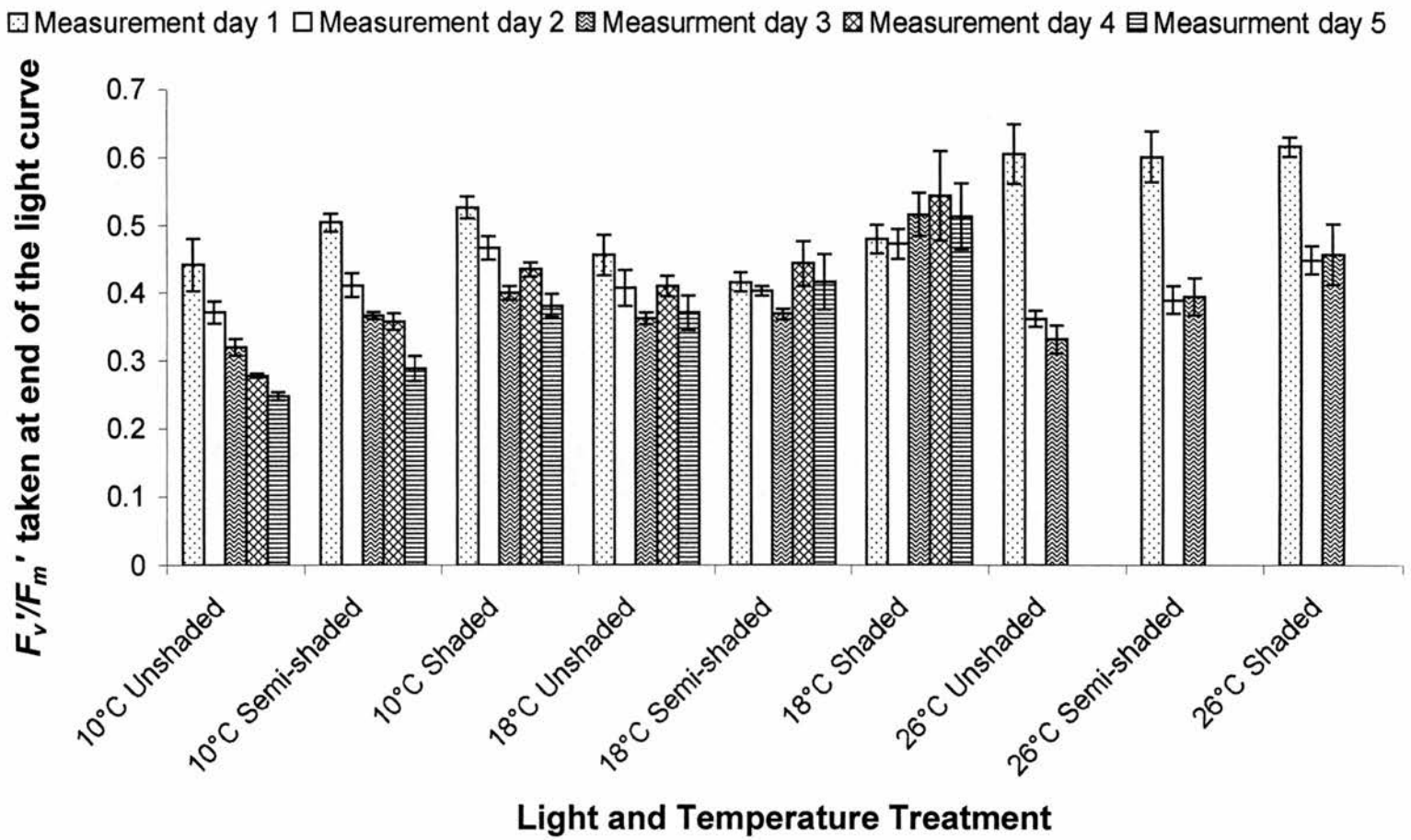


Fig. 4.9 F_v'/F_m' at the end of the light curve, for each experimental treatment (Mean ± S.E.; n=5). P-E curves were taken every 4 days.

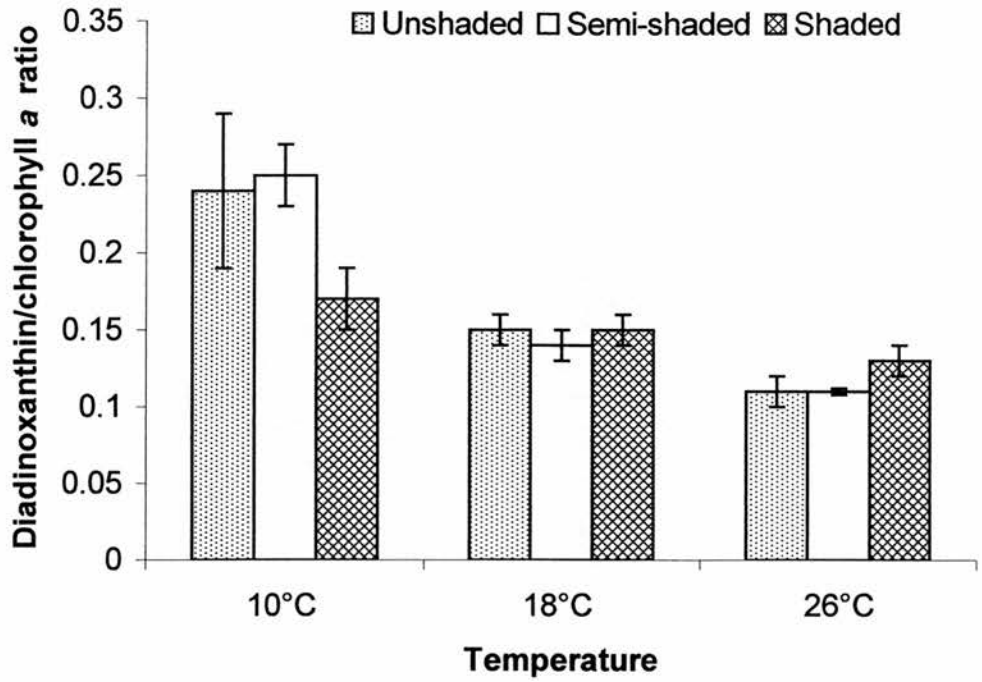


Fig. 4.10 Diadinoxanthin:Chlorophyll *a* ratios after 21 d under the experimental conditions (Mean ± S.E.; n=5 cores in each treatment).

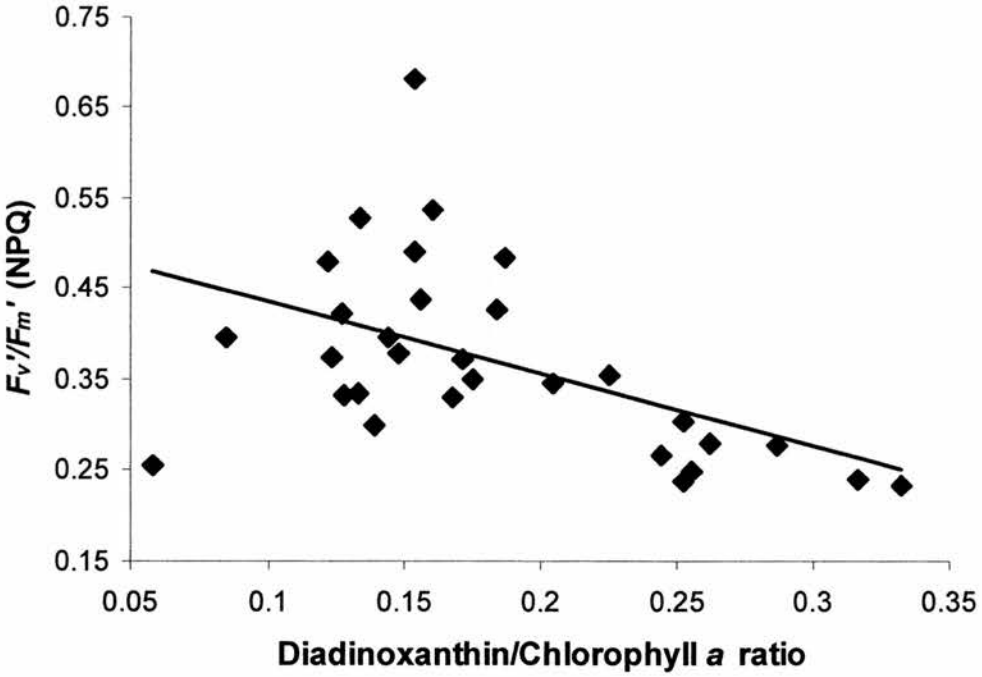


Fig. 4.11 F_v'/F_m' (NPQ) decreases with increasing Diadinoxanthin:Chlorophyll *a* ratios at 10°C and 18°C ($r^2 = 0.25$; $y = -0.79x + 0.51$).

At 10°C, $rETR_{max}$ α^{rETR} and E_K declined over time in the unshaded treatment. On measurement day 1, $rETR_{max}$ and E_K were significantly higher at 26°C, regardless of light treatment, and both parameters decreased over time. Since the light curves did not saturate on measurement day 1 at 26°C, this is likely to be an artificial result. In all 26°C treatments, α^{rETR} was significantly higher on measurement day 1; whilst in all 18°C treatments, it was significantly lower ($F_{30,154} = 5.77$, $p < 0.005$).

For all assemblages, the coefficient of photochemistry (qP) decreased throughout the light curves (data not shown), indicating that Q_A reduction occurred in addition to any increase in non-photochemical quenching (NPQ). Time had a significant effect on F_v'/F_m' taken at the end of a light curve ($F_{30,154} = 10.33$, $p < 0.0005$; Fig. 4.9). F_v'/F_m' is inversely proportional to NPQ. The amount of NPQ required for down-regulation increased over time at 10°C and 26°C, but remained fairly constant at 18°C. Light and temperature treatments did not have a significant effect on F_v'/F_m'.

Diadinoxanthin/chlorophyll *a* ratios remained the same as, or increased above the initial ratio (mean ± S.E.; 0.11 ± 0.004), and decreased as temperature treatments increased. At 10°C, these ratios differed between light treatments, being significantly higher in the unshaded and semi-shaded treatments ($F_{8,36} = 6.15$; $p < 0.001$) (Fig. 4.10). On measurement day 5, NPQ of 10°C and 18°C assemblages increased as the ratio of diadinoxanthin/chlorophyll *a* decreased (Fig. 4.11), although this was a weak negative relationship.

4.4 Discussion

4.4.1 Biofilm development and maintenance

The laboratory-grown 10°C biofilm of this experiment was sustained in a healthy state for 21 d. This is likely to be because net growth and loss were in equilibrium with one another; i.e. the low temperature maintained lower growth rates, whilst the loss processes were minimised by the removal of grazers. High SIMI indices indicated that the assemblage composition had changed very little from the initial assemblage. This may be because temperature conditions in the field, in March, are comparable with this treatment, so that the physiology and composition of the assemblage had already acclimated. These results are in agreement with Defew *et al.* (2002) who maintained a biofilm grown at 10°C for

14 d, and whose assemblage composition illustrated a certain degree of inertia (also see Chapter 3). At 10°C, diadinoxanthin:chlorophyll *a* ratios were significantly lower in the shaded assemblage, indicating that the species acclimated their physiology and used NPQ (which increased with time) in response to the light environment, rather than altering the assemblage composition.

The biofilm grown at 18°C reached higher surface biomass than that of the 10°C biofilm, but after 21 d assemblages were being maintained at similar levels of biomass. Diadinoxanthin:chlorophyll *a* ratios were not significantly different among light treatments, and assemblages responded to the light environment by a significant alteration of the species composition. Even if a species were only a slightly better competitor than others in the assemblage, we would expect it to dominate fairly quickly under conditions that support rapid population growth (e.g. higher temperature). According to this approach, such assemblages are expected to have a low diversity (Huston, 1979). The unshaded 18°C assemblage had the lowest diversity of all treatments and was dominated by *Stauroneis* sp. Shaded conditions at this temperature may not have been favourable to *Stauroneis*, allowing for *Achnanthes* and *Nitzschia* species to dominate, and species richness and diversity to increase. However, the outcome of competition between these species *in situ* may also be influenced by factors such as nutrient concentrations and herbivory.

After 14 d the 26°C biofilms had become very thick. This would have affected light attenuation through the biofilm and sediment (Consalvey, 2002), nutrient concentrations and exchange, and may have affected the diatoms' ability to migrate. It is likely that the 26°C biofilm became severely nutrient-limited after 14 d, which corresponded with the significant decline in surface biomass and health of the biofilm (although nutrients have not been tested in this experiment). In a laboratory experiment involving diatoms, Watermann *et al.* (1999) observed a decline in biomass after 15 d, which they also attributed to nutrient limitation. However, the decline in surface biomass described here may have resulted in an input of nutrients to the system due to diatom senescence. The species that remained alive in the assemblage may then have been able to utilise this source of nutrients in order to establish a new biofilm. Consalvey (2002) maintained a biofilm in the laboratory for 45 d, and LTSEM images

indicated that cyanobacteria had dominated the system after this extended length of time.

Cyanobacteria have been found to be resistant to nutrient stress (Villbrandt *et al.*, 1990), and out-compete diatoms at high temperatures (Watermann *et al.*, 1999; PROMAT, 1997). Cyanobacterial biomass increased with temperature in this experiment, highlighted by an increase in zeaxanthin:chlorophyll *a* ratios. Zeaxanthin:chlorophyll *a* ratios also increased with decreasing light levels at 18°C and 26°. Cyanobacteria are considered shade-adapted organisms (Stal, 1995), often found beneath the diatom layer in estuarine sediments (PROMAT, 1997). The taxonomic shift observed at higher temperatures may also have been mediated by the physiological constraints imposed by higher temperatures on diatom processes (Blanchard *et al.*, 1996; Davison, 1991). For example, although diatoms can actively photosynthesise at temperatures greater than 25°C, they require lower temperatures (up to 10°C air temperature at night) to maintain high rates of cell division (Admiraal, 1977).

4.4.2 Assemblage change and sediment stability

Prior to the decline in surface biomass in 26°C treatments, the biofilms were observed to blister, possibly due to oxygen bubbles, which would also indicate high productivity (Wiltshire *et al.*, 1998). An increase in the roughness of the bed/biofilm increases the likelihood of the bed/biofilm being eroded (Paterson, 1994). In accordance with the findings of Consalvey (2002), the slight current generated by the through-flow tidal system removed small amounts of the blistered biofilm. In the natural environment, waves and the incoming tide could have lifted almost all the biofilm away from the sediment surface. This would have prevented the remaining assemblage from becoming nutrient limited, thus maintaining the health of the biofilm, and may also have allowed another biofilm to develop elsewhere when the cells were deposited on the next ebb tide (Tolhurst, 1999).

Both cyanobacteria and diatoms can affect the erodibility of estuarine sediments. However, on mudflats, filamentous cyanobacteria only build surficial biofilms (Paterson, 1994), so that a diatom biofilm is more resistant to erosion. If increased temperature (which is likely to occur due to global climate change) alters the main functional group of microphytobenthic biofilms from diatoms to

cyanobacteria, this in turn will affect the erosion and deposition of estuarine sediments. However, this response will be mediated by the biofilms' position in the estuary and the sediment size of the area. Compared to the grain size of sand (found towards the mouth of an estuary), the size of diatoms is fairly small so they only form biofilms around single grains. This stabilises the sediment less effectively against erosion than the characteristic patterns of laminated biomass intertwined with siliciclastic grains that networks of cyanobacteria produce (Watermann *et al.*, 1999).

4.4.3 Chlorophyll *a*

Chlorophyll *a* concentration decreased with increasing temperature, indicating a reduction in growth as temperature increased. The relationship of chlorophyll *a* with temperature may have differed due to the difference in species composition (Fig. 4.7) since different sized species contain different amounts of chlorophyll (Blasco *et al.*, 1982), and due to the light and nutrient environment affecting the physiological state of the cells (Davison, 1991; de Jonge, 1980). This relationship would also be affected by the time of sampling (i.e. if chlorophyll *a* had been sampled before the substantial decrease in surface biomass in the 26°C treatments).

The widespread occurrence of direct relationships between pigment content and growth temperature (Geider, 1987) suggest that temperature acclimation of the light-harvesting apparatus is a widespread phenomenon. However, temperature has been shown to have contrasting effects on chlorophyll content. In some cases chlorophyll *a* content was inversely related to growth temperature (Davison, 1991), whilst in others chlorophyll *a* content was shown to increase with increasing temperature (Wolfstein & Stal, 2002; Berges *et al.*, 2002). Such results stress the need to replicate the experiment.

Water contents of sediment from the 26°C cores were significantly lower than other temperature treatments (data not shown), suggesting that compaction of the sediment had occurred (Perkins *et al.*, in press). The inclusion of successively deeper layers of sediment may serve to dilute the biomass and therefore decrease the chlorophyll *a* content (Kelly *et al.*, 2001). This compaction and reduction in the photic zone may have restricted the diatoms ability to migrate away from the sediment surface, and may explain why NPQ increased

over time at 26°C. Diatoms have been shown to use more NPQ when migration is unavailable as a behavioural form of photosynthetic down-regulation (Defew *et al.*, in review; see Chapter 7 for further details).

4.4.4 Measurements of minimum fluorescence

During the last few days of this experiment, F_o^{15} values of the 26°C assemblages were extremely low. Values of minimum fluorescence (F_o^{15}) less than 150 (measured by the FMS2 fluorometer) show a tendency towards high F_v/F_m^{15} ratios (Honeywill, 2001). Whilst this may be a natural phenomenon (i.e. very low biomass microphytobenthic biofilms are less stressed due to a lack of competition for nutrients and light), the mean F_v/F_m^{15} ratio of 0.81 on the final day of measurement would be considered higher than would be expected from natural estuarine benthic diatom biofilms (Honeywill, 2001; Büchel & Wilhelm, 1993).

In order for the fluorescence:chlorophyll *a* ratios to decrease at 26°C, the proportional decrease in F_o^{15} would have to be greater than the proportional decrease in chlorophyll *a*. Light scattering and attenuation of both the excitation beam and fluorescence emitted through the air, biofilm and sediment will decrease the fluorescence detected (Büchel & Wilhelm, 1993; Serôdio *et al.*, 1997; Honeywill, 2001). A decrease in F_o^{15} could be due to NPQ, which increased over time in the 26°C assemblages. An increase in the zeaxanthin:chlorophyll *a* ratios at 26°C indicated an increase in the presence of cyanobacteria. Thus from a mixed assemblage, the decrease in fluorescence to chlorophyll *a* ratios at 26°C can be partly explained by the presence of cyanobacteria, as they exhibit only weak fluorescence from chlorophyll *a* (Sepälä & Balode, 1998; Bryant, 1986). Using data from the Eden Estuary, Honeywill (2001) attributed scatter in the relationship between chlorophyll *a* and F_o^{15} to the presence of cyanobacteria in some samples. Since the surface biomass of the 26°C assemblages was very low after 21 d, a finer chlorophyll *a* sampling resolution (e.g. using the cryolander; Wiltshire *et al.*, 1997) would have improved the correlation between minimum fluorescence (surface biomass) and chlorophyll *a*.

4.4.5 Effects on photosynthesis

Temperature is known to influence the photosynthetic capacity of microphytobenthos, which shows a general trend of increasing with increasing temperature, up to an optimum temperature of approximately 25°C (Blanchard *et al.*, 1997; Blanchard *et al.*, 1996). This was indeed the case within this experiment, in the short-term (days), when $rETR_{max}$ of the 26°C assemblages was double that of assemblages grown at 10°C. These changes in $rETR_{max}$ with temperature are likely to be due to the effect of temperature on the enzymatic complex of inorganic carbon fixation (Blanchard & Guarini, 1996). However, temperature effects on physical processes such as diffusion of electron carriers (e.g. plastoquinone), cellular pH and photorespiration, may also be responsible by affecting enzyme activity directly or via their influence on substrate concentration (Falkowski & Raven, 1997; Davison, 1991; Raven & Geider, 1988). Since the light-saturated rate of photosynthesis is strongly affected by temperature there is an increase in E_K at increased temperature, as was observed in this experiment (Falkowski & Raven, 1997). Harvey (2000) suggests that warmer temperatures may suppress the down regulation of the photosynthetic response, as higher temperatures increase the demand for photosynthetic products. However, temperature did not have a significant effect on the amount of NPQ used by the assemblages in this experiment. In terms of estuarine microphytobenthos, a decrease in down regulation as temperature increases would suggest a higher ETR in warmer habitats, which has not been observed (Perkins *et al.*, 2001).

Over the long term (weeks) temperature effects on $rETR_{max}$ and E_K may have been due to the observed change in species composition (Fig. 4.7 & Table 4.2). The potential for species change is often overlooked. After 21 d of exposure to the experimental treatments, species composition of the assemblages had changed from the initial assemblage. Experimental assemblages could be grouped according to temperature treatments, but light treatments only had an effect on species composition at 18°C. The effects of light may be masked at 10°C and 26°C because these temperatures are towards the extremes of the temperature scale. Consequently, the assemblages have to cope and/or adapt to the physiological stress that these temperatures would impose.

Due to the lack of saturation in the light curves (i.e. an inflexion point at 720 μmol PPFD m^{-2} s^{-1}; previously seen by Perkins *et al.*, 2001 for migratory biofilms *in situ*) on a number of occasions, values of α^{rETR}, $rETR_{max}$ and E_K have to be treated with caution. At high light, the diatoms are likely to have migrated away from the sediment surface, in order to receive less light. The signal from these sub-surface diatoms would contribute to a higher value of F_q'/F_m', which would result in a measured efficiency that was falsely inflated above the true efficiency of the cells at the sediment surface. Consequently the estimated value of rETR would be much higher and light curve saturation may not occur (Defew *et al.*, in review; see Chapter 7 for further details). Microcycling is very likely to be one of the reasons why light has been reported to have a greater effect on cultured diatoms (Verity, 1981) and a much lesser effect on motile benthic diatom assemblages, as was observed in this experiment.

4.5 Conclusions

At 10°C and 18°C the microphytobenthic biofilm was maintained within the 21 d period, although the two assemblages illustrated different strategies of adapting to the light environment. At 10°C, the assemblage acclimated to the light environment by changing the photosynthetic apparatus, whilst at 18°C, the composition of the assemblages altered. However, at 26°C the microphytobenthic biofilm was not maintainable, and cyanobacteria began to increase their abundance and dominate. Such changes in composition could have an effect on the estuarine ecosystem in terms of primary productivity, food webs and sediment stability and transport. This experiment has shown that environmental temperature can mask potential light effects, making it difficult to distinguish between the two variables. When experimenting over a medium term duration (weeks), changes in species composition must be taken into account, particularly when investigating photosynthetic parameters and using minimum fluorescence to estimate biomass.

Chapter 5

Chapter 5: The influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes

Abstract

Grazing (top-down control) and nutrients (bottom-up control) both regulate the biomass and species composition of intertidal benthic diatom assemblages. However, observations of grazing/predation effects on species richness differ under contrasting nutrient conditions, and how temperature influences these relationships has not been investigated. This experiment explored the interactive effects of grazing, nutrients and temperature, and compared the impacts of Corophium volutator *and* Hydrobia ulvae *– two species that differ in their feeding strategies and bioturbation effects. Diatom assemblages were collected from two estuaries that differ in their nutrient status and dominant macrofaunal grazer species. Assemblages were grown without (control) and with grazing activity under contrasting nutrient and temperature regimes.* C. volutator *exerted a strong regulatory influence on epipelic diatoms by reducing their biomass, and preferentially consuming certain dominant taxa, thereby increasing species richness, evenness and diversity. The proportion of epipsammic species increased in the presence* of C. volutator *grazing, at the expense of Navicula species. Biezelingse Ham assemblages grazed by* C. volutator *were not influenced by nutrient or temperature regime, while control assemblages were influenced by temperature. In Zandkreek assemblages, by contrast, differences in the structure of diatom assemblages between the* H. ulvae*-grazed and control treatments were far less pronounced.* H. ulvae *appeared to be a general consumer, grazing subdominant species. Species richness was greater at low temperature, regardless of nutrient treatment. Macrofaunal grazing did not predictably increase or decrease species diversity, but could potentially do both, and may mask the effects of environmental and bottom-up control.*

Chapter 5: The influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes

5.1 Introduction

The factors that determine the abundance and distribution of diatom species in estuarine sediments are still poorly understood. Early theoretical work, such as the 'resource-ratio' and the 'resource-heterogeneity' models, emphasised competition for a 'single most-limiting resource' and 'spatio-temporally variable resources' respectively (Tilman, 1982). However, a major drawback of the early theoretical models was that they did not incorporate the effects of predators. Today, the debate amongst ecologists continues as to whether the primary control is by bottom-up (through available resources) or top-down factors (from predators) (Power, 1992). Environmental variables such as temperature also have an important role to play. Tilman (1999) argues that ecosystem dynamics and functions are regulated by species composition, because species drive ecological processes and have individual traits. This implies that regulatory and selective mechanisms such as competition, predation, and disturbance will affect microphytobenthic processes, including primary productivity, by regulating assemblage composition and biomass. Many studies have shown the effects of hydrodynamics (Paterson & Hagerthey, 2001), grazing (Miller *et al.*, 1996), and nutrient supply (Wulff *et al.*, 2000; Posey *et al.*, 1999) on microphytobenthic biomass. However, few have addressed what effect these factors have on species composition.

There is substantial evidence indicating that benthic diatoms are the primary food resource for many macrofauna and meiofauna taxa inhabiting estuarine sediments (Buffan-Dubau & Carman 2000; Page, 1997). More importantly, some estuarine macrofauna have been shown to regulate microphytobenthic biomass and production (Smith *et al.*, 1996; Underwood & Paterson, 1993; Reise, 1992). Top-down control from predation and/or grazers can be considered a major form of perturbation able to affect both the productivity of an ecosystem (Leibold & Wilbur, 1992) and its species richness (Lubchenco, 1978). On estuarine mudflats, *C. volutator* are generally considered

unselective deposit feeders, although suspension feeding and epipsammic browsing may occur (Gerdol & Hughes, 1994a, b), while *H. ulvae* have been shown to rely on deposit-feeding and browsing (Blanchard *et al.*, 2000; Morrisey, 1988a, b; Lopez & Kofoed, 1980). *H. ulvae* and *C. volutator* are both capable of drastically reducing natural microalgal populations (Coles, 1979). In addition to their feeding strategies, infauna may affect diatom assemblage structure through sediment bioturbation. *C. volutator* transports sediment vertically via burrow construction and irrigation, whereas *H. ulvae* horizontally mix the surface sediment layer (Cadée, 2001).

The few studies that have directly examined the effects of grazers on microphytobenthic species richness have shown that selective grazing by macrofauna can alter species composition with the strength of the effect differing among macrofaunal species (Smith *et al.*, 1996; Reise, 1992). The selective effects may result from differences among diatoms as a viable resource, susceptibility and survival of grazing of individual diatom taxa, as well as the size and/or hunger level of the herbivore (Steinman, 1991). Growth versus resistance trade-offs is a general pattern in plant communities (Tilman, 1982). It is important to recognise that observations of predation effects on species richness are neither clear nor universal and grazing impacts on diversity tend to differ under contrasting nutrient conditions (Proulx & Mazumder, 1998). For example, when grazed by crustaceans and gastropods, Hillebrand *et al.* (2000) found that the diversity of a periphyton community was reduced when nutrient concentrations were low, but enhanced when concentrations were high. Many studies examine top-down and bottom-up affects as independent factors, and how environmental variables (e.g. temperature) interact on the observed patterns has received even less attention.

The objectives of this laboratory study were to:

- Investigate the interactive effects of grazing, nutrients, and temperature upon the biomass, composition and functioning of two intertidal benthic diatom assemblages. Since *C. volutator* and *H. ulvae* differ in their feeding strategies and bioturbation effects, I hypothesised that their impacts on structuring diatom assemblages would differ.

- Determine whether individual estuarine benthic diatom species have unique preferences for certain nutrient and temperature conditions.
- Investigate whether environmentally regulated structural differences in assemblage structure could alter the impacts of grazers or, alternatively, whether grazer impacts could mask environmental effects.

5.2 Methods

Sediment cores were collected on 16 June 2000 from the Biezelingsche-Ham (B-Ham) mudflat, which is located on the north shore of the eutrophic Westerschelde Estuary, and from the Zandkreek mudflat, which is situated on the south shore of the oligotrophic Oosterschelde Estuary. Thirty-two cores (surface area 21cm^2; length 7.6 cm) were collected from the high-shore of both mudflats, in regions of visibly high macrofaunal densities. *C. volutator* (68 ± 5 individuals per core; n=3) was the dominant grazer at the B-Ham mudflat whereas *H. ulvae* (158 ± 20 individuals per core; n=3) dominated the Zandkreek mudflat.

Four tidal tanks (Fig. 5.1) were established in a temperature-controlled room (15°C). Tides were synchronized with the ambient tidal cycle and corresponded to 4 h immersion and 8 h emersion periods. Tanks were illuminated for 16 h d^{-1} with an irradiance of 265 ± 1.9-µmol m^{-2} s^{-1} (mean ± S.E.). Two natural levels of nutrient treatment were used (high and low) to assess nutrient effects. For the high-nutrient treatment, two tidal tanks were filled with filtered (0.45 µm) water from the Westerschelde Estuary. For the low-nutrient treatment, the two remaining tanks were filled with filtered (0.45 µm) water from the Oosterschelde Estuary. Initial and final PO_4, NO_3, NO_2, and NH_4 concentrations were determined using standard automated colorimetric methods (Table 5.1). Temperature effects were assessed using two treatments, high (25°C) and low (18°C), for each nutrient treatment. Every four days, 50% of the water from each tank was removed and replaced to replenish nutrients and replace loss due to evaporation. This caused an increase in salinity.

Four treatments were used to assess the impact of macrofauna on estuarine microphytobenthic diatoms (Fig. 5.2):

Fig. 5.1 Photograph of the experimental set-up

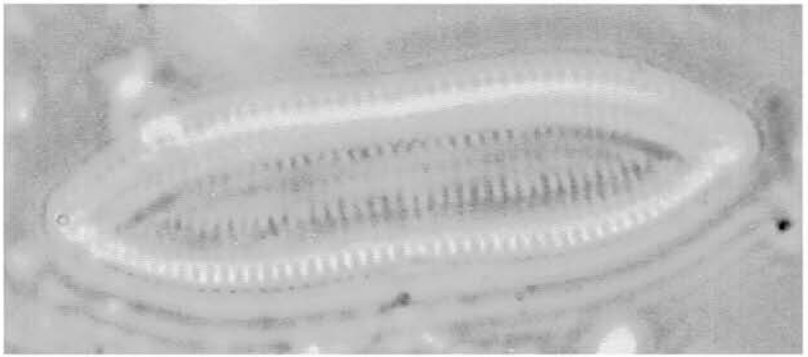
Table 5.1 Initial and final nutrient concentrations, and molar N:P for each of the environmental treatments. Low nutrient water was collected from the Oosterschelde Estuary whereas high nutrient water was collected from the Westerschelde Estuary.

	Oosterschelde (Zandkreek) Low Nutrient				Westerschelde (Biezelinghe Ham) High Nutrient			
	18°C (Low)		25°C (High)		18°C (Low)		25°C (High)	
	Start	End	Start	End	Start	End	Start	End
NH_4 µmol N l^{-1}	6.2	0.2	15.3	6.8	15.2	2.0	22.5	7.4
NO_2 µmol N l^{-1}	2.3	0.3	4.4	1.9	7.8	3.0	14.9	11.3
NO_3 µmol N l^{-1}	57	8.0	70	9.4	204	40	213	108
PO_4 µmol P l^{-1}	2.3	0.4	2.8	0.9	3.4	0.2	3.6	1.0
Molar N:P	28.5	21.3	32.0	20.1	66.8	225	69.6	126

A

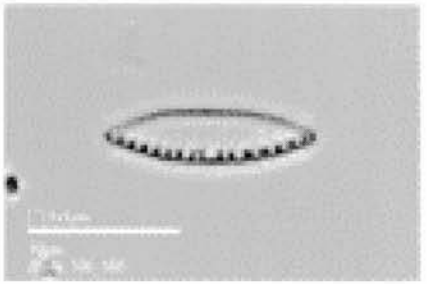
B

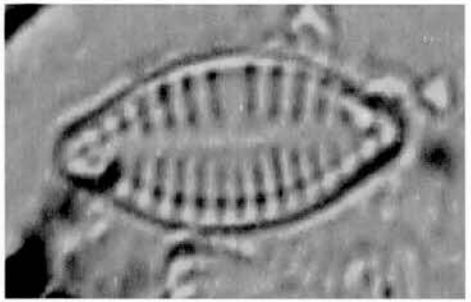
Fig. 5.2 A) *Corophium volutator* grazed core (left) and control B-Ham core (right). B) Control Zandkreek core (left) and *Hydrobia ulvae* grazed core (right).



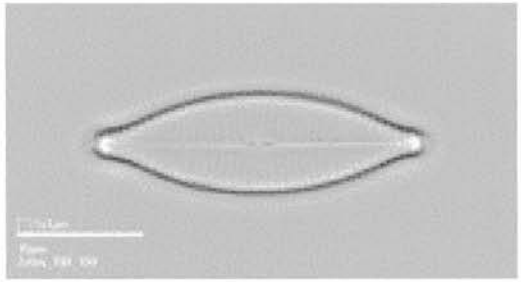
Nitzschia constrictor

Nitzschia frustulum

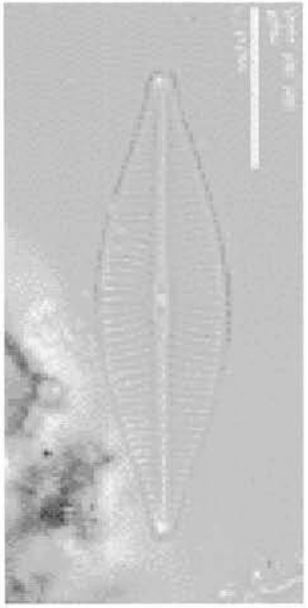


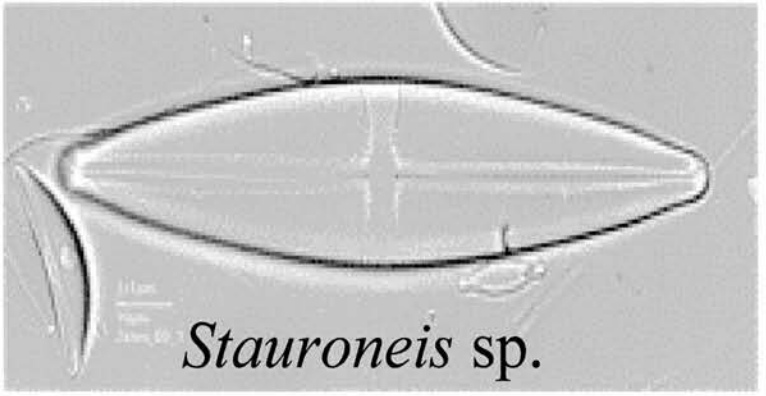


Achnanthes hauckiana



Navicula gregaria &
Navicula phyllepta





Stauroneis sp.

Fig 5.3 Dominant species of B-Ham and Zandkreek diatom assemblages

1) B-Ham cores with *C. volutator*

2) B-Ham control cores (*C. volutator* removed)

3) Zandkreek cores with *H. ulvae*

4) Zandkreek control cores (*H. ulvae* removed)

Each treatment was replicated four times in each of the four tidal tanks; thus, there were 16 cores per tank. Macrofauna were removed by hand from control cores and by placing a 63-μm-mesh disc onto the sediment surface, which facilitated the removal of grazers by driving them to the margins of the core and then onto the mesh, which eased the capture of individuals. Visual observations indicated that the majority of macrofauna were removed within 48 h.

To ensure that microphytobenthic species composition and biomass were similar between macrofauna and control cores at the start of the experiment, a thin sediment slurry layer (4 - 6 mm, and without macrofauna and most meiofauna) from the respective estuary was added to each core. The slurry consisted of algae, small sediment particles, and other organic material, and was obtained by sieving surface sediment through a 150 μm-mesh. Sieved B-Ham sediment was added to B-Ham cores and sieved Zandkreek sediment was added to Zandkreek cores. To prevent the immigration or emigration of macrofauna, a 200 μm-mesh extending 5 cm above the top was wrapped around each core.

Measurements of chlorophyll *a*, F_o^{15}, F_v/F_m^{15}, and EPS were taken when the experiment was terminated on 29 June 2000. Sediment samples were collected using a syringe core. Chlorophyll *a* was determined using HPLC and EPS concentrations determined using the Dubois assay. Fluorometric measurements were made using a PAM diving fluorometer (Walz™). Assemblage composition (Fig. 5.3) was characterised from lens tissue samples, and cell densities were expressed as the number of valves per unit area (cm^{-2}). See Chapter 2 for individual technique methodologies.

5.2.1 Statistics

One-way analysis of variance (ANOVA) was used to determine if measured parameters were significantly different among control and macrofaunal cores (Zar, 1999). Data were log-transformed prior to analysis if this improved the homogeneity of variances. Student-Newman-Keuls (SNK) multiple range tests were used to test for significant ($p<0.05$) differences between grazed and

control treatments. Canonical correspondence analysis (CCA) was used to assess the relationships between the relative abundances of diatom taxa and 12 environmental variables for each assemblage (i.e., B-Ham and Zandkreek). Correlations between relative abundances and environmental variables and the new canonical variable were used as the basis of interpretation. Environmental variables considered in the analysis were macrofaunal presence or absence, chlorophyll *a*, $F_0'^{15}$, temperature, salinity, sediment carbohydrate concentration, final PO_4, NO_3, NO_2, NH_4, and molar N:P ratios. PC-ORD version 3.01 was used for CCA, species richness (S), species evenness (E), and diversity (Shannon Index, H') calculations. The SIMI similarity index was used to compare assemblages between treatments.

5.3 Results

5.3.1 Nutrients

Initial and final nutrient concentrations for each tank are shown in Table 5.1. The high nutrient treatment had NO_3 and molar N:P ratios approximately four and five times higher than the low nutrient treatment. Concentrations of nutrients declined throughout the course of the experiment.

5.3.2 Biezelingsche-Ham Assemblage

Ninety-three diatom species were identified from the B-Ham assemblages. The two canonical axes accounted for 25.6% and 9.4% of the total variance, respectively (Table 5.2). The first canonical axis contrasts assemblages strongly influenced by *C. volutator* (indicated by a high positive correlation; r = 0.968) (Fig. 5.4 & Table 5.2). In addition, $F_0'^{15}$ and chlorophyll *a* were negatively correlated with the first axis indicating that these variables tended to be higher in the absence of *C. volutator*. The second canonical axis contrasts the environmental treatments (Fig. 5.4 & Table 5.2). With respect to the diatom taxa, *Navicula phyllepta, Stauroneis* sp. 1, *Navicula rostellata, Nitzschia frustulum,* and *Navicula digitoradiata* were negatively correlated with the first canonical axis indicating that these taxa were not associated with *C. volutator*. In contrast, *Amphora coffeaeformis* var. *acutiuscula, Nitzschia constricta, Paralia sulcata, Achnanthes hauckiana, Delphineis surirella,* and *Thalassiosira eccentrica* had strong positive correlations with the first canonical axis and thus were associated

Table 5.2 Correlations of the relative abundances of species and environmental data with canonical variables one (CA1) and two (CA2) for each diatom assemblage (Biezelingse-Ham and Zandkreek). Only species whose correlation coefficient exceeded ± 0.300 are shown

Biezelingse-Ham	CA1	CA2	Zandkreek	CA1	CA2
% Variance Explained	25.6	9.4	% Variance Explained	12.9	9.4
Cumulative % Explained	25.6	35.0	Cumulative % Explained	12.9	22.3
Fo^{15}	-0.824	-0.324	Salinity	-0.443	-0.230
Chlorophyll *a*	-0.440	-0.203	PO_4	-0.375	-0.228
Temperature	-0.120	-0.492	Temperature	-0.361	-0.148
PO_4	-0.119	-0.483	Fo^{15}	-0.352	0.385
Salinity	-0.118	-0.442	NH_3	-0.253	-0.094
NH_4	-0.114	-0.517	NO_2	0.208	-0.164
NO_2	-0.064	-0.496	NO_3	0.316	-0.139
NO_3	-0.044	-0.445	Chlorophyll *a*	0.446	0.004
Molar N:P	0.048	-0.044	Carbohydrates	0.472	0.037
Carbohydrates	0.059	-0.313	*Hydrobia ulvae*	0.495	-0.576
Corophium volutator	0.968	-0.302	Molar N:P	0.583	0.219
Navicula phyllepta	-0.746	-0.242	*Nitzschia frustulum*	-0.541	-0.170
Stauroneis sp. 1	-0.662	-0.598	*Stauroneis* sp. 1	-0.517	0.461
Navicula rostellata	-0.646	-0.125	*Navicula* sp. 5	-0.379	0.171
Nitzschia frustulum	-0.603	0.809	*Amphora* sp. 2	-0.348	0.378
Navicula digitoradiata	-0.522	-0.205	*Navicula* sp. 6	-0.328	0.130
Pleurosigma aestuarii	-0.448	-0.137	*Navicula* sp. 11	-0.321	0.128
Achnanthes sp. 2	-0.350	0.206	*Thalassiosira decipiens*	-0.318	0.203
Navicula sp. 11	-0.326	0.292	*Catenela adhaerans*	-0.315	0.064
Synedra ulna	-0.198	0.432	*Navicula cryptocephala*	-0.314	0.126
Navicula gregaria	0.303	-0.170	*Navicula flanatica*	-0.282	0.673
Plagiogramma vanheurckii	0.341	-0.142	*Pleurosigma aestuarii*	-0.025	0.562
Achnanthes sp. 1	0.343	-0.05	*Plagiotropis neovitrea*	0.205	0.528
Entomoneis paludosa	0.347	0.050	*Dimeregramma minor*	0.206	-0.207
Amphora sp. 1	0.363	-0.026	*Cocconeis scutellum*	0.215	-0.108
Cocconeis scutellum	0.381	-0.111	*Navicula vulpina*	0.218	0.541
Plagiotropis neovitrea	0.390	-0.165	*Gyrosigma macrum*	0.244	-0.135
Cyclotella atomus	0.399	-0.094	*Cyclotella meneghiniana*	0.259	-0.266
Actinoptychus senarius	0.426	-0.163	*Achnanthes exigua* var. *heterovalvata*	0.260	-0.072
Cylindrotheca closterium	0.428	-0.177	*Opephora guenter-grassii*	0.263	-0.190
Opephora guenter-grassii	0.435	0.065	*Eunotogramma dubium*	0.272	0.077
Pseudostaurosira perminuta	0.440	-0.152	*Amphora* sp. 1	0.273	-0.301
Navicula sp. 8	0.460	-0.222	*Tryblionella* sp. 1	0.277	-0.277
Cymatosira belgica	0.532	-0.117	*Navicula pygmaea*	0.288	-0.238
Rhaphoneis amphiceros	0.559	-0.166	*Cyclotella atomus*	0.299	-0.247
Cyclotella meneghiniana	0.609	-0.218	*Achnanthes* sp. 2	0.321	-0.054
Cocconeis peltoides	0.655	-0.238	*Opephora pacifica*	0.336	-0.231
Nitzschia recta	0.666	-0.214	*Cocconeis peltoides*	0.395	-0.131
Thalassiosira eccentrica	0.740	-0.256	*Amphora coffeaeformis* var. *acutiuscula*	0.460	-0.227
Achnanthes hauckiana	0.761	-0.161	*Plagiogramma staurophorum*	0.495	-0.304
Delphineis surirella	0.763	-0.194	*Nitzschia dissipata*	0.524	-0.075
Paralia sulcata	0.773	-0.241	*Achnanthes hauckiana*	0.556	-0.145
Nitzschia constricta	0.795	-0.232	*Nitzschia constricta*	0.596	-0.702
Amphora coffeaeformis var. acutiuscula	0.817	-0.168			

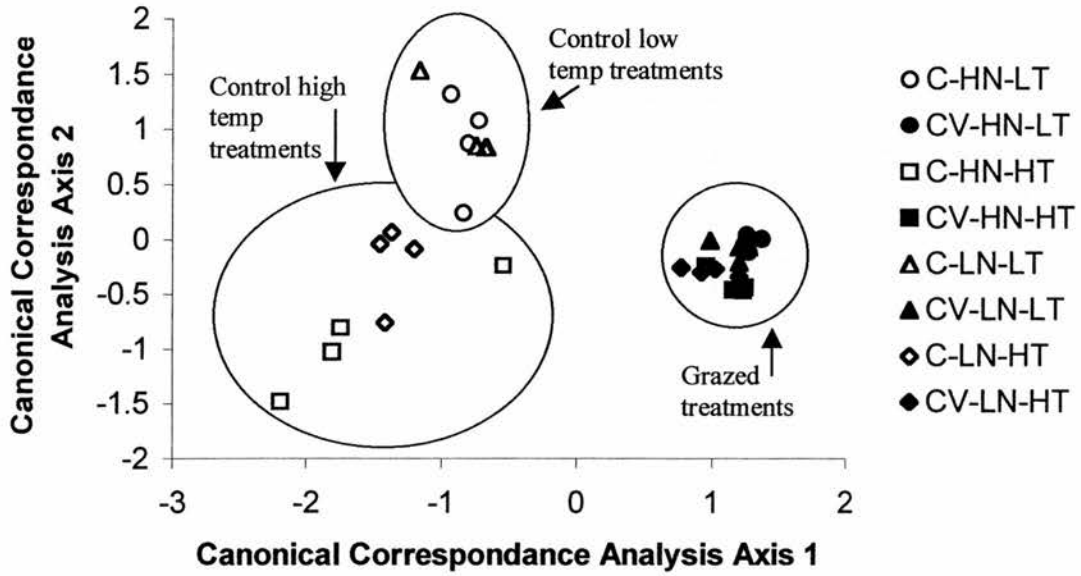


Fig 5.4 CCA plot of B-Ham diatom assemblages in the presence or absence of *C. volutator* and under different environmental conditions. Canonical variables are derived for the relative abundance of diatom taxa. C=control; CV=with *C. volutator*; HN=high nutrient; LN=low nutrient; LT=18°C; HT=25°C

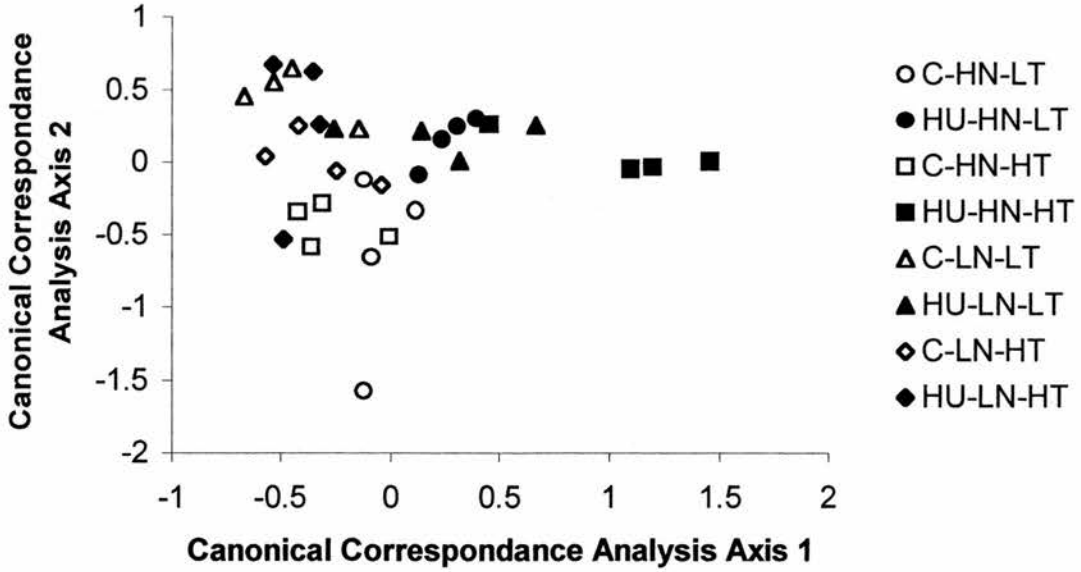


Fig 5.5 CCA plot of Zandkreek diatom assemblages in the presence or absence of *H. ulvae* and under different environmental conditions. Canonical variables are derived for the relative abundance of diatom taxa. C=control; HU=with *H. ulvae*; HN=high nutrient; LN=low nutrient; LT=18°C; HT=25°C

with *C. volutator*. Only two taxa were correlated with the second canonical variable. *Stauroneis* sp. 1 was negatively associated and *N. frustulum* was positively associated with the second axis indicating a preference for high nutrients-high temperature and low nutrient-low temperature, respectively.

Chlorophyll *a* and total cell densities did not differ significantly between *C. volutator* and control assemblages, except for the high nutrients and high temperature treatment, where chlorophyll *a* was three times greater in the control than *C. volutator* grazed assemblage (Table 5.3). F_0^{15} was significantly greater in control than *C. volutator* assemblages for each nutrient-temperature treatment (Table 5.3). F_v/F_m^{15} was significantly lower for control cores under all nutrient and temperature treatments except low nutrient-low temperature. In contrast, EPS concentration did not differ between grazed and control treatments. The production of EPS (total carbohydrate) was significantly greater under high nutrient conditions than under low nutrient conditions (Table 5.3).

5.3.2.1 *C. volutator effects on diatom assemblage structure and composition*

There was very little similarity in assemblage structure between control and *C. volutator* assemblages (Fig. 5.6 & Table 5.4). Species richness was greater for the *C. volutator* treatments than controls, except under conditions of low nutrients and high temperature. Similar higher values were observed for evenness and diversity. The percentages of *Navicula* species were lower whereas the percentages of epipsammic species present were greater for the *C. volutator* treatments than the controls (Table 5.4). F_0^{15} of control assemblages increased under all environmental treatments, with this difference corresponding to higher proportions of motile *Navicula* taxa and lower proportions of epipsammic species (Tables 5.3 & 5.4). This suggests that *C. volutator* negatively affected epipelic diatom biomass and assemblage composition. The following patterns in the relative abundances and cell densities were observed for each treatment combination (Table 5.5):

- Low Nutrient-Low Temperature – The control assemblages were dominated by *N. frustulum*; while the *C. volutator*-grazed assemblages lacked a dominant species. Cell densities of *A. hauckiana*, *D. surirella*, *Navicula gregaria* and *N. constricta* were significantly greater in the grazed assemblage.

Table 5.3 Mean ± SE chlorophyll *a*, cell density, F_o^{15}, F_v/Fm^{15} and total carbohydrate for control and macrofauna treatments for each environmental treatment. One way ANOVA was used to compare between control and animal treatments within environmental treatment. SNK tests were used to identify significant differences ($p<0.05$) between control and grazed treatments. Significant differences are indicated in bold and italics. n=4

	Low Nutrient- Low Temperature					High Nutrient- Low Temperature				
		Biezelingse-Ham		Zandkreek			Biezelingse-Ham		Zandkreek	
	$F_{3, 12, 0.05}$ *p*-value	Control	*Corophium*	Control	*Hydrobia*	$F_{3, 12, 0.05}$ *p*-value	Control	*Corophium*	Control	*Hydrobia*
Chl *a* (mg m^{-2})	2.96 0.075	16.1±3.2	13.0±1.8	9.7±2.9	19.9±1.9	1.77 0.207	36.2±6.6	24.3±5.6	25.0±3.4	36.8±4.4
Cell density (x10^3 cm^{-2})	3.14 0.065	76.7±18.5	87.0±11.3	52.0±18.6	98.9±23.3	2.33 0.126	77.4±28.3	135.6±11.7	27.9±10.6	62.2±22.3
F_o^{15}	18.05 <0.001	***315±41***	***62±4***	182±25	161±9	8.63 0.003	***371±70***	***63±1***	252±26	216±44
F_v/F_m^{15}	3.22 0.062	0.47±0.02	0.54±0.01	0.52±0.01	0.47±0.03	4.64 0.022	***0.42±0.01***	***0.48±0.01***	0.44±0.01	0.42±0.02
Carbohydrates (µg glucose eq. g^{-1})	0.19 0.901	10.2±0.5	10.1±0.4	10.3±0.4	10.5±0.5	1.05 0.407	15.4±0.9	16.2±1.1	14.6±0.6	16.7±1.0
	Low Nutrient- High Temperature					High Nutrient- High Temperature				
		Biezelingse-Ham		Zandkreek			Biezelingse-Ham		Zandkreek	
	$F_{3, 12, 0.05}$ *p*-value	Control	*Corophium*	Control	*Hydrobia*	$F_{3, 12, 0.05}$ *p*-value	Control	*Corophium*	Control	*Hydrobia*
Chl *a* (mg m^{-2})	2.92 0.078	19.3±5.5	12.2±2.5	6.0±2.1	16.0±2.0	3.75 0.041	***42.4±4.9***	***13.2±2.9***	19.6±9.6	26.7±6.7
Cell density (x10^3 cm^{-2})	1.83 0.195	82.7±18.0	119.0±17.5	63.1±13.4	52.3±8.7	2.22 0.138	129.3±39.8	180.2±36.4	124.0±40.8	132.4±15.1
F_o^{15}	18.08 <0.001	***588±65***	***131±20***	288±56	377±16	29.98 <0.001	***1038±112***	***108±5***	***617±98***	***275±32***
F_v/F_m^{15}	17.15 0.001	***0.48±0.01***	***0.58±0.003***	***0.44±0.02***	***0.52±0.01***	20.27 <0.001	***0.45±0.01***	***0.53±0.01***	0.49±0.01	0.51±0.01
Carbohydrates (µg glucose eq. g^{-1})	0.44 0.726	10.3±0.4	10.7±0.5	10.8±0.2	10.2±0.4	0.88 0.477	16.5±1.0	17.1±0.8	18.3±0.6	17.0±0.7

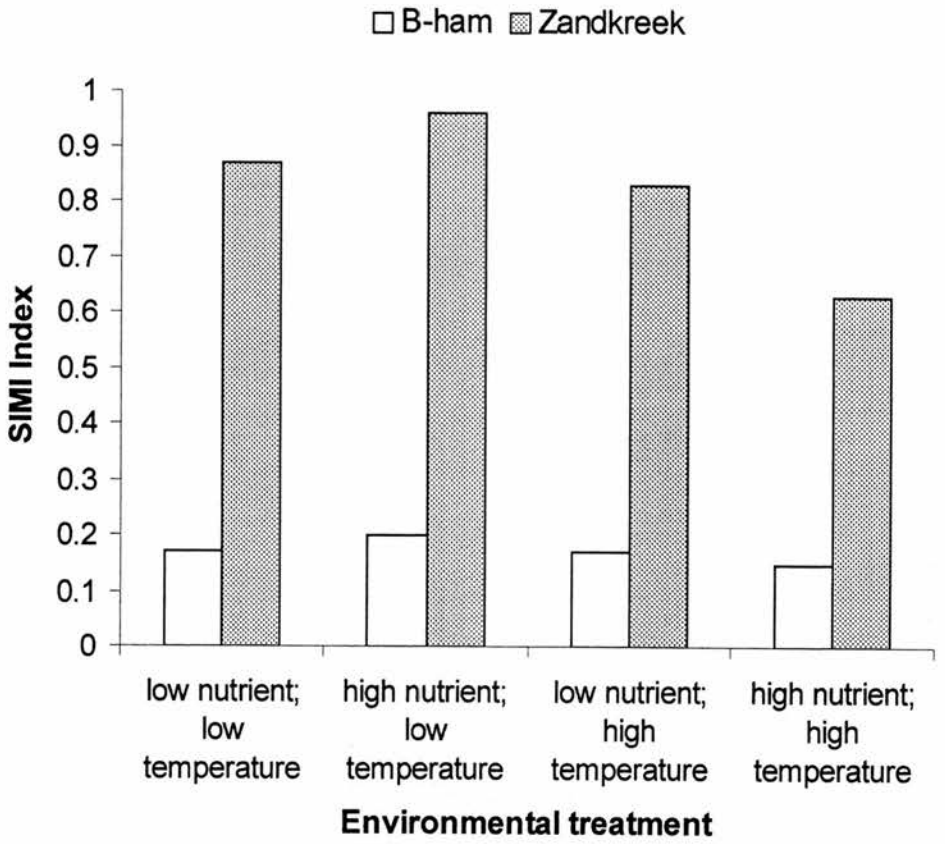


Fig 5.6 SIMI similarity indices comparing control and grazed assemblages (pooled samples) under the four environmental treatments. B-Ham assemblages were grazed by *Corophium volutator* and Zandkreek assemblages were grazed by *Hydrobia ulvae*. An index of 0 meant the samples had no taxa in common, and an index of 1 meant the samples had the same taxa and relative abundance

Table 5.4 Species richness, evenness, and diversity values for control and macrofauna treatments (*C. volutator* or *H. ulvae*) for each environmental treatment. Values are pooled among the four replicates per treatment. The relative abundances of Naviculoid and epipsammic taxa are also presented

	Low Nutrient- Low Temperature				High Nutrient- Low Temperature			
	Biezelingse-Ham		Zandkreek		Biezelingse-Ham		Zandkreek	
	Control	*Corophium*	Control	*Hydrobia*	Control	*Corophium*	Control	*Hydrobia*
Richness (S)	54	57	53	52	47	51	56	52
Evenness (E)	0.61	0.79	0.58	0.70	0.61	0.76	0.76	0.74
Diversity (H')	2.45	3.14	2.29	2.75	2.4	3.0	3.04	2.94
Navicula (%)	25.4	20.2	23.1	29.4	29.7	12.4	41.1	27.1
Epipsammic (%)	16.6	28.2	14.0	24.1	9.7	36.1	15.5	32.2
	Low Nutrient- High Temperature				High Nutrient- High Temperature			
	Biezelingse-Ham		Zandkreek		Biezelingse-Ham		Zandkreek	
	Control	*Corophium*	Control	*Hydrobia*	Control	*Corophium*	Control	*Hydrobia*
Richness (S)	48	43	46	54	38	55	47	49
Evenness (E)	0.61	0.76	0.64	0.60	0.57	0.72	0.68	0.58
Diversity (H')	2.34	2.85	2.44	2.40	2.07	2.88	2.63	2.24
Navicula (%)	36.5	23.2	40.2	26.8	44.5	29.4	33.4	33.4
Epipsammic (%)	9.9	32.5	9.4	12.6	4.2	34.4	12.4	12.4

Table 5.5 Mean ± SE cell densities (x 10^3 cm^{-2}) and relative abundances (%) for selected diatom species for control and *C. volutator* assemblages, under each environmental treatment. n.p. = not present

	Low nutrient Low temperature		Low nutrient High temperature		High nutrient Low temperature		High nutrient High temperature	
	Control	*Corophium*	Control	*Corophium*	Control	*Corophium*	Control	*Corophium*
Achnanthes hauckiana	3.4 ± 0.8 5%	10.3 ± 0.8 12%	2.4 ± 0.6 3%	7.4 ± 1.6 6%	2.1 ± 0.7 3%	22.2 ± 2.4 17%	1.0 ± 0.3 1%	14.9 ±1.4 9%
Amphora coffeaeformis var. acutiscula	1.0 ± 0.6 1%	5.2 ± 0.3 6%	0.06 ± 0.06 <1%	11.2 ± 2.5 9%	0.6 ± 0.4 <1%	7.3 ± 0.5 6%	0.05 ± 0.05 <1%	17.5 ± 6.1 10%
Delpheneis surirella	0.3 ± 0.2 <1%	5.1 ± 1.6 6%	0.2 ± 0.2 <1%	4.1 ± 0.8 4%	0.7 ± 0.6 1%	8.9 ± 2.2 6%	0.2 ± 0.2 <1%	4.7 ± 0.7 3%
Navicula gregaria	2.7 ± 1.1 4%	7.4 ± 1.1 9%	4.6 ± 2.0 5%	13.8 ± 3.5 13%	3.5 ± 1.6 6%	7.7 ± 2.4 6%	9.9 ± 3.9 11%	16.2 ± 2.0 10%
Navicula phyllepta	6.2 ± 2.3 9%	2.2 ± 0.6 3%	18.6 ± 4.9 21%	3.2 ± 0.7 3%	6.4 ± 3.0 11%	1.8 ± 0.5 1%	34.8 ± 14.4 23%	3.0 ± 0.9 2%
Nitzschia constrictor	0.3 ± 0.2 <1%	11.1 ± 1.5 13%	0.6 ± 0.2 <1%	28.0 ± 7.6 23%	1.0 ± 0.7 1%	15.2 ± 1.8 11%	1.2 ± 0.2 1%	51.9 ±18.1 26%
Nitzchia frustulum	39.5 ± 18.1 45%	0.6 ± 0.2 <1%	17.2 ± 6.5 23%	2.8 ± 0.6 2%	41.8 ± 23.6 45%	1.3 ± 0.5 <1%	11.5 ± 5.5 8%	1.9 ±0.7 <1%
Stauroneis sp 1	0.2 ± 0.01 1%	n.p.	20.0 ± 6.5 24%	n.p.	0.3 ± 0.1 <1%	n.p.	49.7 ± 16.6 37%	0.2 ± 0.2 <1%
Thalassiosira eccentrica	0.3 ± 0.1 <1%	7.4 ± 1.5 9%	n.p.	4.1 ± 2.6 3%	0.2 ± 0.1 <1%	13.3 ± 1.0 10%	n.p.	5.3 ± 2.7 3%

- High Nutrients-Low Temperature – The control assemblages were comprised primarily of *N. frustulum* and *N. phyllepta*, although cell densities of these two taxa were not significantly greater than the grazed assemblages. *A. hauckiana*, *N. constricta,* and *T. eccentrica* were significantly higher in the grazed assemblages.
- Low Nutrients-High Temperature – Cell densities and relative abundances of *Stauroneis* sp. 1, *N. frustulum,* and *N. phyllepta* were significantly greater for the control assemblages. *N. constricta, N. gregaria* and *A. coffeaeformis* var. *acutiuscula* dominated the *C. volutator* grazed assemblages in terms of relative abundances. However, only *N. constricta* and *A. coffeaeformis* var. *acutiuscula* had significantly higher cell densities.
- High Nutrients-High Temperature – Cell densities and relative abundances of *Stauroneis* sp. 1 and *N. phyllepta* were significantly higher for the control assemblages. In contrast, relative abundances of *N. constricta*, *A. hauckiana*, *A. coffeaeformis* var. *acutiuscula*, and *N. gregaria* were greater for the grazed assemblages, although only cell densities of *N. constricta* and *A. coffeaeformis* were significantly higher.

5.3.2.2 *Differences in species composition among environmental treatments*

Diatom species composition of control assemblages was strongly influenced by temperature, but not by nutrients (Fig. 5.4). Higher cell densities of *Stauroneis* sp. 1 and *N. phyllepta*, and lower densities of *N. frustulum* represented the major difference between the 25°C and 18°C tanks of the control assemblages (Table 5.5). Salinity co-varied with temperature; thus, differences among assemblages may also be a result of salinity differences. The pooled species richness for control assemblages was greater for low nutrient conditions, regardless of temperature, and greater for low temperature conditions, regardless of nutrient treatment. Species evenness and diversity of control assemblages were not affected by the environmental treatments (Table 5.4). Taxa associated with final nitrate concentrations greater than 40 μmole l^{-1} (18°C) were *Entomoneis paludosa, N. gregaria,* and *Nitzschia dissipata* and taxa associated with nitrate concentrations greater than 100 μmole l^{-1} (25°C) were *E. paludosa,*

N. gregaria, Navicula flanatica, N. phyllepta, N. rostellata, Pleurosigma aestuarii, and *Stauroneis* sp. 1.

Among the nutrient and temperature treatments, *C. volutator*-grazed assemblages had a high degree of similarity, species richness, evenness and diversity (Table 5.4, Fig. 5.4 & Fig. 5.6). Cell densities of *A. coffeaeformis* var. *acutiuscula* and *N. constricta* were greater for the high temperature treatment, while cell densities of *A. hauckiana* and *N. constrictor* were greater when nutrients were high (Table 5.5).

5.3.3 Zandkreek Assemblage

Eighty-five benthic diatom species were identified from the Zandkreek assemblages. SIMI Index values ranged between 0.63 and 0.96, indicating a high degree of similarity between diatom assemblages for control and *H. ulvae* treatments (Fig. 5.6). This similarity was mirrored in the canonical correspondence analysis in which the first two canonical axes accounted for 12.9% and 9.4% of the total variance, respectively (Table 5.2). The first canonical axis was negatively correlated with salinity and positively correlated with molar N:P suggesting a nutrient and temperature effect. *H. ulvae* was negatively and F_0^{15} was positively correlated with the second canonical axis. *N. frustulum* and *Stauroneis* sp. 1 were negatively correlated with the first canonical axis, while *N. constricta, A. hauckiana,* and *N. dissipata,* were positively correlated with the first canonical axis, suggesting preferential differences in salinity tolerances and nutrient requirements. *N. constricta* was negatively correlated whereas *N. flanatica, P. aestuarii,* and *Plagiotropis neovitrea* were positively correlated with the second canonical axis indicating a weak grazing effect.

Benthic algal biomass measured as chlorophyll *a*, F_o^{15}, and cell densities did not differ significantly between *H. ulvae* and control treatments, except for the high nutrients and high temperature treatment, where F_o^{15} was approximately 2.3 times greater in the control than *H. ulvae* treatment (Table 5.3). Compared to B-Ham control assemblages, the surface biofilm (F_o^{15}) in Zandkreek control assemblages was not as substantial (Table 5.3), with the exception again being the high nutrient-high temperature tank, where high densities of *N. phyllepta* and *Stauroneis* sp.1 maintained a surface biofilm. F_v/F_m^{15} and EPS values were

Table 5.6 Mean ± SE cell densities (x 10^3 cm^{-2}) and relative abundances (%) for selected diatom species for control and *H.ulvae* assemblages, under each environmental treatment. n.p. = not present

	Low nutrient Low temperature		Low nutrient High temperature		High nutrient Low temperature		High nutrient High temperature	
	Control	*Hydrobia*	Control	*Hydrobia*	Control	*Hydrobia*	Control	*Hydrobia*
Achnanthes hauckiana	1.3 ± 0.3 3%	9.4 ± 2.0 10%	1.1 ± 0.8 4%	1.7 ± 0.5 2%	1.6 ± 0.9 6%	7.4 ± 2.2 14%	12.5 ± 1093 6%	9.5 ± 4.6 7%
Cocconeis peltoides	0.4 ± 0.3 <1%	0.6 ± 0.2 <1%	0.3 ± 0.1 <1%	0.3 ± 0.2 <1%	0.5 ± 0.3 2%	1.6 ± 0.6 3%	0.04 ± 0.04 <1%	0.4 ± 0.1 <1%
Navicula gregaria	1.9 ± 0.4 5%	13.0 ± 4.0 13%	8.0 ± 1.7 14%	4.5 ± 1.9 9%	3.2 ± 1.0 12%	3.8 ± 1.6 6%	12.0 ± 4.6 15%	21.9 ± 2.3 18%
Navicula phyllepta	4.0 ± 1.1 9%	11.2 ± 3.8 10%	9.9 ± 3.7 14%	5.5 ± 1.7 11%	3.8 ± 1.5 14%	8.8 ± 3.7 14%	31.5 ± 15.6 21%	16.0 ± 4.6 12%
Nitzschia constrictor	0.7 ± 0.4 1%	7.4 ± 3.8 7%	1.7 ± 1.0 3%	0.9 ± 0.3 2%	1.0 ± 0.3 4%	3.1 ± 1.8 4%	2.3 ± 0.9 2%	40.3 ± 5.4 31%
Nitzschia dissipata	0.09 ± 0.09 <1%	2.3 ± 1.1 4%	0.03 ± 0.03 <1%	0.3 ± 0.1 <1%	0.1 ± 0.1 <1%	4.9 ± 1.6 6%	1.0 ± 0.9 <1%	0.6 ± 0.3 <1%
Nitzchia frustulum	28.9 ± 12.2 50%	26.7 ± 11.1 26%	20.6 ± 7.1 34%	22.7 ± 10.3 41%	5.2 ± 3.4 16%	10.5 ± 4.0 17%	16.9 ± 7.0 14%	23.0 ± 8.8 17%
Opephora guenter-grassii	1.6 ± 1.2 2%	2.5 ± 1.6 4%	1.0 ± 0.4 2%	1.3 ± 0.7 3%	0.03 ± 0.03 <1%	4.6 ± 3.2 6%	0.9 ± 0.6 <1%	1.2 ± 1.0 <1%
Rhaphoneis amphiceros	0.05 ± 0.05 <1%	0.8 ± 0.5 <1%	0.04 ± 0.04 <1%	0.06 ± 0.06 <1%	0.3 ± 0.1 1%	0.1 ± 0.1 <1%	0.3 ± 0.2 <1%	0.4 ± 0.2 <1%
Stauroneis sp 1	0.1 ± 0.04 <1%	0.1 ± 0.1 <1%	2.5 ± 0.7 4%	4.8 ± 3.5 10%	0.4 ± 0.2 2%	n.p.	17.0 ± 8.1 12%	0.2 ± 0.1 <1%

similar between control and grazed assemblages with the exception of the low nutrient-high temperature tank where the F_v/F_m[15] of grazed assemblages was significantly higher than control assemblages (Table 5.3). As was found for B-Ham assemblages, total carbohydrate concentrations in high nutrient blocks were higher than low nutrient blocks for both control and *H. ulvae* treatments, whereas temperature had no effect (Table 5.3).

5.3.3.1 *H. ulvae effects on diatom assemblage structure and composition*

There was a high degree of similarity between diatom assemblages grown in the presence and absence of *H. ulvae*, in both the type and proportion of diatom species present, regardless of environmental treatment (Figs. 5.5 & 5.6). Within each environmental treatment, the presence of *H. ulvae* did not have a significant effect on species richness, diversity, evenness, the percentage of *Navicula,* or percentage of epipsammic species present (Table 5.4). The following patterns in the relative abundances and cell densities were observed for each treatment combination (Table 5.6):

- Low Nutrients-Low Temperature – *N. frustulum* cell densities were similar between treatments but the relative abundance of *N. frustulum* in the control assemblages were double that of the *H. ulvae* assemblages. *A. hauckiana, N. gregaria, N. dissipata, N. phyllepta* and *N. constricta* were dominant species of the *H. ulvae* assemblages, but only cell densities of *A. hauckiana, N. gregaria,* and *N. dissipata* were significantly greater.
- High Nutrients-Low Temperature – *A. hauckiana, N. gregaria, N. phyllepta, N. constricta,* and *N. frustulum* were the dominant species of both assemblages, having relative abundances greater than 10%. Cell densities of these taxa did not differ significantly between treatments with the exception of *A. hauckiana*. Cell densities of *Cocconeis peltoides* and *Opephora guenter-grassii* were significantly greater, and cell densities of *Rhaphoneis amphiceros* and *Stauroneis* sp. 1 were significantly less for the *H. ulvae* treatment, although in general, these taxa comprised less than 2% of the total relative abundance.
- Low Nutrients-High Temperature - *N. frustulum* was the dominant species of the control and *H. ulvae* assemblages. *N. gregaria, N.*

phyllepta, and *Stauroneis* sp. 1 also made up a significant proportion of the relative abundances (>10%) of both assemblages, and had similar cell densities. The proportion of *Stauroneis* sp. 1 was greater in the presence of *H. ulvae*.

- High Nutrients-High Temperature - *N. constricta* dominated the *H. ulvae* assemblage, where cell densities were 20 times greater than controls. Cell densities of *Stauroneis* sp. 1 were two orders of magnitude greater for the control assemblage than *H. ulvae*-grazed assemblage. *A. hauckiana, N. gregaria, N. phyllepta,* and *N. frustulum* had high relative abundances (>10%) in both assemblages, but the cell densities of these taxa did not differ significantly between the control and *H. ulvae* assemblages.

5.3.3.2 Differences in species composition among environmental treatments

Zandkreek diatom assemblages were weakly influenced by nutrients (Fig. 5.5). In general, high *N. frustulum* densities were associated with low nutrient conditions. Species richness of control assemblages was greater at the lower temperature than the higher one, regardless of nutrient treatment (Table 5.4). However, the species evenness and diversity of control assemblages did not follow a temperature or nutrient pattern (Table 5.4). Species evenness and diversity of *H. ulvae* assemblages was lowest for the high temperature treatments, and species richness was similar between nutrient treatments (Table 5.4).

5.4 Discussion

The results presented here imply that top-down effects (i.e. direct consumption of microalgae or bioturbation) are predator (grazer) specific and differ in the degree to which they change the biotic and abiotic characteristics of the ecosystem. Additionally, bottom-up effects vary depending on the strength of top-down factors.

It has been suggested that most of the grazing pressure on diatoms is directed towards the larger epipelon, while the small-sized epipsammon are less affected (Reise, 1992). Grazing pressure should, therefore, increase the percentage composition of epipsammic species. Our results support this

hypothesis, with the epipsammic fraction either increasing or occasionally remaining the same in the presence of grazers.

In the absence of grazing, competition for nutrients between species generally reduces diversity (Begon *et al.*, 1990; Huston, 1979). For example, McClatchie *et al.*, (1982) found that grazer exclusion decreased the number of species in a mudflat diatom assemblage. Grazer removal from B-Ham sediments decreased diversity and species richness under all environmental conditions, except for the increased species richness of the control assemblage under low nutrient-high temperature conditions. In contrast, species richness and diversity illustrated a variety of responses upon grazer removal from Zandkreek sediments.

Predation/grazing *per se* does not have a predictable effect on diversity. Predation/grazing can actually reduce diversity if certain prey is eaten to the exclusion of all others or if virtually everything is eaten. However, the predator/grazer that always selects the most abundant prey (compensatory mortality) will inevitably increase diversity, and most of the numerous examples of predation increasing diversity are of this type (Lubchenco, 1978). However, the predator need not be selective since any type of density-independent mortality applied to an assemblage will tend to prevent competitive exclusion. If the competitive equilibrium is prevented by predation, the actual outcome of competition may be completely different from that predicted by the dynamic equilibrium model (Huston, 1979).

Proulx & Mazumder (1998) suggested that grazer effects are confounded by the trophic state, and found that under oligotrophic conditions, grazers reduced plant species richness, but under eutrophic conditions, grazers increased, decreased, or did not affect species richness, depending on the study. For high nutrient treatments, species richness and diversity of B-Ham assemblages grazed by *C. volutator* were greater than the corresponding control assemblage. *C. volutator* grazing increased diversity under low nutrient conditions, contrary to the findings of Proulx & Mazumder (1998). Zandkreek assemblages grazed by *H. ulvae* did not follow a nutrient-related pattern.

5.4.1 C. *volutator* effects

Field and laboratory studies have shown that benthic diatoms are an important component of *C. volutator*'s diet, and *C. volutator* feeding can significantly reduce biomass (Gerdol & Hughes, 1994b; Stuart *et al.*, 1985) and influence diatom species composition (Smith *et al.*, 1996). The mandibles, prior to ingestion, crush diatom frustules, and intact valves are rarely encountered in gut content analyses. Confirmation of diatom ingestion comes from the uptake of ^{14}C labelled diatoms, the presence of chlorophyll *a* in the gut (Gerdol & Hughes, 1994b) and the ratios of gut digestive enzymes (Stuart *et al.*, 1985). Whilst I did not confirm the ingestion of benthic diatoms by *C. volutator*, I have assumed that the significant differences in biomass and species composition between grazed and control assemblages were due to grazing/ingestion by *C. volutator*.

Gerdol & Hughes (1994b) recorded significant reductions in chlorophyll *a* and cell numbers at *C. volutator* densities as low as 9000 m^{-2}. Despite amphipod density being equivalent to approximately 32 000 individuals m^{-2} in this study, significant differences in chlorophyll *a* and total cell densities between control and *C. volutator* grazed assemblages were not found (with the exception of chlorophyll *a* concentrations for the high nutrient-high temperature treatment). However, F_o^{15} values were significantly greater for control assemblages, indicating a higher surface biomass in the absence of *C. volutator* grazing. The similarity in overall chlorophyll *a* concentrations between control and *C. volutator*-grazed assemblages may have occurred due to variation in the depth distribution of chlorophyll *a*. The bioturbatory activity of *C. volutator* may prevent a stable biofilm forming at the surface by regularly redistributing chlorophyll *a*. This will influence any measure of surface biomass, such as F_o^{15}, but be much less apparent in measurements of total chlorophyll *a* unless the depth of sampling is at an extremely high resolution (Kelly *et al.*, 2001; Wiltshire, 2000; Wiltshire *et al.*, 1997). To date, there are no studies comparing distributions of chlorophyll *a* between sediments with low and high densities of infauna.

Assemblages grazed by *C. volutator* had higher species evenness compared to control assemblages under the same environmental conditions, indicating that the dominant species were preferentially selected (Lubchenco, 1978). As a generalisation, selective predation may be expected to induce higher

community diversity if the preferred prey is competitively dominant (Begon *et al.*, 1990). I infer that *N. frustulum*, *Stauroneis* sp. 1, and *N. phyllepta* may have been the preferred prey of *C. volutator*, since all were competitively dominant species in control assemblages, but represented only minor components of the diatom assemblages in which *C. volutator* was present. Among the environmental treatments, the structures of diatom assemblages exposed to the effects of *C. volutator* were remarkably similar. In all four *C. volutator* grazed assemblages, *A. hauckiana* and *N. constricta* were common, which may indicate that these two taxa are less susceptible to grazing. Algae such as diatoms may resist herbivory by having a large size (Lubchenco & Gaines, 1981) or conversely by being small and prostrate on the sediment surface, and avoiding predation due to the morphological constraints of grazer mouthparts (Steinman *et al.*, 1987). These results show that at sufficiently high densities, *C. volutator* can regulate the assemblage composition of intertidal benthic diatoms and override the potential effects of the nutrient and temperature conditions established in this experiment.

Results from this experiment are in agreement with fieldwork of Hagerthey *et al.* (in review). On the Eden Estuary, Scotland, they compared sites grazed by *C. volutator* with sites treated with a biocide in order to remove macrofaunal grazing pressure. Results discussed here found *A. hauckiana* to have greater dominance in grazed cores, whilst *N. frustulum* had greater dominance in control cores. Similarly, Hagerthey *et al.* (in review) found *Achnanthes conspicua* dominance to be greater at grazed sites, and *Nitzschia pusilla* dominance to be greater at biocide-treated sites.

F_v/F_m^{15} was significantly lower for control assemblages than grazed assemblages. A reduced F_v/F_m^{15} ratio is associated with stationary phase (Perkins pers. comm.) and nutrient limitation (Geider *et al.*, 1993). Control assemblages had developed a thick biofilm by the end of the experiment, and consequently, F_v/F_m^{15} values may have been lower due to nutrient limitation. Cells in the *C. volutator* grazed assemblage would most likely be in the exponential phase of growth, which is associated with higher F_v/F_m^{15} ratios. Based on these physiological measurements and proxy measurements of biomass, it is possible to infer the effect of grazing on the functioning of the biofilm (Blanchard, pers. comm.). Blanchard et al. (2001) found a significant negative relationship, which

states that the higher the biomass level, the lower the net production. Therefore, since grazing reduced the amount of biomass, and kept the biofilm in a state of growth, production is likely to be higher in *C. volutator* grazed assemblages than in control assemblages, where biomass may have reached the 'biotic capacity' of the system, and consequently, production would be substantially reduced.

A change in species composition between control and *C. volutator* grazed assemblages did not affect the amount of total carbohydrate produced. This suggests that the potential of sediments to erode will be affected by top-down control on the biomass present, but that the actual assemblage composition will be substantially less important. However, assemblage structure could still have an effect on the type/composition of EPS produced, which may have implications for sediment erosion.

5.4.2 *H. ulvae* effects

H. ulvae has been shown to consume sediment microalgae (Herman *et al.*, 2000; Blanchard *et al.*, 2000; Morrisey, 1988a, b). However, the effects of *H. ulvae* on the sediment-dwelling algae are unclear and complicated by grazing intensity and bioturbation (Levinton & Bianchi, 1981). In this study, densities of *H. ulvae* were high (equivalent to 75 000 individuals m^{-2}) but did not appear to impart a strong regulatory function on the structure of Zandkreek diatom assemblages. This may be due to negative density-dependent effects, whereby the growth rate (and therefore grazing rate) of *H. ulvae* is reduced as snail density increases (Morrisey, 1987). Blanchard *et al.* (2000) reported that above a density of 25 000 snails m^{-2}, ingestion rates for individual snails declined from 26.6±1.1 to 22.4±1.0 ng Chl *a* $snail^{-1}$ hr^{-1}. A greater proportion of biomass was consumed at high temperatures, which agrees with the results of Hylleberg (1975) who found *H. ulvae* had maximal ingestion at the combination of high salinity (30) and high temperature (30°C). Morrisey (1988a) suggested that a substantial fraction of microalgae grazed by *H. ulvae* was not assimilated, and in this experiment *H. ulvae* did not significantly reduce the abundance or cell density of any diatom taxa. *H. ulvae* may prefer to graze subdominant species, since diversity was generally lower for grazed assemblages compared to control assemblages under the same conditions (Swamikannu & Hoagland, 1989).

H. ulvae grazed assemblages were weakly affected by the nutrient conditions (Fig. 5.5), and particularly under high nutrient conditions, the density of several taxa, including *A. hauckiana*, *A. coffeaeformis var. acutiscula*, *N. gregaria* and *N. constrictor* increased relative to control assemblages. Gastropod excreta may also have had a role to play by enriching and fertilising the diatom populations and the sediments (López-Figueroa & Niell, 1987).

5.4.3 Environmental effects

Benthic algal growth is stimulated by increased temperature (see mini-review by Davison, 1991) and nutrients (Posey *et al.*, 1999). More importantly, nutrients are hypothesized to impart a strong regulatory influence on algal dynamics (competition) and hence species composition. A major tenet of this hypothesis is that species differ in their resource requirements (taxonomic tradeoffs). In laboratory cores, and in the absence of perturbations such as grazing and wave action, species composition of an estuarine benthic diatom assemblage is likely to be the outcome of interspecific competition. High diversity would be expected from treatments supporting low growth rates (i.e. low nutrient environments and low temperature regimes), where competitive interactions are kept to a minimum. In contrast, conditions supporting high growth rates and thus rapid population growth (i.e. high nutrient environments and high temperature regimes) would be expected to have a lower diversity, and often have a single species that unequivocally dominates.

When nutrients were reduced in the control B-Ham assemblages (producing conditions that are expected to reduce growth rates and increase diversity), species richness increased by ten species, diversity increased slightly, but cell densities were not significantly different. High nutrient concentrations within the B-Ham sediment may have buffered the diatoms against the treatments of reduced nutrient concentrations in the water supply. Diversity of the nutrient-poor control Zandkreek assemblages would be expected to reduce when nutrient supply was increased, unless diversity is already low due to a nutrient deficiency (Huston, 1979). In this experiment, diversity and evenness values increased. Shannon indices were 2.29 and 3.04, and evenness values were 0.58 and 0.76 for low and high nutrient regimes respectively.

Growth rates are expected to increase when temperature is increased, and thus under the same nutrient regime, diversity would be expected to decline. This was the case for all treatments except when temperature was increased for the Zandkreek assemblage grown under low nutrient conditions. In this case, whilst species richness did actually decrease, diversity increased slightly.

The composition of diatom assemblages in the absence of grazers varied in response to the environmental conditions. Species composition of control B-Ham assemblages were more strongly influenced by temperature (Fig. 5.4), while control Zandkreek assemblages were more strongly influenced by nutrients (Fig. 5.5). This indicates that adaptations of diatom taxa to the different interspecific competitive environments are potentially important when grazing pressure is minimal.

Underwood & Provot (2000) demonstrated that the form of nitrogen and salinity affected the growth rates of four common intertidal diatoms. *N. phyllepta* was the only taxa studied by Underwood & Provot (2000) that was also present in the present study. The highest densities of *N. phyllepta* observed corresponded to the environmental optima proposed by Underwood and Provot (2000) and therefore support their conclusion (*N. phyllepta* optimal growth rates are between 25-150 μM NH_4, 25-250 μM NO_3, and salinity 10-20). In this study, few taxa from either the B-Ham or Zandkreek assemblages showed strong responses to either nutrients or temperature (Table 5.2).

Little is known with respect to the effect of nutrient conditions on polysaccharide production by epipelic diatoms. Experiments using *Cylindrotheca closterium* suggest that exopolysaccharide accumulation is stimulated under low nutrient conditions, but is dependent on the type of nutrient depletion (Staats *et al.*, 2000). Results of this experiment found total carbohydrate to be higher under the high nutrient conditions, regardless of grazing pressure and temperature. In a healthy biofilm, high nutrients are likely to increase the biomass and thus the total EPS produced will indeed increase, but the rate of production is not likely to increase, so that the amount of carbohydrate produced per unit biomass will decrease or remain the same. Nutrient and temperature blocks were found to be insignificant with regards to the total amount of biomass produced per unit biomass (data not presented).

5.5 Conclusions

Macrofaunal species that graze on estuarine sediment populations do not simply increase or decrease species diversity of the diatoms, but can potentially do both. The precise effect depends on the relationships between food preferences, diatom competitive abilities, relative resistance to grazing, and on the intensity of the grazing pressure. Within this context, the dietary spectrum of the individual grazers has an important role to play, and was obviously different between the grazers. *C. volutator* preferentially consumed certain dominant diatom species, while *H. ulvae* appeared to be a more general consumer. This is in contrast to results obtained by Morrisey (1988a), where it was concluded that the similar amphipod *Corophium arenarium* had a broader dietary range than that of *H. ulvae*. The spread of grazing pressure may also have a part to play in the diversity-grazing response. For example, herbivores exerting a spatially more heterogeneous grazing pressure may cause a more pronounced response than herbivores that spread the pressure more evenly (Sommer, 1999; Olff & Ritchie, 1998). *C. volutator* exerted a strong regulatory influence on the species composition of benthic diatom biofilms, and masked the potential regulatory effects of environmental conditions. This contrasted with *H. ulvae* where grazing effects were far less pronounced, and assemblages were weakly influenced by nutrients. Both species bioturbate the sediment and therefore intensity of bioturbation cannot be excluded as a potentially important mechanism regulating biofilm species composition. The estuarine milieu is by definition a harsh environment, where environmental and chemical gradients can vary steeply on both spatial and temporal scales. The lack of a nutrient and temperature response for the majority of taxa in the absence of grazers indicates that these estuarine diatom species are well adapted for a broad range of nitrogen concentrations, salinity, and temperatures.

5.6 Publication

Data from this study have been published in Marine Ecology Progress Series. See Hagerthey, S.E., Defew, E.C., & Paterson, D.M. (2002). Influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes. *Mar Ecol Prog Ser* **245**: 47-59.

Chapter 6

Chapter 6: Does enhanced carbon dioxide concentration influence carbon utilisation and species composition of estuarine microphytobenthos?

Abstract

The assessment of the effects of anthropogenic CO_2 increase on estuarine sediment microalgae has received relatively little attention compared to the intense research on CO_2 related responses of the terrestrial biosphere. As yet there is little evidence of CO_2 limiting photosynthesis in benthic estuarine diatom biofilms (Underwood & Kromkamp, 1999). This chapter describes the influence of increased atmospheric CO_2 concentration on the biomass, production of extracellular polymeric substances, relative electron transport rate (rETR) and assemblage composition of natural estuarine benthic diatom assemblages. The hypotheses tested were: 1) an enhancement of CO_2 concentration would increase primary productivity and hence increase the biomass and EPS production of the benthic diatoms, and 2) competitive interactions would alter the assemblage structure from that initially sampled due to a potential change in the limiting resource and density dependant factors. Natural cores were divided between tidal tanks maintained in 2 chambers. The CO_2 concentration of one chamber was maintained at a mean of 365 ppm (ambient CO_2 conditions) and the other chamber was maintained at a mean of 560 ppm (enhanced CO_2 conditions). Biomass, light curve parameters (the maximum rates of ETR, maximum light utilisation coefficients and light saturation parameters) and species composition did not differ significantly between experimental assemblages grown at ambient (365 ppm) and enhanced (560 ppm) CO_2 concentrations. I hypothesised that natural biofilms are unlikely to become carbon limited because, unlike culture conditions, intact biofilms are not enclosed and consequently have access to carbon, both from the atmosphere and from the sediment pore water.

Chapter 6: Does enhanced carbon dioxide concentration influence carbon utilisation and species composition of estuarine microphytobenthos?

6.1 Introduction

The marine and estuarine environments differ from freshwater in both salinity and alkalinity. This may give rise to CO_2 limitation in photoautotrophs, since under these conditions the CO_2 / HCO_3^- equilibrium is shifted towards HCO_3^- formation, due to a decrease in proton concentration at a higher pH. Estuarine benthic diatoms can take CO_2 from the atmosphere, and HCO_3^- from pore water during tidal emersion and from the overlying water during immersion.

Algal photosynthesis is dependent on the uptake and assimilation of carbon dioxide (CO_2). Carbon fixed during photosynthesis is primarily assimilated by the Calvin Cycle enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which can only use CO_2 as a substrate (Reinfelder *et al.*, 2000). Multiple processes, including light, and the supply of CO_2 and other nutrients, regulate Rubisco activity (Falkowski & Raven, 1997; MacIntyre *et al.*, 1996b). Algae can overcome low CO_2 concentrations by the process of carbon-concentrating mechanisms (CCM's) (Burkhardt *et al.*, 2001; Riebesell, 2000; Kaplan and Reinhold, 1999). Some involve carbonic anhydrase, a zinc-containing metalloprotein enzyme, that catalyses the interconversion of CO_2 and bicarbonate ions (HCO_3^-) (Moroney & Somanchi, 1999)

If the atmospheric concentration of CO_2 were to increase, as predicted by the IPCC (2001), the probability of a CO_2 molecule binding with the Rubisco enzyme would also increase. This may reduce photorespiration (Davison, 1991), and net primary productivity and carbon input to the ecosystem would be enhanced. However, this will also depend upon other limitations to primary productivity, such as nutrient availability, irradiance, and factors such as pollutants and toxins (Harvey, 2000; Körner & Bazzaz, 1996). In addition, an increase in a limiting nutrient may cause a shift in the composition and diversity of the microphytobenthic assemblage. If CO_2 was previously limiting, then increased atmospheric CO_2 concentrations may lead to primary production being limited by a different resource. If that were to occur, then the best competitors

(those with lowest half saturation coefficients; i.e. the substrate concentration at which the growth rate is at half it maximal rate) for the new limiting resource would initially be selected for, and out-compete other species less well adapted to obtaining that resource (Tilman, 1993). The issue of increased atmospheric CO_2 is therefore highly important, as it may enhance microphytobenthic primary productivity and also alter the species most dominant within the benthic algal assemblage.

The addition of bicarbonate to stationary phase cultures of *Cylindrotheca closterium* in non-light limiting conditions has shown that these cultures had become carbon limited (Defew *et al.*, submitted). The rate of carbon uptake was enhanced by bicarbonate addition and then reduced by the use of both extra- and intracellular carbonic anhydrase enzyme inhibitors. The effects of the inhibitors were fed back through the electron transport chain, resulting in down regulation of electron transport through reduced light harvesting (α^{ETR}) and reduced maximum electron transport rate (ETR_{max}) (Defew *et al.*, submitted). As yet there is little evidence of CO_2 limiting photosynthesis in benthic estuarine diatom biofilms (Underwood & Kromkamp, 1999), and the assessment of the effects of anthropogenic CO_2 increases on estuarine sediment microalgae has received relatively little attention compared to the intense research on CO_2 related responses of the terrestrial biosphere. Kromkamp *et al.* (1998) suggested that microphytobenthos were not limited by CO_2 over a tidal emersion period, when the bulk of primary productivity occurs, since maximum photosynthetic efficiency (F_v/F_m) measured in undisturbed sediment cores did not decrease between the beginning and late in the emersion period.

The aim of this chapter was to examine the influence of increased atmospheric CO_2 concentration on natural estuarine benthic diatom assemblages. Measurements of algal biomass, extracellular polymeric substances, relative electron transport rate (rETR) and assemblage species composition were made. The hypotheses tested were that:

- An enhancement of CO_2 concentration would increase primary productivity and hence increase the biomass and EPS production of the benthic diatoms.

- Competitive interactions would alter the assemblage structure from that initially sampled due to density dependent factors and a potential change in the limiting resource.

6.2 Methods

In May, October and November 2001, natural sediment cores (surface area 21cm^2; depth 7.6 cm) were collected from the upper intertidal area of the Eden Estuary (56°22'N, 2°50'W). For each run of the experiment (hereafter a replicate), 3 cores were used for initial measurements, and 20 cores were distributed equally between 4 incubation tanks. Enclosed clear perspex chambers (Chamber A and Chamber B both 1m x 1m x 1m) were used to house the tidal tanks. Two tanks were placed into Chamber A and two into Chamber B. For each replicate experiment, the carbon dioxide concentration of one chamber was maintained at a mean of 365 ppm (ambient CO_2 conditions) and the other chamber was maintained at a mean of 560 ppm (enhanced CO_2 conditions) (Table 6.1). A natural tidal regime was simulated (using filtered coastal water of 22 salinity). Every four days, 50% of the water from each tank was replaced in order to replenish nutrients and replace loss due to evaporation. The chambers were located inside a greenhouse under an ambient light regime, whilst air temperature was controlled between 5°C - 15°C with a 6 h sinusoidal cycle.

Syringe cores were used to collect initial measurements of chlorophyll *a* concentration, and total and colloidal carbohydrate concentrations. EPS measurements were not made for replicates 3 and 4 due to sample loss. Assemblage composition was determined from lens tissue samples. Initial measurements were taken from 3 individual cores, 1 d after collecting the sediment. After 7 d (replicates 1 and 2) and 14 d (replicates 3 and 4), these same parameters were measured on all experimental cores. Three replicate P-E curves (1 minute intervals after each incremental increase in light level) were also measured for each experimental treatment on day 1 and upon termination of the experiment. Relative electron transport rate (rETR), maximum theoretical electron transport rate ($rETR_{max}$), maximum light utilisation coefficient (α^{rETR}), and light saturation parameters (E_K) were measured. On intact biofilms, fluorescence measurements were made using an FMS2 fluorometer

Table 6.1 Details of the experimental set-up and experiment duration.

Date and duration of experiment	Replicate	Chamber A	Chamber B
May 2001 (1 week)	1	Ambient CO_2 [365ppm]	Enhanced CO_2 [560ppm]
May 2001 (1 week)	2	Enhanced CO_2 [560ppm]	Ambient CO_2 [365ppm]
October 2001 (2 weeks)	3	Ambient CO_2 [365ppm]	Enhanced CO_2 [560ppm]
November 2001 (2 weeks)	4	Enhanced CO_2 [560ppm]	Ambient CO_2 [365ppm]

Table 6.2 Total and colloidal carbohydrate concentrations ($\mu g\ g^{-1}$) per sediment sample and per unit biomass. Concentrations for initial assemblages, ambient (365 ppm CO_2) and enhanced (560 ppm CO_2) assemblages of replicates 1 and 2.

		Total carbohydrate ($\mu g\ g^{-1}$)	Total Carbohydrate per unit biomass ($\mu g\ g^{-1}$)	Colloidal carbohydrate ($\mu g\ g^{-1}$)	Colloidal Carbohydrate per unit biomass ($\mu g\ g^{-1}$)
Replicate 1	Initial Assemblage	6.8 ± 0.7	0.08 ± 0.01	2.8 ± 0.1	0.03 ± 0.004
	Ambient Assemblage	8.3 ± 0.8	0.27 ± 0.19	2.6 ± 0.07	0.09 ± 0.06
	Enhanced Assemblage	10.1 ± 0.5	0.36 ± 0.18	2.7 ± 0.1	0.10 ± 0.05
Replicate 2	Initial Assemblage	7.9 ± 1.2	0.07 ± 0.01	2.7 ± 0.1	0.02 ± 0.002
	Ambient Assemblage	9.7 ± 0.4	0.05 ± 0.01	2.6 ± 0.05	0.01 ± 0.002
	Enhanced Assemblage	10.0 ± 0.7	0.05 ± 0.01	2.7 ± 0.1	0.01 ± 0.001

(Hansatech™) at the time of low tide. See Chapter 2 for methodologies of the individual techniques.

6.2.1 Statistics

One-way analysis of variance (ANOVA) and post-hoc Tukey tests were used to determine if measured parameters were significantly different between treatments (Zar, 1999). Assemblage analysis was carried out using Reciprocal Averaging (RA) Ordination and the SIMI similarity index (Medlin, 1983). Further details can be obtained from Chapter 2, section 2.9.

6.3 Results

6.3.1 Photophysiological parameters

Differences in light response curves between the initial and experimental assemblages were compared (Fig. 6.1 and Fig. 6.2). Despite the ambient assemblage of replicate 2 having a lower ETR than the initial or enhanced assemblage (Fig. 6.1B), the maximum rates of ETR ($rETR_{max}$; Fig. 6.2A), maximum light utilisation coefficients (α^{rETR}; Fig. 6.2B) and the light saturation parameters (E_K; Fig. 6.2C) did not differ significantly between the initial assemblage or between experimental assemblages for replicates 1 and 2. In replicate 3, $rETR_{max}$ of the initial assemblage was significantly higher than $rETR_{max}$ of the ambient and enhanced assemblages (Figs. 6.1C and 6.2A; $F_{2,6} = 8.79$; $p < 0.05$). The same pattern was also observed in replicate 4, (Figs. 6.1D and 6.2A), but again the variation was not significant. In replicates 3 and 4, E_K of the initial assemblage was significantly higher than E_K of both the experimental assemblages (Fig. 6.2C; $F_{2,6} = 12.31$; $p = 0.008$ replicate 3 and $F_{2,6} = 8.72$; $p = 0.017$ replicate 4). Light curve parameters did not differ between ambient and enhanced assemblages of replicates 3 and 4 (Fig. 6.2). None of the replicates showed a significant difference in any light curve parameter as a result of enhanced CO_2 level.

6.3.2 Sediment analyses

Chlorophyll *a* did not differ significantly between the initial and experimental sediments (Fig. 6.3). In replicate 1, there was no change in chlorophyll *a* over the 7 d of the experiment, but chlorophyll *a* increased over 7 d

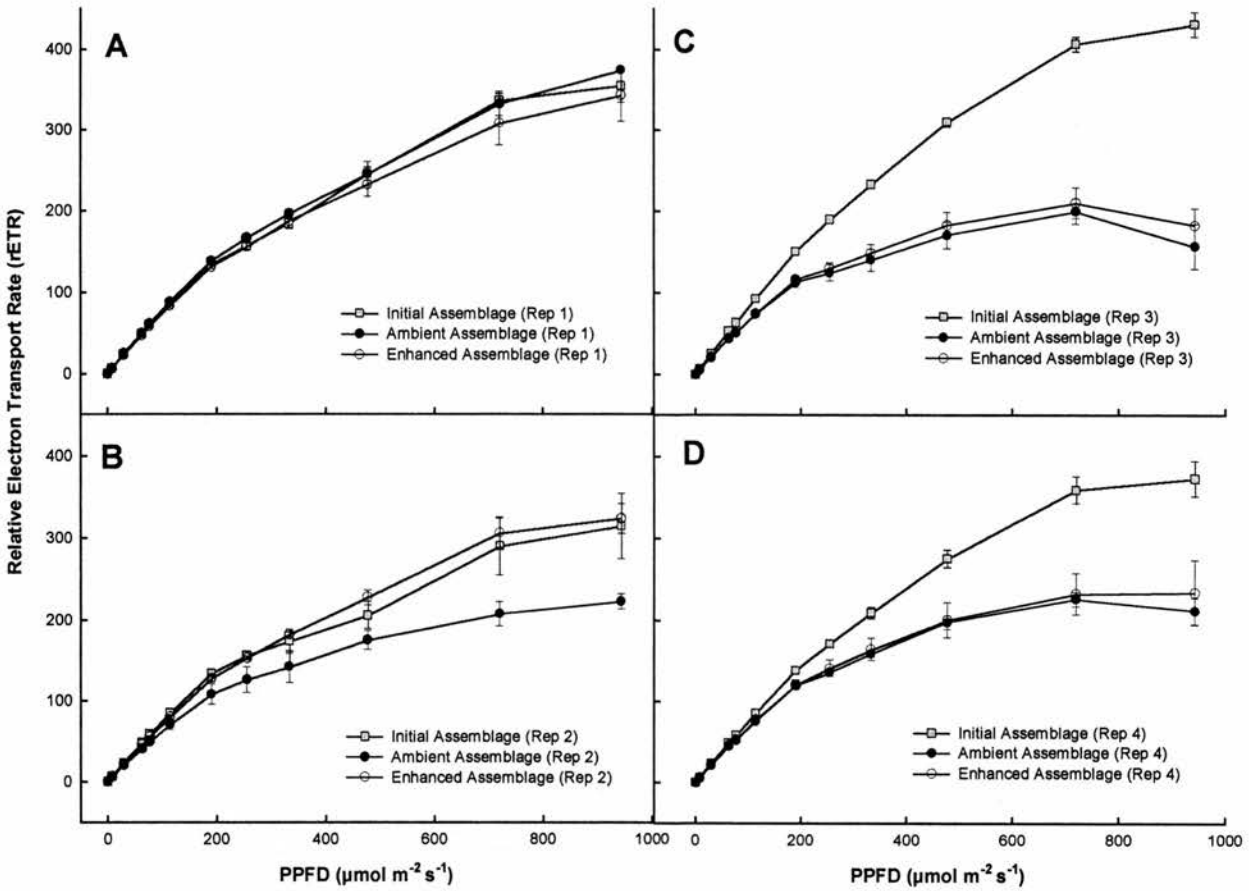


Fig. 6.1 Light response curves for initial, ambient (365 ppm) and enhanced (560 ppm) assemblages: (A) Experimental Replicate 1; (B) Experimental Replicate 2; (C) Experimental Replicate 3; (D) Experimental Replicate 4. (Mean ± SE; n=3).

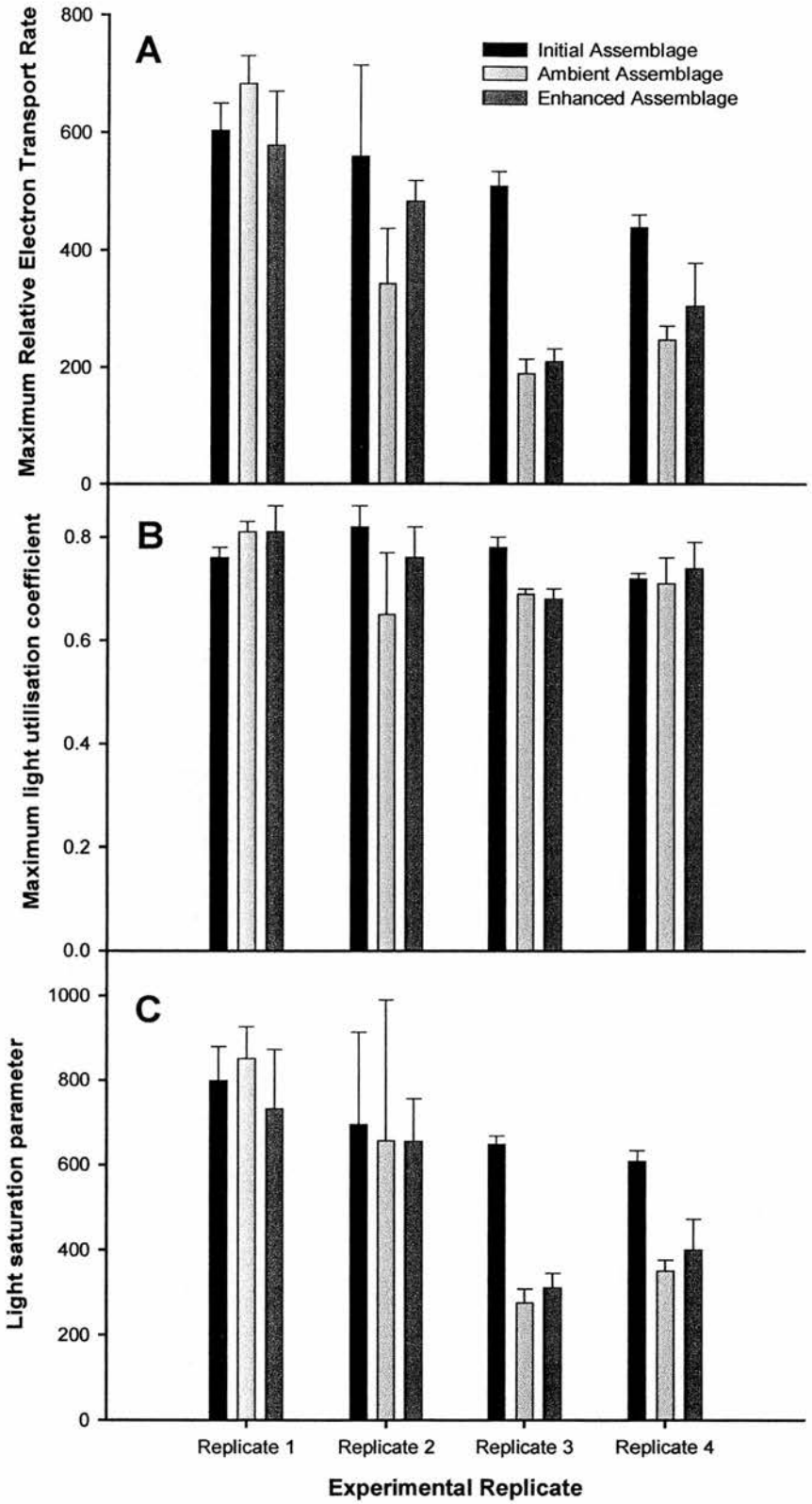


Fig. 6.2 (A) Maximum relative electron transport rates ($rETR_{max}$), (B) maximum light utilisation coefficients (α^{rETR}) and (C) the light saturation parameters (E_K) for initial, ambient (365 ppm) and enhanced (560 ppm) assemblages of each experimental replicate (Mean ± SE; n=3).

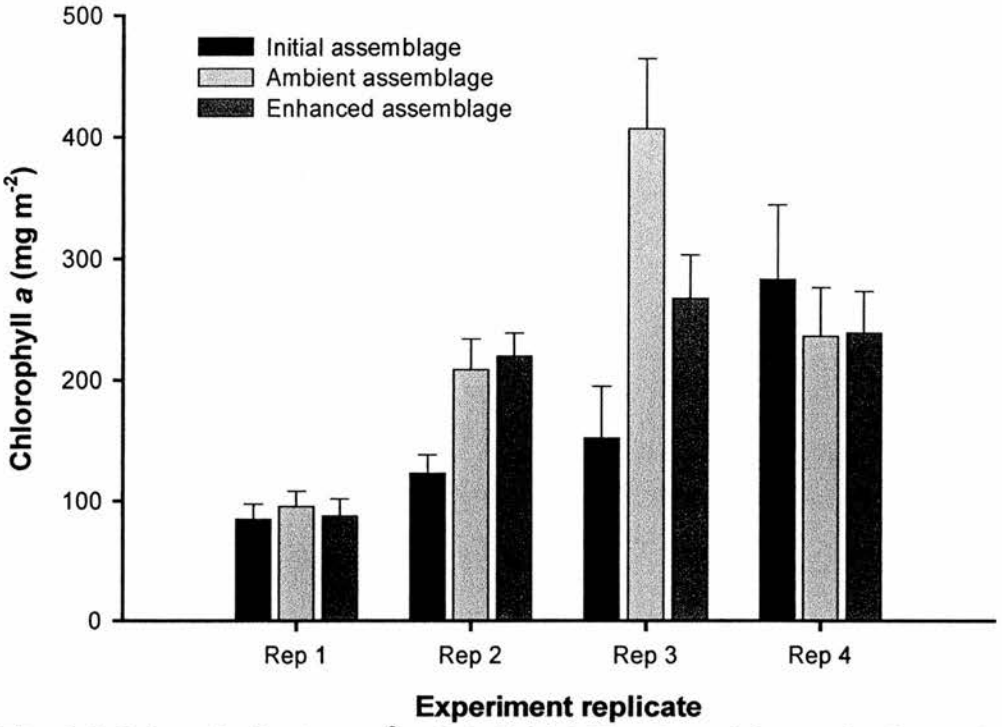


Fig. 6.3 Chlorophyll *a* (mg m^{-2}) of the initial (day 1), ambient and enhanced sediments after 7 d (replicates 1 & 2) and 14 d (replicates 3 & 4). (Mean ± SE; n=3 for initial sediments and n=5 for experimental sediments).

Fig. 6.4 (see over page) Characterisation of diatom assemblages. (A) Replicates 1 and 2 (7 d experimental duration). (B) Replicates 3 and 4 (14 d experimental duration). Data is pooled from 3 replicate samples in each treatment. Reciprocal averaging and selected species scores for diatom assemblages from the initial and experimental sediments. R1-4 = replicate 1-4; I = initial A = ambient [CO_2]; E = enhanced [CO_2].

achdel=*Achnanthes delicatula*, acheng=*A. engelbrechtii* achlan=*A. lanceolata*, ampcof=*Amphora coffeaeformis*, ampcom=*A. commutata*, ampcop=*A. copulata*, ampcova=*A. coffeaeformis var. acutiscula*, ampmar=*A. marina*, ampspa=*Amphora* species a, ampspb=*Amphora* species b, cocdis=*Cocconeis disculus*, cocpel= *C. peltoides*, cocpla=*C. placentula*, cycstr=*Cyclotella striata*, delmin=*Delpheneis minutissima*, delsur=*D. surirella*, diavul=*Diatoma vulgare*, entpal=*Entomoneis paludosa*, gyrfas=*Gyrosigma fasciola*, gyrwan=*G. wansbeckii*, navdig=*Navicula digitoradiata*, navfla=*N. flanatica*, navgre=*N. gregaria*, navphy=*N. phyllepta*, navrhy=*N. rhynchocephala*, navsal=*N. salinarum*, navsta=*N. stankovicii*, navvul=*N. vulpina*, navsp5= Unknown *Navicula* species 5, navsp9=Unknown *Navicula* species 9, navs10=Unknown *Navicula* species 10, nitbil=*Nitzschia bilobata*, nitdis=*N. dissipata*, nitepi=*N. epithemioides*, nitfru=*N. frustulum*, nitrec=*N. recta*, nitsp3=*Nitzschia* species 3, nitsp4=*Nitzschia* species 4, opemar=*Opephora marina*, opesch=*O. schwartzii*, opespa=*Opephora* species 1, planeo=*Plagiotropis neovitrea*, pleang=*Pleurosigma angulatum*, psapan=*Psammodictyon pandiformis*, psapun=*Psammothidium punctulatum*, rhanit=*Rhaphoneis nitida*, rhocur=*Rhoicosphenia curvata*, staamp=*Stauroneis amphioxys*, surgem=*Surirella gemma*, surova=*S. ovalis*, tryapi=*Tryblionella apiculata*, trypun=*T. punctata*, tryvis=*T. visurgis*, unksp0=unknown species 0, unksp1=unknown species 1.

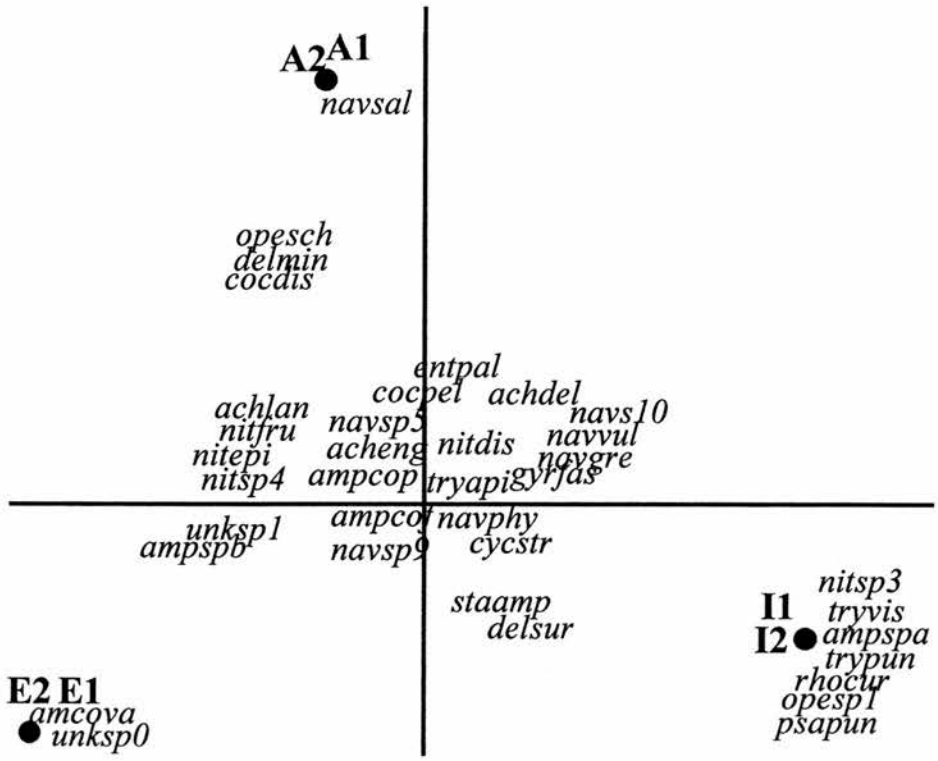


Fig. 6.4A

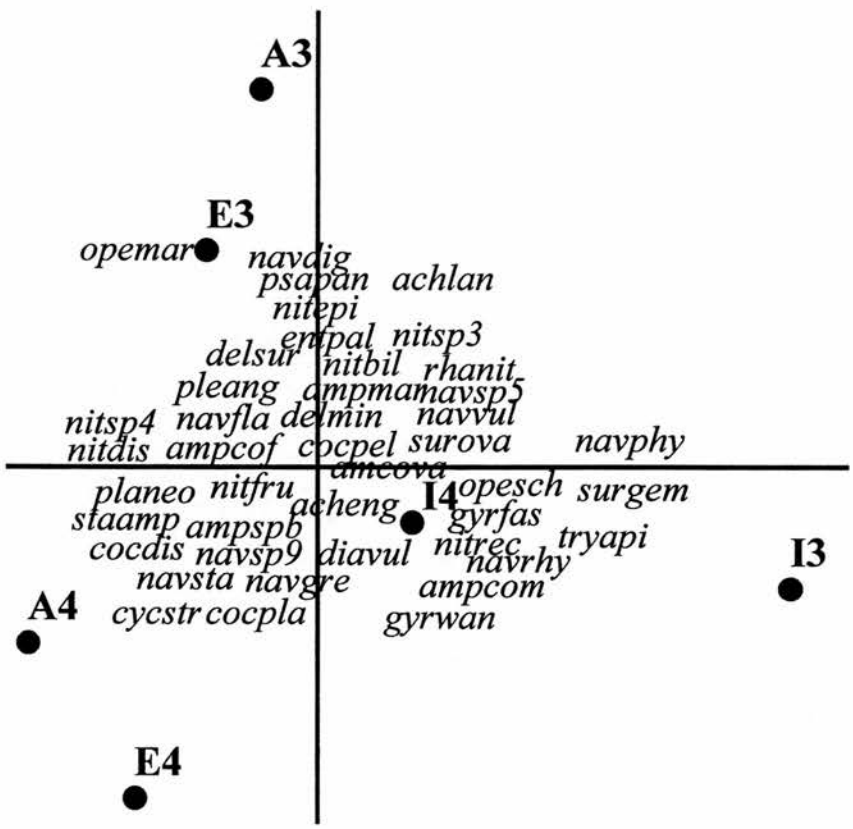


Fig. 6.4B

Table 6.3 SIMI similarity indices comparing the initial and experimental diatom assemblages. An index of 0 indicates samples had no taxa in common, and an index of 1 indicates samples had the same taxa and relative abundance.

		Initial Assemblage	Ambient Assemblage
Ambient Assemblage	Replicate 1	0.89	X
	Replicate 2	0.94	X
	Replicate 3	0.74	X
	Replicate 4	0.85	X
Enhanced Assemblage	Replicate 1	0.69	0.92
	Replicate 2	0.98	0.94
	Replicate 3	0.76	0.87
	Replicate 4	0.78	0.96

Table 6.4 Species richness, evenness, and diversity values for the initial and experimental diatom assemblages of each experimental replicate (after 7 d for replicates 1 & 2 and after 14 d for replicates 3 & 4.) Values are from pooled data (n=3).

	Species richness	Species evenness	Species diversity (H')
Replicate 1			
Initial assemblage	57	0.65	2.61
Ambient assemblage	43	0.72	2.69
Enhanced assemblage	50	0.72	2.83
Replicate 2			
Initial assemblage	49	0.63	2.45
Ambient assemblage	38	0.64	2.34
Enhanced assemblage	53	0.66	2.63
Replicate 3			
Initial assemblage	60	0.76	3.09
Ambient assemblage	60	0.72	2.95
Enhanced assemblage	63	0.73	3.04
Replicate 4			
Initial assemblage	59	0.71	2.89
Ambient assemblage	64	0.69	2.88
Enhanced assemblage	63	0.70	2.89

in replicate 2. In replicate 3, there was a large increase in chlorophyll *a* over 14 d in the ambient assemblage, but not in the enhanced assemblage. In replicate 4, there was no change in chlorophyll *a* over the 14 d of the incubation. Thus enhanced CO_2 did not significantly alter the biomass of the diatomaceous biofilms.

Total and colloidal carbohydrate concentrations ($\mu g\ g^{-1}$ and per unit biomass) did not differ significantly between experimental treatments or between replicates 1 and 2 (Table 6.2). In replicate 1, total carbohydrate concentrations ($\mu g\ g^{-1}$) of the enhanced assemblage increased above the initial values ($F_{2,20} = 3.41$; $p = 0.053$).

6.3.3 Assemblage composition

Reciprocal Averaging Analysis (Fig. 6.4 A, B) and high SIMI values (ranging from 0.69 – 0.98; Table 6.3) indicated that species composition of all assemblages had changed slightly between the initial and experimental assemblages, but had barely changed between the two CO_2 concentrations. Replicates 1 and 2 were dominated by *Achnanthes engelbrechtii* and *Navicula gregaria*, and the ambient assemblage of replicate 2 also had a high percentage of *Navicula* species 5. Experimental replicates 3 and 4 were dominated by *A. engelbrechtii* and *Pleurosigma angulatum*, whilst the initial assemblage of these replicates was dominated by *Tryblionella apiculata*. In all experimental replicates, species richness, evenness and diversity of assemblages were similar between the initial and experimental assemblages (Table 6.4).

6.4 Discussion

6.4.1 Effects of enhanced CO_2 concentration

Net primary productivity, biomass accumulation, and carbon input to ecosystems are usually enhanced by elevated CO_2 (although this will be dependent on the extent and type of other limitations) (Reich *et al.*, 2001; Riebesell *et al.*, 1993). Data from the intact sediment cores indicated that an enhancement of photosynthetic rate (measured as electron transport rate using chlorophyll fluorescence) did not occur in natural estuarine benthic diatom assemblages grown under enhanced CO_2 concentration, indicating that these assemblages were not carbon limited. Higher relative electron transport rates of

the initial assemblages, compared to experimental assemblages, of replicates 3 and 4 may have been due to a higher proportion of *T. apiculata* in the initial diatom assemblages, whilst the experimental assemblages contained higher proportions of *A. engelbrechtii* and *P.angulatum*. Likewise, the slightly lower ETR of ambient assemblage replicate 2, compared to the enhanced and initial assemblages could have been due to a greater proportion of *Navicula* species 5. In no case using natural diatom assemblages did enhanced CO_2 increase the chlorophyll *a* concentration by the end of the incubation, indicating that elevated atmospheric CO_2 concentrations did not stimulate growth. The amount of total carbohydrate was significantly higher at enhanced CO_2 concentrations in replicate 1, but this was not due to an enhancement of growth or photosynthesis.

Since Rubisco affinities for CO_2 differ among taxa (Falkowski & Raven, 1997), rising CO_2 concentration, shifts in carbon supply rates or changes in the ratio of carbon to other essential resources (i.e. C:N or C:P) could alter species composition of diatom assemblages. Algal species with high carbon demand (and higher half saturation constants for carbon dioxide) and a low surface area to volume ratio (which potentially have lower rates of nutrient uptake), as well as species lacking a carbon concentrating mechanism would be expected to benefit. A shift in assemblage structure towards larger sized cells may occur in a bottom-up (resource dependent) controlled assemblage (Wolf-Gladrow *et al.*, 1999). This was not observed in this experiment, in terms of the average size of the species present, although actual cell size measurements were not taken. Whilst the dominant species changed between some assemblages (see 6.3.3), high SIMI values indicated that the assemblages were generally very similar in the species present and their proportional representation. Species richness was slightly reduced under ambient experimental conditions in replicates 1 and 2, although this pattern was not found in replicates 3 and 4. Species evenness and diversity did not differ between treatments in any replicates.

6.4.2 Mechanisms of preventing carbon limitation

The ability of epipelic diatoms to vertically migrate in the sediment has been hypothesised as a possible mechanism for avoiding CO_2 limitation (Kromkamp *et al.*, 1998), or it may be that in estuarine benthic diatoms, Rubisco activity is already saturated at ambient carbon dioxide levels, and so when light

is not limiting, Rubisco is itself the limiting step. Another possibility is that, similar to the cultures of *C. closterium* and *N. salinarum* (Defew *et al.*, submitted), other estuarine benthic diatom species use carbonic anhydrase in order to utilise bicarbonate as an exogenous inorganic carbon source for photosynthesis, which would tend to diminish or abolish potential effects of CO_2 limitation (Wolf-Gladrow *et al.*, 1999; Dixon & Merrett, 1988). Ecological factors such as photoperiod and pH are likely to be important in determining carbonic anhydrase activities in marine unicellular protists (Hobson *et al.*, 2001). Investigations of short-term photosynthetic responses to changes in inorganic carbon supply for organisms grown under 'natural' conditions suggest that most of the marine phytoplankton is not carbon limited (Falkowski & Raven, 1997), and the results of this first study suggest that this could also be the case for natural estuarine epipelic diatoms. In general, aquatic photoautotrophs that can actively transport inorganic carbon suppress the oxygenase and stimulate the carboxylase activities of Rubisco, so that photosynthesis is saturated with respect to inorganic carbon at the levels found in seawater (Falkowski & Raven, 1997).

6.5 Conclusions

Whilst bicarbonate addition may relieve carbon limitation in stationary phase diatom cultures (Defew *et al.*, submitted), carbon limitation was not observed for intact biofilms *ex situ*. Carbon limitation in cultures probably results due to reduced gas exchange between the culture medium and the atmosphere, due to enclosure to maintain axenic cultures. Conversely, intact biofilms are not enclosed and so have access to carbon, both from the atmosphere and from the sediment pore water, presumably exploiting carbonic anhydrase for utilisation of bicarbonate. As a result, biofilms are unlikely to become carbon limited, and therefore the functioning (i.e. the growth, EPS production and photosynthesis) and structure are unlikely to change. An exception may be when the biofilm is thick enough to reduce diffusion of carbon dioxide to deeper layers, although in such cases light limitation may be more important. In contrast to terrestrial systems, increases in atmospheric CO_2 levels are unlikely to increase rates of production in estuarine microphytobenthic biofilms. This may be an important finding in terms of predicting the effects of global change scenarios on coastal regions.

Chapter 7

Chapter 7: Diatom Migration affects photophysiological parameters measured by chlorophyll fluorescence

Abstract

Estuarine diatoms have developed strategies to maintain high rates of primary production, whilst protecting against potentially damaging light levels. These strategies include non-photochemical quenching (NPQ) mechanisms and the ability to migrate within the photic zone. In studies of estuarine microphytobenthic assemblages, the rapid and non-intrusive technique of Pulse Amplitude Modulated (PAM) fluorometry has commonly been used to measure a variety of parameters including NPQ, F_q'/F_m', F_v/F_m and relative Electron Transport Rate (rETR). Using two benthic diatom assemblages from the Eden Estuary, Scotland, and one assemblage from Arlesford Creek, Essex, dark and light adaptation prior to light response curves with a dark recovery phase were used to investigate the effects of vertical migration on fluorescence measurements. During the 15 min dark-adaptation period, diatom cells had migrated away from the sediment surface, decreasing the minimum (F_o) and maximum (F_m) yields and altering the relationship between biomass and F_o. Migration during the recovery phase also affected these fluorescence yields and hence the rate of NPQ reversal. Downward vertical migration during light response curves was observed as light levels increased. This resulted in the over-estimation of efficiency, and hence rETR, by as much as 100% compared to biofilms in which migration was inhibited (with the use of phytotrays). Using assemblages from the Eden Estuary, phytotrays were shown to be a useful tool for determining the over-estimation of rETR. However, the assemblage from Arlesford Creek, Essex, did not behave as expected. Photosynthesis of a non-migratory assemblage did not saturate, and instead illustrated an inflexion point. This highlighted our incomplete understanding of the fluorescence characteristics of estuarine benthic diatoms, and underlines the need for further investigation into the contributions of different mechanisms of down-regulation (i.e. behavioural and/or physiological).

Chapter 7: Diatom migration affects photophysiological parameters measured by chlorophyll fluorescence

7.1 Introduction

Pulse Amplitude Modulated (PAM) fluorometry is a rapid and non-intrusive technique (Schreiber *et al.*, 1986). On microphytobenthic biofilms, this method has been used to measure: photosynthetic efficiency (Perkins *et al.*, 2002, 2001; Kromkamp *et al.*, 1998; Hofstraat *et al.*, 1994); the effects of stress and nutrient limitation (Underwood *et al.*, 1999; Geider *et al.*, 1993); a proxy for biomass (Serôdio *et al.*, 2001; 1997; Honeywill *et al.*, in press); primary production from photosynthesis-irradiance curves (P-E curves) (Perkins *et al.*, 2001; 2002; Barranguet & Kromkamp, 2000; Miles & Sundbäck, 2000; Serôdio & Catarino, 2000; Barranguet *et al.*, 1998; Hartig *et al.*, 1998); and photochemical and non-photochemical quenching (qP and NPQ) (White & Critchley, 1999).

Diatoms are able to maintain high rates of primary production, whilst protecting themselves against potentially damaging light levels using both behavioural (i.e. migration down into the sediment) and physiological (i.e. NPQ) strategies (Perkins *et al.*, 2002; 2001; Underwood & Kromkamp, 1999). Vertical migration down into the sediment has been observed during both the dark-adaptation period and light response curve. As a result, this may have affected the photophysiological parameters measured by PAM fluorometry that are reported in chapters 3, 4 and 6.

The probability of chlorophyll *a* excitation energy being dissipated as fluorescence can be affected by whether the first 'stable' electron acceptor in PSII (the primary quinone acceptor or Q_A) is oxidised or reduced, the type and extent of NPQ, and vertical migration. When Q_A is oxidised, it can accept electrons from P_{680}, and if kept oxidised will continue to quench fluorescence, resulting in a reduction of the *F'* signal (fluorescence yield in the light-adapted state prior to the saturating beam). However, if the reoxidation of Q_A is limited (i.e. it is not fully oxidised) fluorescence yield will increase, resulting in an increase of the *F'* fluorescence yield. Activation of NPQ will reduce the fluorescence yield of *F'* and F_m' (maximum fluorescence yield in the light-

adapted state during light saturation), whilst NPQ reversal will increase the fluorescence yield of F' and F_m'. Downward migration during the dark-adaptation period (Defew, pers. obs) and/or in response to high light (Perkins *et al.*, 2001; Underwood & Kromkamp, 1999) will affect the measurement of F_v/F_m and F_o[15]. Downward migration can also reduce fluorescence yields at F' and F_m' which will falsely increase measurements of F_q'/F_m' thereby affecting measurements of relative Electron Transport Rate (rETR) (Perkins *et al.*, 2002; 2001; Kromkamp *et al.*, 1998), and may also alter the balance of the surface and sub-surface signal.

The extent to which different diatom species use migration and/or NPQ has not been extensively studied in estuarine benthic species. In order to study this, the photophysiology of both light- and dark-adapted sediment biofilms, were compared with biofilms of identical species composition whose cells were unable to migrate. Non-migratory biofilms were established by creating thin biofilms on the surface of small polypropylene membrane rafts called 'phytotrays'. (The 0.3 μm pores of these rafts allowed for the exchange of nutrients between the cells and the filtered seawater upon which the rafts float). The effects of vertical migration on fluorescence measurements of estuarine benthic diatom photophysiology (i.e. NPQ, F_q'/F_m', F_v/F_m and rETR) were investigated.

The aims of this experiment were to:

- Establish the use of phytotrays (small polypropylene membrane rafts) in the preparation of non-migratory biofilms.
- Investigate the extent to which rETR is over-estimated from fluorescence measurements due to vertical migration.

7.2 Methods

Three assemblages that differed in their species composition were used to assess photophysiology. Assemblages 1 and 2 (A1 and A2) were collected from the Eden Estuary in December 2001. Each Eden assemblage was used for migratory and non-migratory treatments. Assemblage 3 (A3) was collected from Arlesford Creek, Essex (51°50.2' N, 0°59.5'E) in March 2001, and was used only for the non-migratory treatment.

The non-migrating biofilm of A1 was assessed using cells that had been collected by lens tissue, removed and concentrated by centrifugation (1500 rpm for 15 min) and placed on the phytotray in a small volume of filtered seawater. For the non-migratory biofilm of A2, moistened lens tissues were placed directly onto the phytotray, and for A3, diatom cells were shaken from lens tissues and left to settle out into the bottom of a test-tube for 1 h before being placed on the phytotray in a small volume of filtered seawater.

This change in methodology occurred on consideration that the centrifugation process caused disruption to components of the photosynthetic apparatus, such as the thylakoid membranes, and due to differences in the collection and transportation of cells. The patterns in the data described here were replicated (n=3) within each treatment, giving confidence that the patterns observed were true treatment effects.

7.2.1 Fluorescence measurements

To assess the photophysiology of the microphytobenthos, chlorophyll *a* fluorescence measurements were made on previously dark- and light- adapted samples (hereon after referred to as DA and LA samples respectively). Dark-adapted measurements were made on biofilms that had been dark-adapted for 15 min (Honeywill *et al.*, 2002; Perkins *et al.*, 2001; Serôdio *et al.*, 2001; Barranguet & Kromkamp, 2000; Serôdio *et al.*, 1997;), whilst light-adapted samples were exposed to ambient irradiance levels of 300 μmol PPFD m^{-2} s^{-1} for 15 min prior to fluorescence measurements.

'Rapid' light curves were performed with incremental increases in light level (10 to 1315 μmol PPFD m^{-2} s^{-1}) after 30 s at each level. Rapid light curves were used in an attempt to reduce the potential effect of migration as much as possible. Light-adapted fluorescence measurements of the photochemical capacity at PSII (F_q'/F_m') were made, and rETR, a proxy measurement of primary productivity, was calculated as the product of F_q'/F_m' and PPFD. Values of the maximum theoretical electron transport rate ($rETR_{max}$), the maximum light utilisation coefficient (α^{rETR}), and the light saturation parameter (E_K) were calculated from P-E curves. In order to observe the relaxation of the PSII reaction centres after completion of a light curve, a 15 min recovery phase was carried out in darkness, where PSII efficiency (F_v/F_m) of the biofilm was

monitored every minute. The photochemical quenching coefficient, qP and the extent to which photochemistry was down-regulated by NPQ were also quantified. Each treatment had 3 replicates. (See Chapter 2, section 7 for equations relating to the calculation of the photosynthetic parameters).

7.2.2 Statistics

Data are presented as mean values ± standard errors. Data were checked for normal distribution. Non-normal data were transformed by either log or arcsine functions. Significant differences in fluorescence parameter values, and between migratory and non-migratory treatments, assemblage composition, and light- and dark-adapted treatments, were determined using the t-test, 1-way ANOVA, and post-hoc Tukey tests (Zar, 1999; Fowler *et al.*, 1998).

7.3 Results

7.3.1 Species composition

Gross assemblage composition was estimated from cell counts. Diatom assemblage A1 was dominated by *Navicula gregaria* (32%), *Gyrosigma fasciola* (34%) and small Naviculoids (20%); diatom assemblage A2 was dominated by *G. fasciola* (55%) and *Pleurosigma angulatum* (27%); and diatom assemblage A3 was dominated by *N. gregaria* (27%) and small Naviculoids (45%).

7.3.2 Photosynthesis-Irradiance curves from the Eden Estuary Assemblages

Relative electron transport rates (rETR) of migratory biofilms were higher than those of non-migratory biofilms (Figs. 7.1A & 7.1B and Table 7.1). Maximum theoretical electron transport rates ($rETR_{max}$) of migratory biofilms were significantly higher than non-migratory biofilms ($F_{7,16} = 8.70$, $p = <0.001$), whilst the light saturation coefficients (E_K) of migratory biofilms were significantly higher than the non-migratory biofilms for A2 ($F_{7,16} = 8.65$, $p = <0.001$), but not for A1. The maximum light utilisation coefficients (α) of migratory biofilms were significantly higher than the non-migratory biofilms for A1 but not for A2, and α of migratory A1 biofilms were significantly higher than migratory A2 biofilms ($F_{7,16} = 40.41$, $p = <0.001$). Photosynthetic parameters

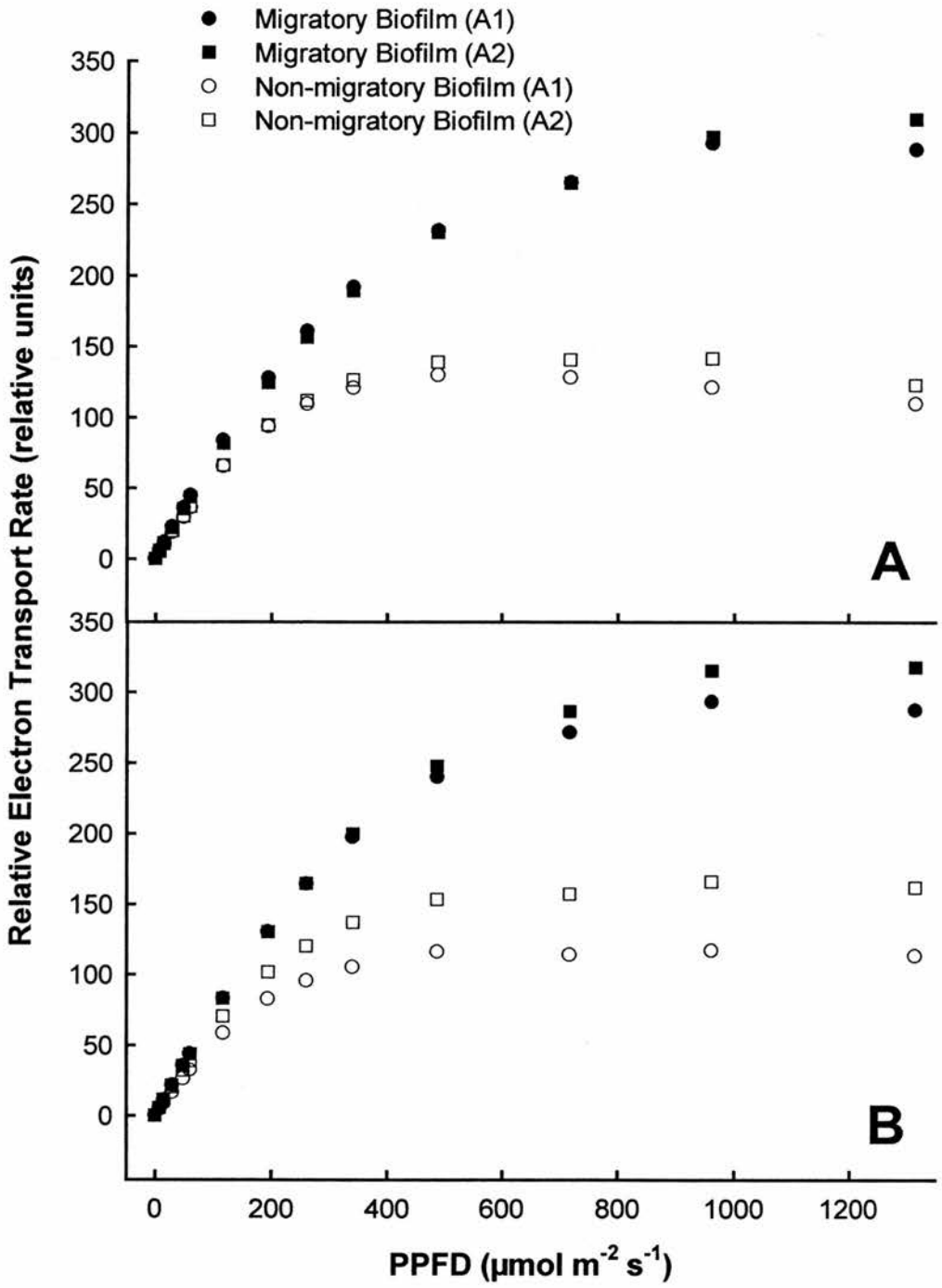


Fig. 7.1 Light response curves (mean values) obtained from previously dark-adapted (A) and previously light-adapted (B) migratory and non-migratory Eden Estuary assemblages (n = 3).

Table 7.1 Photosynthetic parameters of previously dark-adapted (DA) and previously light-adapted (LA) migratory and non-migratory Eden Estuary assemblages. rETRmax = maximum theoretical electron transport rate, α = maximum light utilisation coefficient and E_K = light saturation parameter. Values are the mean parameter estimates ± SE, and n = 3 for each experimental treatment.

	Light Treatment	$rETR_{max}$ (Relative Units)	α^{rETR} (Relative Units)	$E_K{}^{rETR}$ (µmol m-2 s-1)
Migratory Biofilm (Assemblage 1)	DA	324.3 ± 61.2	0.72 ± 0.04	446.0 ± 64.1
	LA	311.0 ± 6.4	0.73 ± 0.0	428.0 ± 7.0
Migratory Biofilm (Assemblage 2)	DA	361.3 ± 24.7	0.76 ± 0.01	474.0 ± 36.5
	LA	356.7 ± 28.6	0.74 ± 0.02	483.0 ± 23.1
Non-migratory Biofilm (Assemblage 1)	DA	122.7 ± 19.2	0.50 ± 0.0	245.3 ± 38.3
	LA	124.3 ± 4.4	0.56 ± 0.01	222.3 ± 10.0
Non-migratory Biofilm (Assemblage 2)	DA	136.3 ± 40.2	0.55 ± 0.03	243.7 ± 60.8
	LA	171.0 ± 56.7	0.61 ± 0.07	266.0 ± 62.6

($rETR_{max}$, E_K and α) did not differ significantly between previously DA and LA treatments.

7.3.3 Changes in efficiency and photochemical capacity at PSII

Initial values of F_v/F_m (previously DA cells) and F_q'/F_m' (previously LA cells) of migratory biofilms were significantly higher than non-migratory biofilms of A1, but not A2 ($F_{3,8} = 4.83$; $p = 0.033$ and $F_{3,8} = 5.62$; $p = 0.023$ for F_v/F_m and F_q'/F_m' respectively). Initial values of F_q'/F_m' from previously LA non-migratory A1 biofilms were significantly lower than the corresponding dark-adapted initial F_v/F_m values ($F_{7,16} = 6.17$; $p = 0.001$).

F_q'/F_m' decreased throughout the light curves, proportional to the increase in PPFD, for both migratory and non-migratory biofilms. The recovery of F_v/F_m showed a biphasic response, and all biofilms had a rapid initial increase in F_v/F_m within the first minute of dark-adapted recovery, followed by a second slower phase (Figs. 7.2A & 7.2B).

At the end of the recovery period, F_v/F_m values from previously DA migratory biofilms were significantly higher than the non-migratory biofilms ($F_{3,8} = 13.61$; $p = 0.002$), and whilst F_v/F_m of migratory biofilms had recovered to the original mean values, non-migratory biofilms had only recovered to between 69-78% of the original mean values. At the end of the recovery period, values of F_v/F_m from previously LA migratory biofilms and the non-migratory A1 biofilm were equal to the original mean F_q'/F_m' value, whilst F_v/F_m of the non-migratory A2 biofilm had only returned to 88% of the original mean F_q'/F_m' value. (The "original mean value" is defined here as the F_v/F_m or F_q'/F_m' value obtained at the beginning of the light curve).

7.3.4 Changes in minimum fluorescence yield. (F' and F_o)

The F' yield of previously DA migratory biofilms doubled between 0 and 340 µmol PPFD m^{-2} s^{-1}, after which yields decreased until the end of the light curve. At the end of the light curve, the mean percentage change in F', compared to the initial mean F_o[15], was greater for the migratory biofilm of A1 than A2. For non-migratory biofilms, F' from A1 increased gradually throughout the duration of the light curve, whilst F' from A2 increased between 0 and 195 µmol PPFD

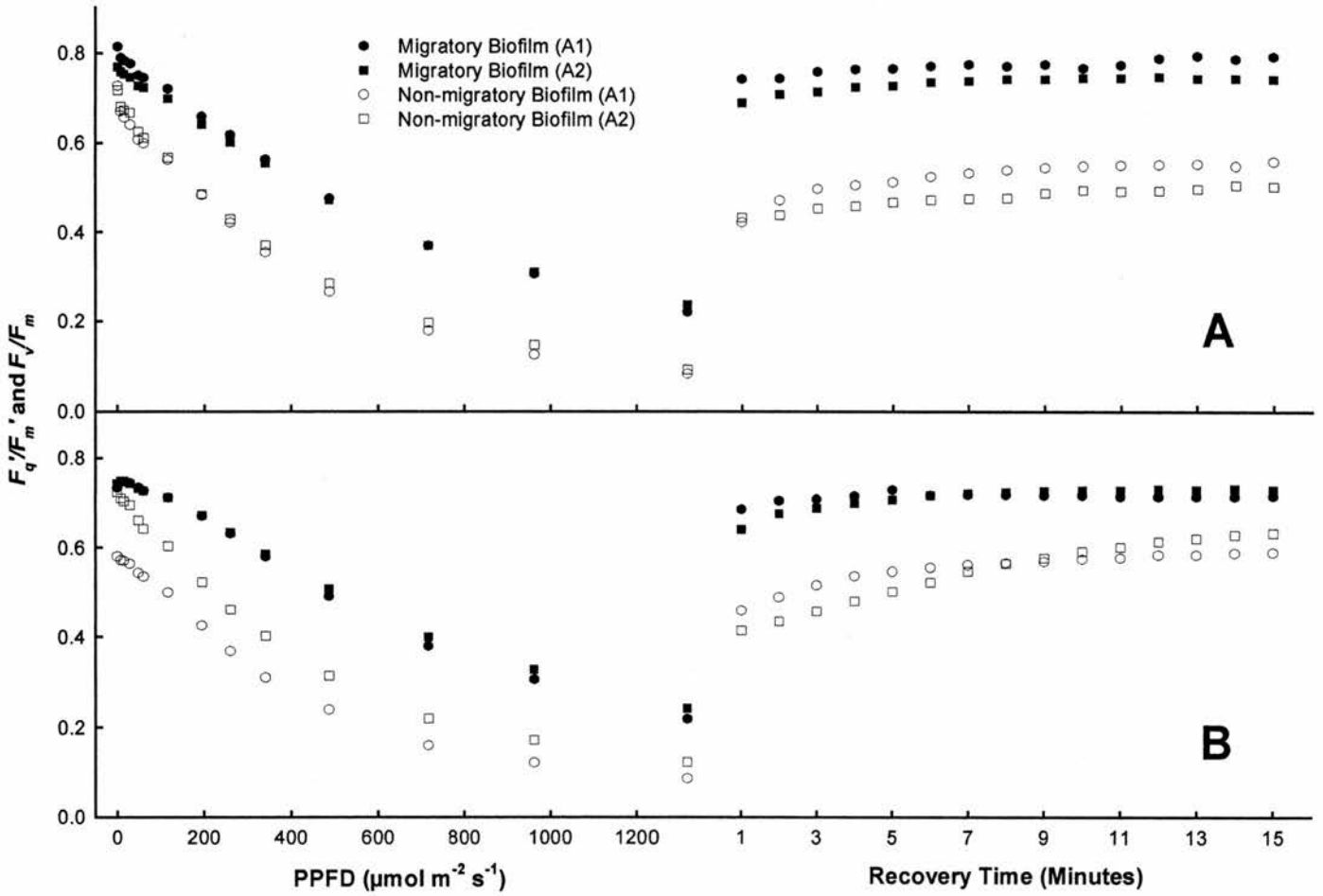


Fig 7.2 Changes in F_q'/F_m' and F_v/F_m during the light response curves and the 15 min recovery period. Previously dark-adapted (A) and light-adapted (B).

m^{-2} s^{-1} and then decreased to below the initial mean F_o^{15} value until the end of the light curve (Fig. 7.3A). F_o recovered with a biphasic response, and after 15 min recovery, values of F_o were below original mean F_o^{15} values (Table 7.2). In all biofilms, F_o decreased rapidly within the first minute of recovery to values below the initial mean F_o^{15} value. For the remaining 14 min, F_o of migratory and non-migratory A1 biofilms continued to slowly decrease. F_o of migratory A2 biofilms remained fairly constant until an increase in the final 5 min, whilst F_o of the non-migratory A2 biofilms remained constant (Fig. 7.3A).

F' of previously LA migratory biofilms decreased slightly at the beginning of the light curve before increasing between 30 and 90 µmol PPFD m^{-2} s^{-1}, after which *F'* decreased until the end of the light curve. At the end of the light curve, the mean percentage change in *F'* was significantly greater for the migratory biofilm of A1 than A2 ($t = 17.18$; $p < 0.001$; $df = 3$). *F'* of the non-migratory A1 biofilms increased between 0 and 195 µmol PPFD m^{-2} s^{-1}, decreased between 195 and 340 µmol PPFD m^{-2} s^{-1}, and then increased again for the remainder of the light curve, whilst *F'* of the non-migratory A2 biofilms increased slightly at the beginning of the light curve, and then gradually decreased throughout the duration of the light curve. F_o recovered with a biphasic response. Values decreased rapidly within the first minute to values below the original mean *F'* value, and then increased for the remainder of the recovery period (Fig. 7.3B). At the end of the recovery period, F_o values were below the original mean *F'* values (Table 7.2).

7.3.5 Changes in maximum fluorescence yield. (F_m' *and* F_m)

F_m' of previously DA migratory biofilms increased up to 120 µmol PPFD m^{-2} s^{-1}, after which F_m' decreased until the end of the light curve. F_m' decreased sigmoidally through the light curve for previously DA non-migratory biofilms. F_m for all biofilms recovered with a biphasic response. Values increased rapidly within the first minute and then continued to increase slowly, except for the migratory A1 biofilms, which had an initial increase, followed by a slower decrease for the remainder of the recovery period (Fig. 7.4A). At the end of the recovery period, F_m values from all migratory and non-migratory biofilms were below their respective original mean F_m^{15} values, although this difference was

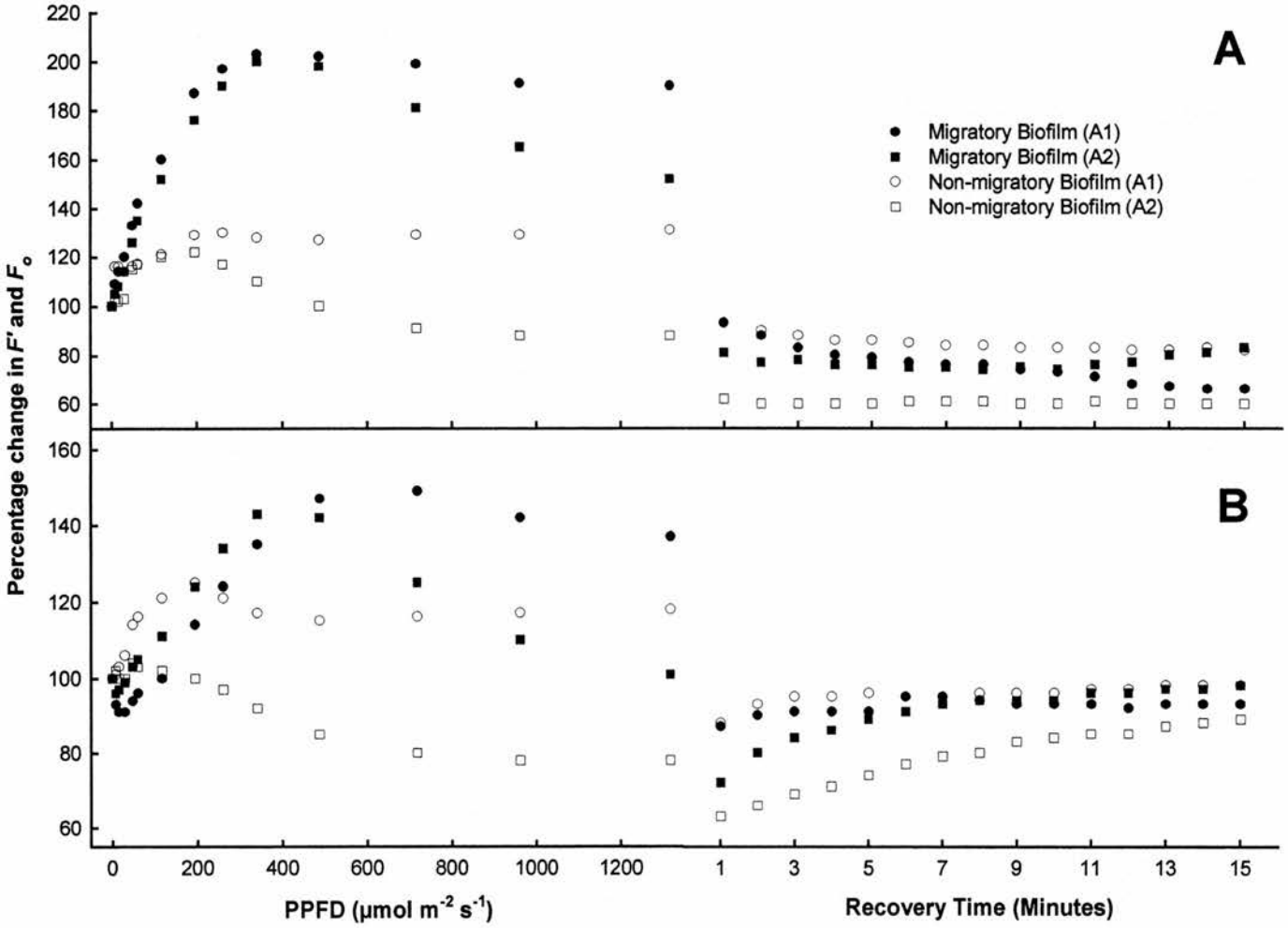


Fig. 7.3 Changes in F' and F_o during the light response curves and the 15 min recovery period. Previously dark- (A) and light-adapted (B)

Table 7.2 Selected values of F_o^{15}, F_o, F', F_m^{15}, F_m and F_m' taken during the light response curves and recovery periods. Values were taken from previously dark-adapted (DA) and previously light-adapted (LA) migratory and non-migratory Eden Estuary assemblages. Values are the mean parameter estimates ± SE, and n = 3 for each experimental treatment.

Biofilm and Light Treatment		Initial light curve value Fo^{15} F'	F_m^{15} F_m'	Final light curve value F'	F_m'	Initial Recovery value Fo	F_m	Final Recovery value Fo	F_m
Migratory Biofilm	DA	157 ± 91	746 ± 417	298 ± 172	362 ± 216	145 ± 84	486 ± 216	103 ± 60	402 ± 178
(Assemblage 1)	LA	281 ± 76	1036 ± 257	385 ± 100	492 ± 125	246 ± 82	793± 277	260 ± 85	895 ± 232
Migratory Biofilm	DA	182 ± 30	779 ± 103	277 ± 27	362 ± 34	147 ± 18	471 ± 56	151 ± 30	578 ± 106
(Assemblage 2)	LA	343 ± 93	1308 ± 319	346 ± 91	252 ± 113	246 ± 50	668± 78	335 ± 85	1219 ± 286
Non-migratory Biofilm	DA	608 ± 92	2212 ± 316	797 ± 157	867 ± 164	563 ± 98	976 ± 181	496 ± 101	1173 ± 323
(Assemblage 1)	LA	637 ± 133	1570 ± 342	754 ± 91	824 ± 97	563 ± 141	1031± 234	626 ± 142	1584 ± 192
Non-migratory Biofilm	DA	717 ± 74	2560 ± 301	634 ± 37	703 ± 62	448 ± 29	796 ± 71	432 ± 38	881 ± 121
(Assemblage 2)	LA	542 ± 164	1896 ± 473	423 ± 114	474 ± 117	334 ± 126	541± 142	481 ± 173	1251 ± 398

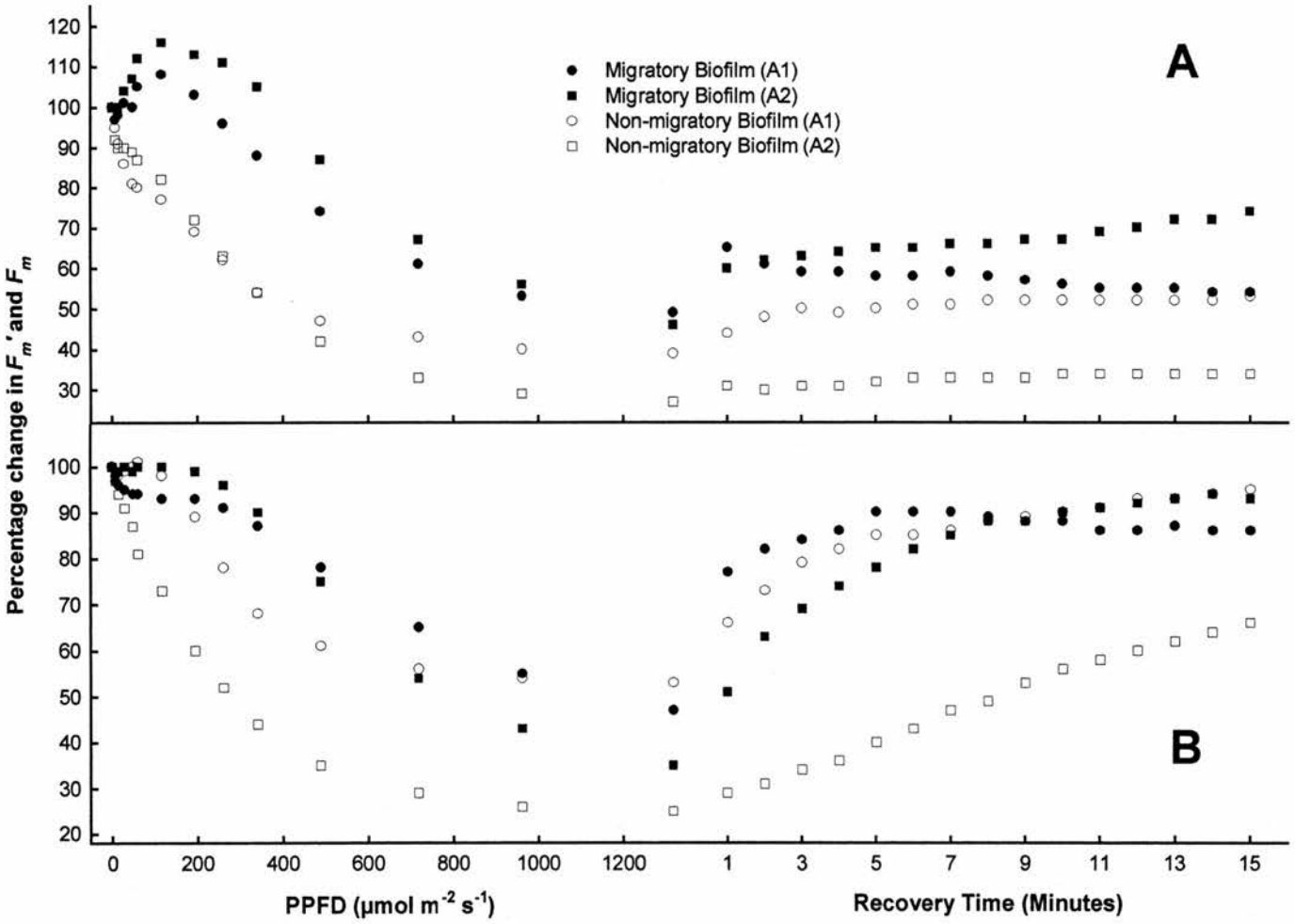


Fig. 7.4 Changes in F_m' and F_m during the light response curves and the 15 min recovery period. Previously dark- (A) and light-adapted (B).

only significant for the non-migratory A2 biofilms ($F_{15,32} = 3.58$; $p = < 0.001$) (Table 7.2).

F_m' of previously LA migratory biofilms remained constant between 0 and 195 µmol PPFD m^{-2} s^{-1}, after which F_m' decreased until the end of the light curve. F_m' of the previously LA non-migratory biofilms decreased throughout the duration of the light curve. During the dark recovery phase, the general trend was an increase in F_m for all biofilms, and the least increase occurred for the non-migratory A2 biofilm, whose mean F_m value had only returned to 56% of the original mean F_m' value by the end of the recovery period (Fig. 7.4B and Table 7.2).

7.3.6 Changes in photochemical and non-photochemical quenching

For all biofilms, whether previously DA or LA, the coefficient of photochemistry (qP) decreased throughout the light curves (data not shown). This indicated that Q_A reduction occurred in addition to any increase in NPQ as the actinic light level increased.

A change in F_v'/F_m' is inversely proportional to NPQ. NPQ of all previously DA and LA biofilms increased throughout the light curve (Figs. 7.5A & 7.5B). At the end of the light curve, levels of NPQ from both previously DA and LA non-migratory biofilms were significantly higher than corresponding migratory biofilms. At the end of the light curve, previously DA migratory biofilms had significantly higher levels of NPQ than previously LA migratory biofilms ($F_{7,16} = 19.29$, $p = <0.001$), whilst levels of NPQ did not vary significantly between DA and LA non-migratory biofilms.

During the recovery period, changes in NPQ of previously DA biofilms illustrated a biphasic response, being rapidly reversed within the first minute, followed by a second slower phase of reversal. This occurred for all biofilms except the migratory A1 biofilms whose F_v'/F_m' values slowly decreased for the remainder of the recovery period (i.e. NPQ slowly increased) (Fig 7.5A). F_v'/F_m' values of previously LA biofilms slowly increased throughout the recovery period, and a biphasic response was far less obvious (Fig. 7.5B). For previously DA biofilms, NPQ had not been fully reversed to mean original levels at the end of the recovery period (Fig. 7.5A). For previously LA biofilms, NPQ of the non-migratory A1 and migratory A2 biofilms had been fully reversed by the end of

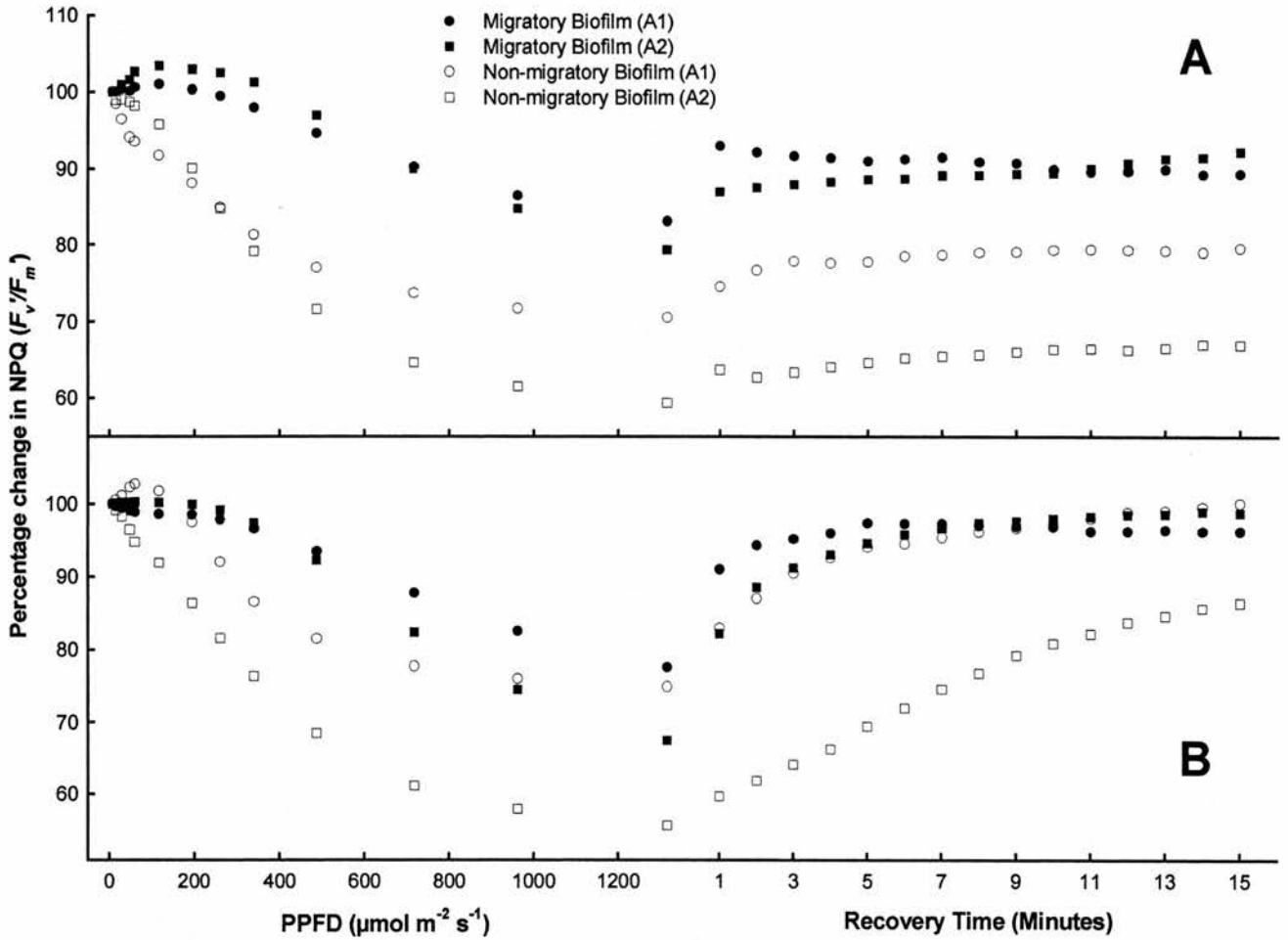


Fig. 7.5 Changes in photochemical quenching (F_q'/F_v') during the light response curves and the 15-min recovery period. Previously dark- (A) and light-adapted (B)

the recovery period, whilst NPQ of the non-migratory A2 and the migratory A1 biofilms had not been fully reversed (Fig. 7.5B).

At the end of the recovery period, F_v'/F_m' values of previously DA migratory biofilms were significantly higher (and therefore NPQ levels were significantly lower) than corresponding non-migratory biofilms. NPQ levels of previously LA assemblages did not differ significantly between migratory and non-migratory treatments, and only the previously DA non-migratory A2 biofilms had significantly lower F_v'/F_m' values than when previously LA ($F_{7,16}$ = 7.34, p = < 0.001).

7.3.7 Essex Assemblage

Phytotrays were used with an assemblage taken from Arlesford Creek in Essex, in order to determine whether the rETR of a different diatom assemblage whose ability to migrate was removed would also saturate. The rETR of non-migratory biofilms from Arlesford Creek failed to saturate, and instead illustrated an inflexion point at 720 µmol PPFD m^{-2} s^{-1}. This occurred for both DA and LA biofilms (Fig. 7.6).

F_q'/F_m' of both DA and LA non-migratory biofilms decreased throughout the light curve, and the gradient of the two slopes decreased after 720 µmol PPFD m^{-2} s^{-1}. The gradients of the two slopes were significantly different for the previously LA non-migratory biofilms (t = 3.188; p = 0.05; df = 3) but not for the previously DA non-migratory biofilms. The recovery of PSII efficiency illustrated a biphasic response, having a rapid increase within the first minute of recovery, followed by a second, slower phase. At the end of the recovery period, mean F_v/F_m of the previously LA non-migratory biofilms had returned to original mean F_q'/F_m' values, whilst the mean F_v/F_m of previously DA non-migratory biofilms had only returned to 75% of the original mean F_v/F_m value (Fig. 7.6).

F' of previously DA and LA non-migratory biofilms increased between 0 and 195 µmol PPFD m^{-2} s^{-1} after which F' decreased until the end of the light curve. F_o of the previously DA non-migratory biofilm fluctuated during the recovery phase, whilst F_o of the previously LA non-migratory biofilm increased throughout the recovery period and had returned to the original mean F' value at the end of the 15 min dark recovery period (Fig. 7.7). F_m' of the previously DA non-migratory biofilm decreased throughout the duration of the light curve,

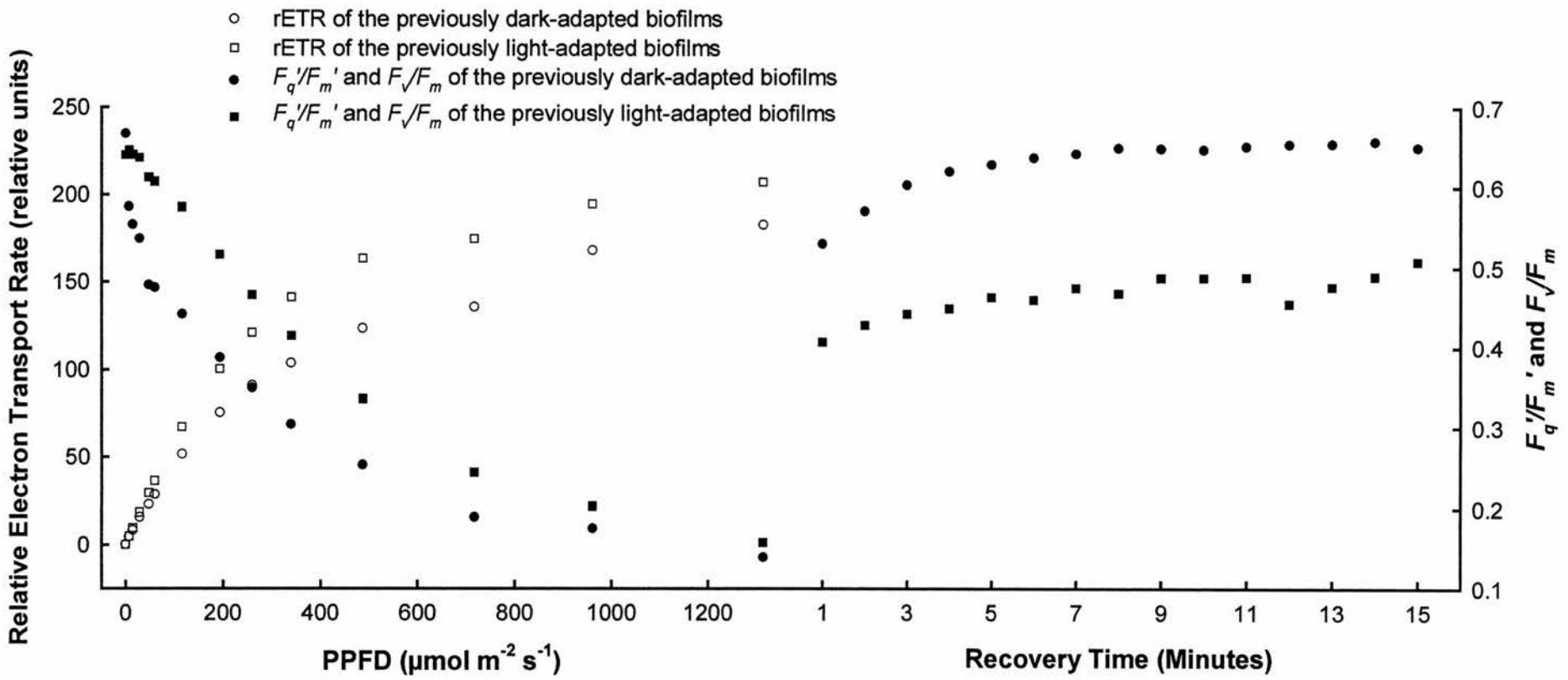


Fig. 7.6 Light response curves and changes in F_q'/F_m' and F_v/F_m during the light response curves and the 15 min recovery period, obtained from previously dark- and light-adapted non-migratory Arlesford Creek assemblages.

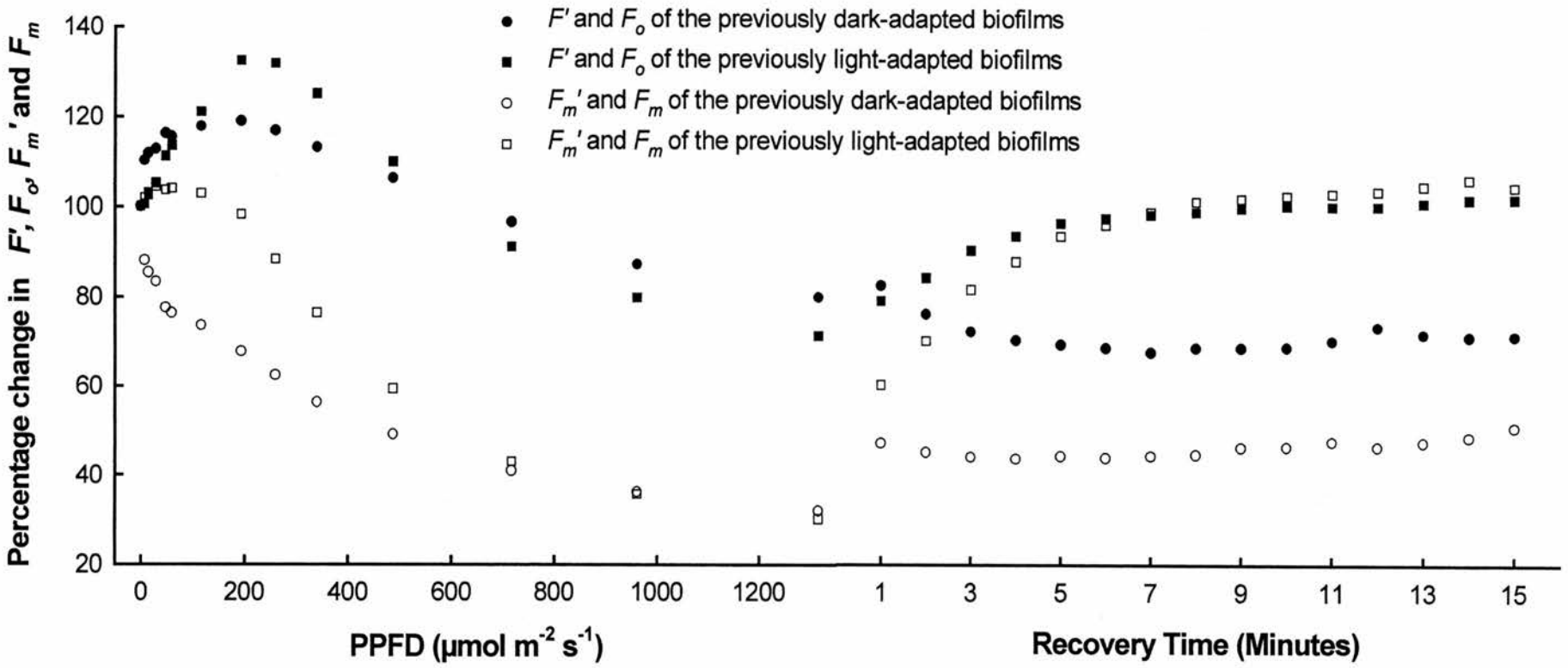


Fig. 7.7 Changes in F', F_o, F_m' and F_m during the light response curves and the 15 min recovery period, obtained from previously dark- and light-adapted non-migratory Arlesford Creek assemblages.

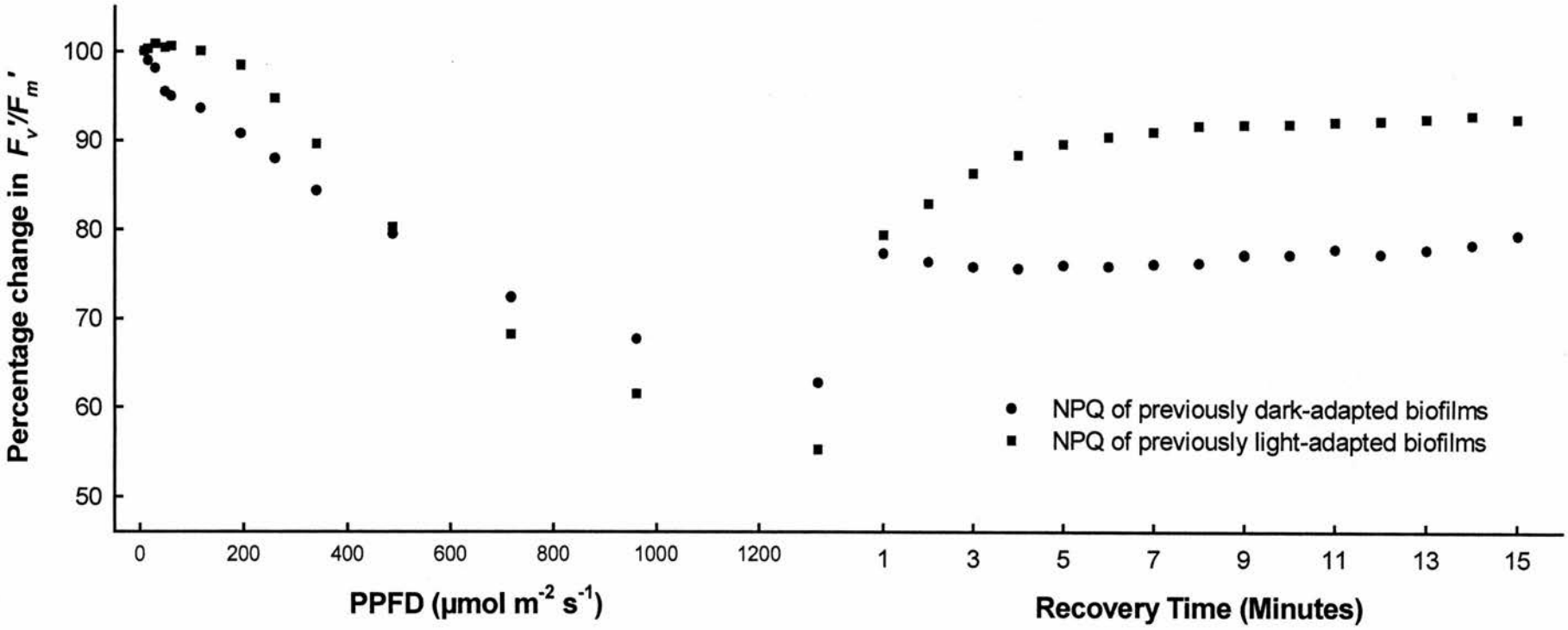


Fig. 7.8 Percentage change in non-photochemical quenching (F_v'/F_m') during the light response curves and the 15 min recovery period, obtained from previously dark- and light-adapted non-migratory Arlesford Creek assemblages.

whilst F_m' of the previously LA non-migratory biofilm increased initially before decreasing for the remainder of the light curve. Recovery of F_m for the previously DA non-migratory biofilm illustrated the biphasic response, increasing rapidly within the first minute, and then slowly increased for the remainder of the recovery period to reach 50% of the original mean F_m value. F_m of the previously LA non-migratory biofilm increased throughout the recovery period to reach 104% of the original F_m' value (Fig. 7.7).

The coefficient of photochemistry (qP) decreased throughout the light curves for both previously DA and LA non-migratory biofilms (data not shown). F_v'/F_m' of both DA and LA non-migratory biofilms decreased throughout the light curve (and hence NPQ of the biofilms increased), and the rate of increase in NPQ significantly decreased after the inflexion. The gradient of the two slopes significantly decreased after 720 μmol PPFD m^{-2} s^{-1} ($t = 9.280$; $p = 0.01$; $df = 3$ and $t = 14.302$; $p = 0.01$; $df = 3$ for previously DA and LA non-migratory biofilms respectively). During the recovery period, changes in NPQ illustrated a biphasic response, being rapidly reversed within the first minute, followed by a second slower phase of reversal. NPQ levels had returned to 80% and 100% of the original levels at the end of the recovery period for DA and LA non-migratory biofilms respectively (Fig. 7.8).

7.4 Discussion

7.4.1 Over-estimation of rETR

By comparing migratory and non-migratory biofilms from the Eden Estuary, this study has demonstrated that fluorescence measurements of the quantum efficiency of photochemistry at PSII, and calculated relative electron transport rates (rETR) from natural biofilms, can be over-estimated by as much as 100%. Prevention of migration resulted in a known level of light (PPFD) actually being received by the cells, and led to saturation and an accurate determination of rETR, and hence $rETR_{max}$, which is essential if the photosynthesis-irradiance relationship is to be used in primary productivity studies, such as for comparison with carbon fluxes and oxygen evolution. A comparison between the results of these rapid curves and oxygen or carbon data is *per se* flawed since rapid P-E curves do not allow full equilibration of the photosynthetic apparatus at the individual PAR values. Despite this, rapid light

curves have been routinely used (e.g. Perkins *et al.*, 2001; 2002; Barranguet & Kromkamp, 2000; Serôdio & Catarino, 2000; Barranguet *et al.*, 1998; Hartig *et al.*, 1998) and allow a compromise between minimising the effects of migration, whilst still achieving a stable *F'*.

Values of F_o^{15} were higher in non-migratory biofilms because these cells were unable to migrate into the sediment during the dark-adaptation period, and hence signal strength is higher than intact biofilms where migration into the sediment occurred. F_o^{15} values in the range of 542-717 using an FMS2 would correspond to chlorophyll *a* concentrations of 55-70 mg m^{-2} (according to the model of Honeywill *et al.*, 2002), and since the Eden Estuary can achieve concentrations up to 160 mg m^{-2}, I conclude that the phytotray biofilm was not thick enough to induce self-shading and signal reabsorption. Future experiments should incorporate actual measurements of chlorophyll *a*.

7.4.2 PSII efficiency

PSII efficiency decreased throughout the light curves. A decrease in calculated PSII efficiency can be the result of an increase in *F'* fluorescence yield due to Q_A reduction, and/or a decrease in F_m' fluorescence yield (and hence a decrease in F_q') due to NPQ. PSII efficiency of Eden Estuary biofilms decreased due to a decrease in F_m' (whilst *F'* yield varied throughout the light-curve.) *F'* may appear stable when Q_A reduction is balanced by NPQ.

It would be expected that at the beginning of the light curve, F_q'/F_m' values of previously LA non-migratory biofilms would be lower than corresponding F_v/F_m values of previously DA non-migratory biofilms due to a higher level of NPQ and the closure of some of the reaction centres in the previously LA biofilms. This occurred for the non-migratory A1 biofilms, but not for the non-migratory A2 biofilms. The reasons for this are unclear. PSII efficiency of non-migratory biofilms would also be expected to start at a lower value than corresponding migratory biofilms due to the absence of sub-surface cells (i.e. cells deeper in the sediment that have higher efficiencies since they are exposed to lower light levels; Perkins *et al.*, 2002), and because they are receiving the full level of PPFD since there is no shading from sediment grains. This was indeed true for the previously LA non-migratory biofilms of A1, but

not for A2. However, PSII efficiency of previously DA treatments was not significantly different between migratory and non-migratory biofilms.

The ability of estuarine benthic diatoms to migrate away from the sediment surface, particularly at high PPFD, means that these 'sub-surface' cells are receiving a lower PPFD. The sub-surface signal from the migratory biofilms acted to give a higher value of F_q'/F_m' due to these cells being in a lower light level than those cells at the sediment surface. As a result, the measured efficiency was falsely inflated above the true efficiency of the cells at the sediment surface. Consequently the estimated value of rETR was much higher than the true value of rETR, as measured from the corresponding non-migratory biofilms. The interpretation of fluorescence data can be further complicated by the possibility of cells micro-cycling within the biofilms (Perkins *et al.*, 2002; Underwood & Kromkamp, 1999). Higher operating efficiencies may also be measured due to sub-surface cells migrating down into the sediment and being replaced by sub-surface cells that have higher operating efficiencies. This possibility is precluded by the use of phytotrays. The amount of sub-surface signal incorporated into the fluorescence yields is difficult to determine without knowing the measuring depth of the fluorometer, the precise light field within the sediment and the vertical position of the cells. These results support the conclusions of Perkins *et al.* (2002) who determined that realistic estimation of rETR within intact biofilms would require high resolution imaging of fluorescence.

7.4.3 The dark-adaptation period

F_v/F_m values of the migratory A1 biofilms were significantly higher than the corresponding non-migratory biofilms, and F' and F_m' yields of previously DA migratory biofilms (A1 and A2) increased up to 120 µmol PPFD m^{-2} s^{-1}. This suggests that during the dark-adaptation period the cells had migrated into the sediment, and that during the initial phases of the light curve they were migrating up to the sediment surface in response to light. Cells of previously LA migratory biofilms also migrated towards the sediment surface during the initial phases of the P-E curves. This clearly demonstrates vertical migration occurring during short periods (i.e. minutes) of dark adaptation and during the light curves, which will have important implications when interpreting fluorescence data from intact biofilms.

A 15 min dark-adaptation period has been used to minimise changes in the biofilm assemblage structure resulting from vertical migration, whilst achieving a stable minimal fluorescence yield (termed F_o^{15}) (Honeywill, 2001; Perkins *et al.*, 2001; Barranguet & Kromkamp, 2000). However, these results show that measurements of relative photosynthetic efficiency and electron transport rate are greatly influenced by the length of dark-adaptation, the cells' ability to migrate, and the light history of the sample (which differed between A1 and A2 since the maximum light utilisation coefficients of A1 were significantly higher than A2, suggesting that A1 was a more shade-adapted assemblage than A2).

7.4.4 Migration and NPQ

Between 120 and 340 μmol PPFD m^{-2} s^{-1}, the fluorescence yield at F' of migratory biofilms continued to increase, indicating Q_A reduction, whilst F_m' decreased as NPQ increased. Once PPFD became saturating, both F' and F_m' decreased, indicating that the migratory biofilms used a combination of vertical migration into the sediment and NPQ activation to avoid over-excitation and photo-damage. The photosynthetic performance and the extent to which a diatom will use NPQ and/or migration vary among diatom species, making it critical that the composition of the diatom assemblage is known (Perkins *et al.*, 2001; 2002; Underwood *et al.*, 1999; Kromkamp *et al.*, 1998; Blanchard *et al.*, 1996). For example, Perkins *et al.* (in prep) found down-regulation strategies differed between *Nitzschia amphioxys*, which had an increase in NPQ prior to migration, and *Pleurosigma angulatum*, which had little NPQ and migrated away from high light. In contrast, A2 from the Eden estuary, whose second most dominant species was also *P. angulatum*, showed large amounts of NPQ. This highlights the need for further research into what NPQ strategies individual diatom species use, and when they use them.

During the dark recovery period, PSII efficiencies recovered with a biphasic response. Within the first minute of recovery, F_o decreased due to Q_A oxidation and F_m increased due to rapid NPQ reversal. During the remainder of the recovery period, PSII efficiencies of the migratory biofilms were restored due to a combination of three mechanisms: 1) migration down into the sediment; 2) Q_A oxidation; and 3) slow NPQ reversal (F_o and F_m slowly decreased). The

previously DA migratory A2 biofilms differed slightly from the A1 biofilms, in that there was less Q_A oxidation, and a greater amount of NPQ to reverse. Also, in the last 5 min of recovery, cells began to migrate upwards towards the sediment surface (F_o and F_m slowly increased). This was not observed in the phytotrays, indicating that cells become photo-damaged in the absence of migration. The proportion that each mechanism is involved in the recovery of PSII operating efficiencies is difficult to determine for natural biofilms, although the use of phytotrays in conjunction with natural biofilms allows us to observe the extent of migration down into the sediment, and how this could potentially contribute to the maintenance of photochemical efficiency in the biofilm.

7.4.5 The biphasic response

The biphasic response seen in these experiments during the recovery phase has also been illustrated in the green algae *Dunaliella tertiolecta*, when most of the recovery in NPQ and PSII efficiency took place within the first minute after darkening. The same response was not found for the marine diatom *Phaeodactylum tricornutum*, although NPQ of this species was quickly reversed (Casper-Lindley & Björkman, 1998). The rapid onset and reversal of NPQ was also observed for the planktonic diatom, *Phaeodactylum tricornutum* (Lavaud *et al.*, 2002a,b). One of the main sites of NPQ action are the PSII antennae, which exhibit high diversity amongst algae, with regard to both pigment and protein composition (Ting & Owens, 1993). Thus differences among diatom species and/or assemblages are also quite likely, and may go some way to explaining differences in the regulation of NPQ.

7.4.6 Potential photodamage

PSII efficiencies and NPQ of non-migratory biofilms had not completely recovered at the end of 15 min. This suggests that some photo-damage may have occurred, and that NPQ alone could not provide adequate photoprotection when non-migratory biofilms were subjected to saturating PPFD. During the recovery phase, NPQ reversal in the first minute is likely to be due to the rapid relaxation of 'energy-dependent' quenching (q_E) as the intrathylakoid H^+ concentration decreases. Further slower relaxation will then be due to other processes such as the de-phosphorylation of light harvesting complex II (q_T) and the reversal of

photosynthetic inhibition (q_I). Exposure to a short dark period can allow cells to recover from dynamic photoinhibition (Falkowski & Raven, 1997). The dark interval is required to relax q_E and q_T, while q_I is not readily reversible, and a longer period of recovery is needed for regeneration of damaged PSII reaction centres induced by chronic photoinhibition (Krause & Weis, 1991). Whilst all NPQ had been reversed in migratory biofilms by the end of the recovery period, NPQ in non-migratory biofilms was still being slowly reversed. This suggests that down-regulation mechanisms of photoinhibitory fluorescence quenching were still being reversed, and potentially provides further evidence that cells on phytotrays had been photo-damaged. However, alternative cyclic photochemical processes, not necessarily related to fluorescence quenching, also control PSII efficiency. These are cyclic phosphorylation or chlororespiration, or perhaps a combination of these, which involve the electron pathway around PSI, and are thought to be involved in balancing the levels of ATP and NADPH. Since these pathways are cyclic they do not contribute to Q_A oxidation, but may influence NPQ through the modification of the proton gradient. Consequently, it is not a direct quenching method, but may be an indirect one dependent upon other factors influencing NPQ (Krause & Weis, 1991 and references therein; Caron *et al.*, 1987).

7.4.7 Photoinhibition

A central issue related to the effect of light fluctuations on productivity is the occurrence of photoinhibition in response to high light. Whilst there are many examples of photoinhibition occurring in natural phytoplankton communities (i.e. Macedo *et al.*, 2001; Falkowski & Raven, 1997; Kirk, 1994), this phenomenon (in microphytobenthic communities) is rarely reported for natural microphytobenthic assemblages, although it has been reported for cultured assemblages (Admiraal, 1984). These results support the conclusions of Kromkamp *et al.* (1998), and emphasize that the migratory strategy of estuarine epipelic diatoms, and the ability of cells to cycle within a biofilm according to the light level at the sediment surface, serve as an effective means of avoiding photo-damage.

Several studies agree that microphytobenthos are particularly resistant to strong illumination (Blanchard & Montagna, 1992; Rasmussen *et al.*, 1983), and

I suggest that migration is a likely mechanism of avoiding photoinhibition and photo-damage. Therefore, the high PPFD to which the non-migratory cells of A3 were exposed in the final stages of the P-E curve would have previously been thought to produce photoinhibition and/or photo-damage. However, this did not appear to be the case. Instead, photosynthesis failed to saturate, and an inflexion point at 720 μmol PPFD m^{-2} s^{-1} was observed (previously seen by Perkins *et al.*, 2001 for migratory biofilms *in situ*). It is not known what mechanisms contributed to this observation. One potential explanation is that a short-term decrease in the effective absorption cross section (a*) of PSII could, in principle, have altered the P-E curve without affecting the maximum quantum efficiency of photochemistry in PSII, allowing for the optimisation of photosynthetic efficiency by reversibly adjusting light-harvesting to match photosynthetic capacity (Olaizola *et al.*, 1994). Calculated photosynthetic electron transport rate would be lower if a* was measured from an assemblage and used to calculate rETR. However, a* is a difficult parameter to measure from diatoms on a sediment system (Perkins *et al.*, 2002; Henley, 1993).

7.4.8 The xanthophyll cycle

PSII efficiencies and NPQ of Essex assemblages were returned to original values for LA cells and to a lesser extent in DA cells. I hypothesise that this may be due to a secondary level of NPQ resulting from the accumulation of diatoxanthin, independent of the diadinoxanthin pool (Olaizola *et al.*, 1994), although other mechanisms of photoprotection, such as chlororespiration (Dijkman & Kroon, 2002; Ting & Owens, 1993), an oxygen dependent electron transport and pseudo-cyclic electron transport (Caron *et al.*, 1987), may be involved in down-regulation. Previously LA assemblages may have had time to photoacclimate, and consequently may have had a larger pool of xanthophyll cycle pigments, or a reduced cellular content of chlorophyll and accessory pigments (Anning *et al.*, 2000 and references therein). If the pool of xanthophylls was larger, then a larger amount of diadinoxanthin would have been available for de-epoxidation, allowing the previously LA cells to cope better with the high PPFD and regain maximum theoretical PSII efficiency by the end of the recovery period.

7.5 Conclusions

This study demonstrates that in order to use fluorescence to estimate primary productivity (rETR) of estuarine benthic biofilms, it is imperative to know and take into account the following points: 1) the position of the cells within the vertical profile of the sediment photic zone; 2) the irradiance levels actually received by the cells; 3) how quickly the cells migrate away from the light source; 4) what species are present in the assemblage. Using assemblages from the Eden Estuary, phytotrays were proven as a useful tool for determining the over-estimation of rETR and highlighted that rETR data detailed in chapters 3, 4 and 6 may have been grossly over-estimated. However, the assemblage from Arlesford Creek, Essex, highlighted that we still have a lot to learn regarding the estuarine benthic diatoms, and further research is required to determine the fluorescence characteristics of a large number of taxonomically diverse species, so that the interpretation of their responses to other factors, such as nutrient limitation and temperature, which can also affect the physiological state or species composition of an assemblage, can be more accurately examined.

Chapter 8

Chapter 8: Can the stability of intertidal sediments be predicted from proxy parameters? An *in situ* investigation.

Abstract

The ability to predict how intertidal sediments are likely to respond to climatic and anthropogenic change is essential for developing successful management schemes. Although a number of sedimentological and biological factors are known to influence the stability of intertidal cohesive mudflats, their complex interactions are still poorly understood. Attempts to find a single easily measurable sediment variable that could be used to predict cohesive sediment stability have so far been unsuccessful. The extensive ground-truthing of the BIOPTIS project involved the synoptic mapping of many sediment properties on two large grids in the Eden Estuary, Scotland, and the measurements of erosion threshold presented here, made using the Cohesive Strength Meter (CSM), represent the largest single data set of in situ *erosion threshold measurements made to date, covering a range of sediment types and conditions. Erosion threshold had a weak dependence with chlorophyll a, which explained 40% of the variation in the data. Surface sediment types and sediment water content (traditionally considered the primary controls of stability on cohesive sediments) did not help explain the variation within the data set, nor did the presence of selected macrofaunal species. Data from muddy sand sites of the Eden Estuary highlighted the dangers of extrapolating the predictive relationship of Riethmüller et al. (1998) above chlorophyll a concentrations of 60 mg m^{-2}. As it stands, the model was not found to be generally applicable and is likely to be site and possibly technique specific. The data presented here suggest that a multivariate approach, including microphytobenthic and macrofaunal species composition, as well as biological and physical parameters, may produce an estimated range of critical erosion threshold values, but that relationships derived are likely to be site specific. At present, time-consuming field measurements will still have to be relied upon to predict which areas of coastline will be susceptible to erosion.*

Chapter 8: Can the stability of intertidal sediments be predicted from proxy parameters? An *in situ* investigation.

8.1 Introduction

For many years, the influence of sediment-dwelling biota was largely ignored, with the belief that it was the physical properties of the sediment that influenced sediment stability. Today, however, the importance of the microbiota in intertidal sediments is well documented (see Paterson, 1997; 1994 for reviews). Extracellular Polymeric Substances (EPS) are generally considered to be responsible for the stabilising effect, and are generated by many intertidal organisms, including diatoms (Riethmüller *et al.*, 2000; Paterson, 1997; Yallop *et al.*, 1994; Black, 1992; Holland *et al.*, 1974) and selected macrofaunal species (Austin *et al.*, 1999; Willows *et al.*, 1998; Blanchard *et al.*, 1997; Meadows *et al.*, 1990). The importance of EPS in the erosion and deposition of intertidal sediments has been highlighted in Chapter 1. EPS can increase the critical shear stress for erosion of the sediment by forming an elastic matrix between sediment grains (Fig. 8.1) leading to biostabilisation, and by altering the physio-chemical properties. They may also act by smoothing the sediment surface, thereby reducing the current-induced shear stress, and by trapping fine particles (Amos *et al.*, 1998; Paterson, 1994; Paterson *et al.*, 1994; Underwood & Paterson, 1993; Grant *et al.*, 1986).

An increase in our capability to predict sediment erosion on estuarine mudflats is important from both an ecosystem and management perspective, since changes in water depth and a steady loss of bed material could have substantial implications for coastal protection, ecosystem function and benthic species composition, abundance and primary productivity. One of the key objectives in the field of sediment transport research is to be able to generate maps of sediment stability over an entire mudflat, so that the natural variability in sediment stability and areas susceptible to erosion can be determined. This will become an important tool for combating the effects of sea-level rise on coastal erosion, and an example of this can be seen in the Venice Lagoon (EU TIDE project).

Data from previous chapters have shown that variables such as light, temperature, grazing and carbon dioxide can all have an impact upon the structure and functioning of estuarine microphytobenthic biofilms, and such changes have the potential to have a direct effect on the stability of the sediment, possibly by way of changes in EPS production and/or composition. If it is possible to predict how climate change will influence variables such as diatom biomass and EPS concentrations, we may then be able to model how climate change will affect the stability over a whole mudflat. However, in order to make rapid field measurements and decrease the time taken to produce such predictions, it would be desirable to establish predictive relationships of sediment stability to proxy parameters. Ideally, these parameters will be more easily mapped in the field or by remote sensing techniques.

Whilst EPS is an important variable in the biostabilisation process, it cannot be used as a proxy parameter for stability in large scale mapping, since it is not yet possible to discern EPS from Remote Sensing. However, studies have shown that concentrations of colloidal carbohydrates in sediments are closely related to the content of chlorophyll *a,* taken as a biomass proxy for diatoms, although the variability is large (Staats *et al.*, 2001; Underwood & Smith, 1998; Underwood *et al.*, 1995). Since chlorophyll *a* content of the surface sediment can be easily measured (i.e. by using the contact corer) and, in principle, quantitatively estimated using optical remote sensing techniques, chlorophyll *a* may provide an alternative parameter for estimating and mapping critical erosion threshold (Riethmüller *et al.*, 2000). Indeed, in an extensive study along a shore normal transect, Paterson *et al.* (2000) found chlorophyll *a* to correlate best with sediment stability.

This experiment aimed to:

- Investigate the possibility of using chlorophyll *a* as a proxy parameter for predicting the erodibility of intertidal mudflats.
- Critically test the published model of Riethmüller *et al.* (1998), comparing its predictions of the erodibility of muddy-sand sediments of the Eden Estuary against CSM measurements made during the BIOPTIS project.

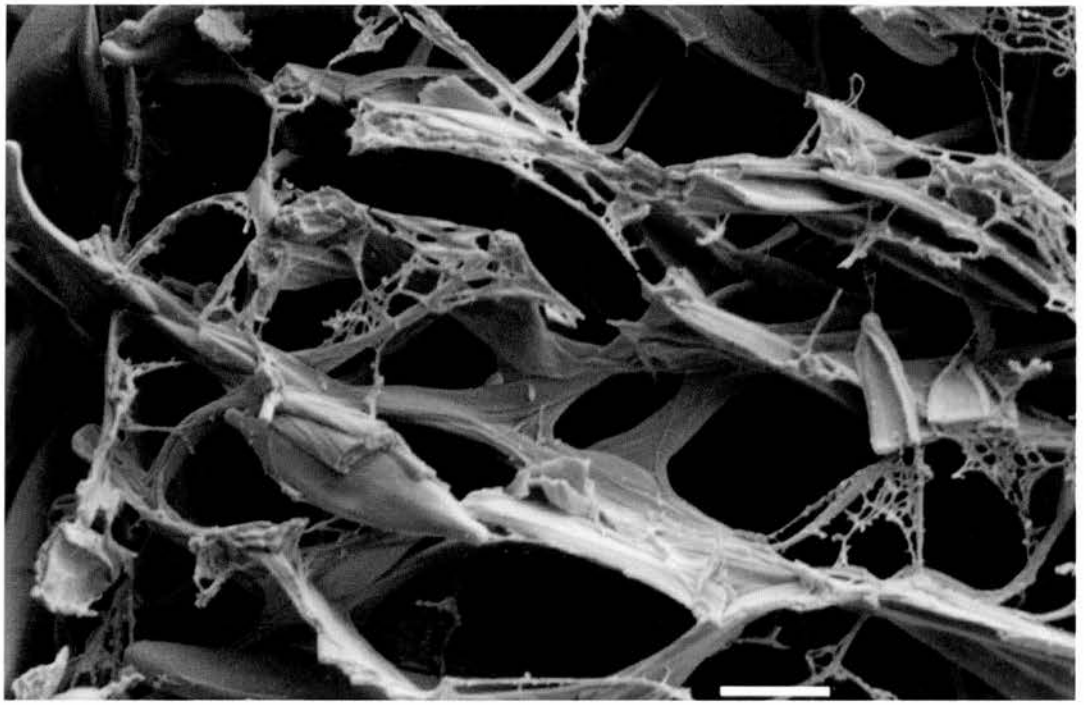


Fig. 8.1 LTSEM of EPS in natural sediments from the Eden Estuary. Fracture face showing thick strands of EPS associated with a diatom biofilm that help bind the sediment particles together, and increase the elasticity of the bed. Bar marker = 10 μm.

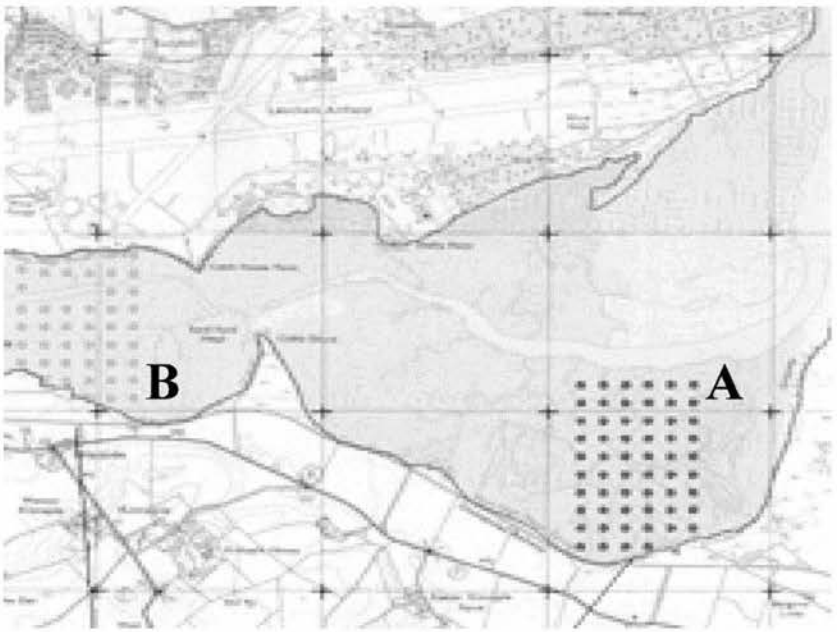


Fig. 8.2 Grid A (900m x 500m) consisted of 52 grid nodes, spaced 100m apart, running from the top shore down to the channel of the River Eden. Grid B (800m x 500m), was further upstream, and consisted of 46 grid nodes, spaced 100m apart, with the channel of the River Eden running through a portion of the grid.

This investigation differs from previous studies in that a greater range and spatial coverage of chlorophyll *a* contents were measured using millimetre scale accuracy. Also the use of the Cohesive Strength Meter (CSM) enabled replicate measurements of *in situ* sediment surface stability (up to 9.08 Nm^{-2}) to be made on a spatial scale covering several hundred meters in just a few days. Initial studies by Riethmüller *et al.* (2000; 1998) and Austin *et al.* (1999) used the EROMES erosion device, which has several draw-backs including sediment disturbance, since cores have to be removed from the mudflat and taken back to the laboratory (Tolhurst *et al.*, 2000a).

8.2 Methods

The intertidal mudflats of the Eden Estuary, Scotland were sampled during the last two weeks of August 1999. Two grids were established: Grid A (900m x 500m) consisted of 52 grid nodes, spaced 100m apart, running from the top shore down to the channel of the river Eden; Grid B (800m x 500m), which was further upstream, consisted of 46 grid nodes, spaced 100m apart, with the channel of the river Eden running through a portion of the grid (Fig. 8.2).

Three replicate measurements of grain size, chlorophyll *a*, water content, organic content and sediment stability were made at each grid node (see Chapter 2 for individual methodologies). In this investigation, sediment grain size data were subdivided into five sediment classes by fine-grain fraction (< 63μm): a) clay-mud (fine grain fraction >85%); b) mud (fine grain fraction 50–85%); c) muddy sand (fine grain fraction 25–50%); d) sand and sandy mud (fine grain fraction 10–25%); e) sand (fine grain fraction <10%). This classification was after Figge *et al.*, 1980 cited in Riethmüller *et al.*, 1998. Sediments whose erosion thresholds were above 2 Nm^{-2} were considered to be relatively stable. One core was taken from each grid node for macrofaunal species composition. Substrata lying beneath plant canopies are not available for assessment by remote sensing, and consequently, grid nodes dominated by *Enteromorpha* were not used for the analysis presented here (but are discussed in Chapter 9).

8.2.1 Statistics

Contour maps of sediment erosion thresholds for grids A and B were produced using the surface mapping software, Surfer (version 6.04). SPSS

(version 10) was used for multivariate statistical analysis. Pearson correlation analysis was used to investigate the effects of microphytobenthos on sediment stability. Data were subdivided and analyzed according to: 1) the spatial position, 2) percentage fine grain fraction (<63 μm) (sand and sandy-mud sites 10-25% and muddy-sand sites 25-50%) and 3) chlorophyll *a* (low chlorophyll *a* <99 mg m^{-2}; high chlorophyll *a* >100 mg m^{-2}) and water content (low water content <40%; high water content >40%). One-way ANOVA and post-hoc Tukey tests were used to test for significant differences between erosion thresholds of these subdivisions. Stepwise multiple linear regressions were applied to the sediment stability data in order to establish the influences of chlorophyll *a*, water content, fine-grain fraction (% of sediment <63μm), organic content, and selected macrofaunal abundance on erosion threshold. In the analysis, a total of 129 measurements were considered.

8.3 Testing the published model of Riethmüller *et al.* (1998)

The chlorophyll *a* data were used to test the published model of Riethmüller *et al.*, (1998). This model suggests chlorophyll *a* concentrations could be used to predict the erosion thresholds of muddy-sand sites:

Erosion threshold = 0.0084*[chlorophyll *a*]+0.34 (Equation 8.1)

Erosion thresholds predicted by this model were calculated and then compared to actual erosion thresholds measured with the CSM, to determine how robust the model was over large spatial scales and a greater range of chlorophyll *a* concentrations. It should be noted that whilst measured erosion threshold is highly device dependent (Tolhurst *et al.*, 2000a), data from the EROMES and CSM devices have been evaluated and found to be comparable (Tolhurst *et al.*, 2000b).

8.4 Results

8.4.1 Chlorophyll *a* vs. erosion threshold

Sediment erodibility exhibited great variation, both within and between the two grids (Fig. 8.3), with Grid A exhibiting greater heterogeneity of sediment stability than Grid B. 33% of Grid A sites were considered relatively unstable

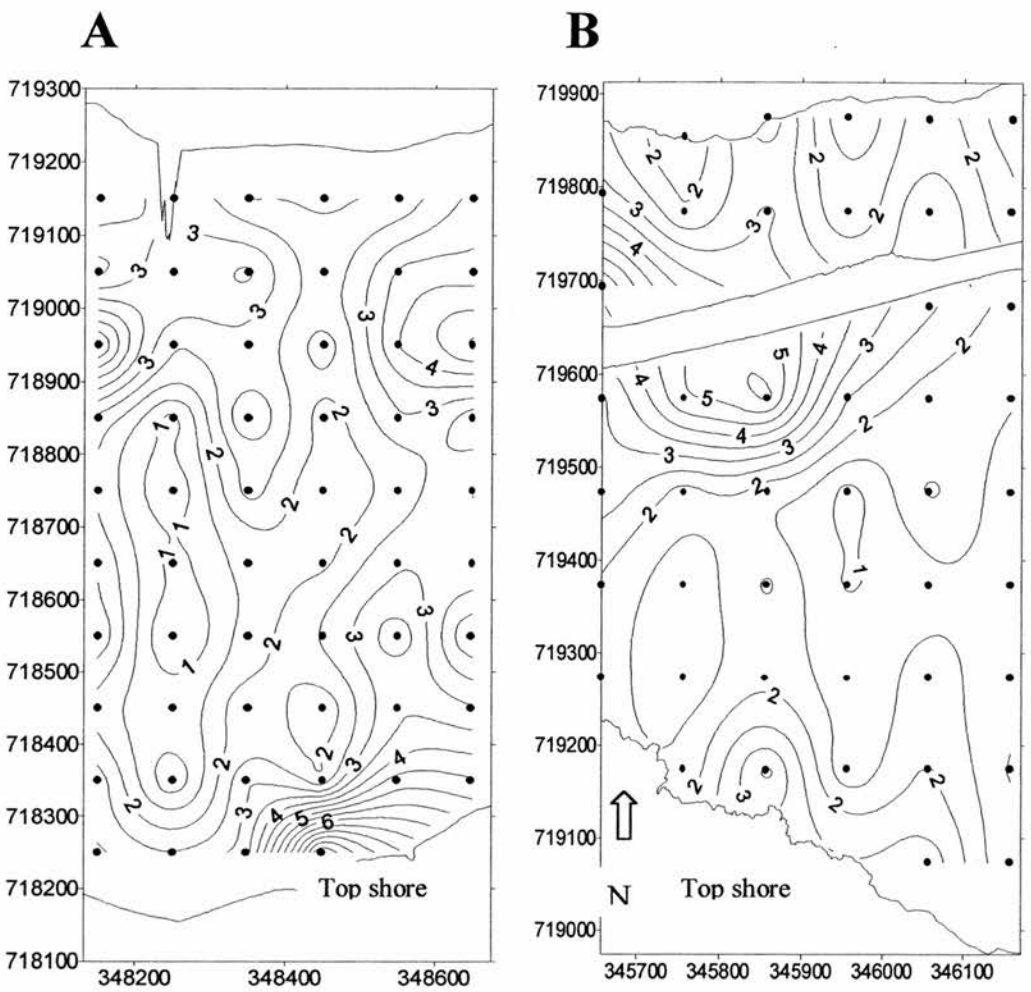


Fig. 8.3 Contour maps of surface sediment stability on Grid A and Grid B of the Eden Estuary. Contour lines represent erosion thresholds (Nm^{-2}) and x,y, axes represent Northings and Eastings (m).

Table 8.1 The dependency of the relationship between erosion threshold and chl *a* (low chl <100 mg m^{-2}; mid chl 100-200 mg m^{-2}; high chl >200 mg m^{-2}).

Chlorophyll *a* concentration (mg m^{-2})	Number of sites	Mean erosion threshold value (Nm^{-2})	Standard error of the mean (Nm^{-2})	Minimum erosion threshold value (Nm^{-2})	Maximum erosion threshold value (Nm^{-2})
Low Chl *a* <100 mg m^{-2}	90	1.82	0.09	0.58	4.25
Mid Chl *a* 100-200 mg m^{-2}	58	2.94	0.22	0.58	9.08
High Chl *a* >200 mg m^{-2}	9	5.24	1.66	1.66	9.08

(erosion thresholds < 2 Nm^{-2}), whilst 75% of Grid B sites were considered relatively unstable.

Overall, erosion threshold increased with increasing chlorophyll *a* (Fig. 8.4). However, the association of erosion threshold with chlorophyll *a* was weak (r^2_{total}=0.34), and the scattering of the data suggests the influence of additional factors. Erosion thresholds from Grid A had a stronger dependence on chlorophyll *a* than Grid B (r^2_{GridA}=0.42 and r^2_{GridB}=0.18) (Fig. 8.4). When sites were segregated by chlorophyll *a* concentration (low chlorophyll *a* <100 mg m^{-2}; mid chlorophyll *a* 100-200 mg m^{-2}; high chlorophyll *a* >200 mg m^{-2}), mean erosion thresholds were significantly different between groups ($F_{2,154}$ = 23.24; p < 0.001) (Table 8.1).

8.4.2 Influence of grain size

All Grid A sites used in this correlation were sand and sandy-mud sites, whilst 70% of sites from Grid B were sand and sandy-mud and 30% of sites were muddy-sand. Variation in the data set was not significantly reduced when the data were subdivided by fine-grain fraction (Fig. 8.5). Erosion threshold increased with increasing chlorophyll *a* for both sand and sandy-mud sites (r^2 = 0.37) and muddy-sand sites (r^2 = 0.40).

8.4.3 Influence of water content

A weak association (r^2 = 0.13) between water content and chlorophyll *a* was observed, and taken as a whole, water content increased with increasing chlorophyll *a* (Fig. 8.6). Sites were segregated by water content (low water content <40%; high water content >40%) and chlorophyll *a* concentration (low chlorophyll *a* <99 mg m^{-2}; high chlorophyll *a* >100 mg m^{-2}). For sand and sandy-mud sites and muddy-sand sites, there were no significant differences in erosion threshold based on water content alone. For sand and sandy-mud sites with low water content, erosion thresholds for high chlorophyll *a* sites were significantly greater than erosion thresholds for low chlorophyll *a* sites ($F_{3,97}$ = 11.22; p < 0.001), whilst erosion thresholds for high water content sites did not differ significantly between high and low chlorophyll *a* sites. In contrast, muddy-sand sites with high water content had erosion thresholds that were significantly greater for high chlorophyll *a* sites than erosion thresholds of low chlorophyll *a*

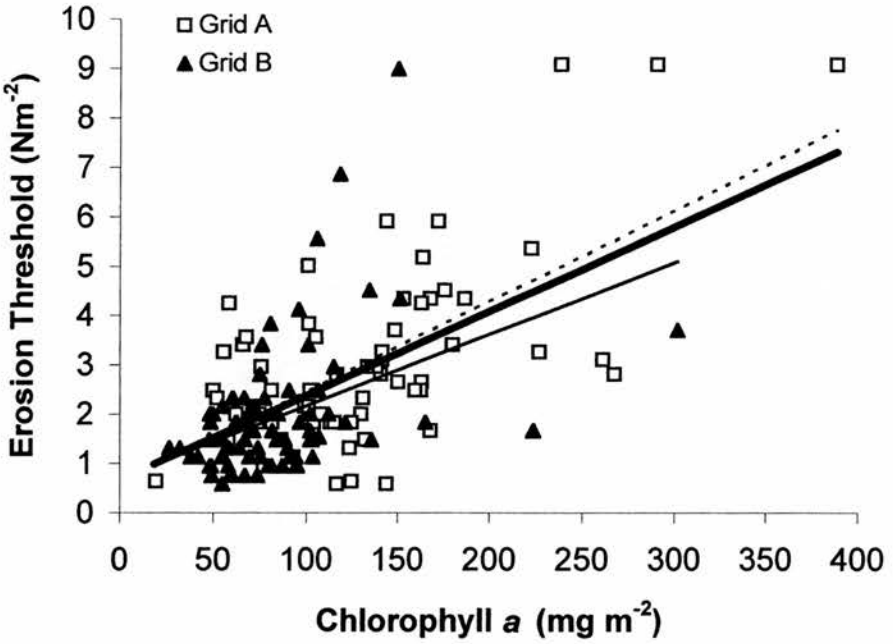


Fig 8.4 Total critical erosion thresholds plotted against their corresponding chlorophyll *a* concentration (thick solid line; $r^2 = 0.34$; $y = 0.017x + 0.67$). Erosion thresholds of Grid A (dashed line; $r^2 = 0.42$; $y = 0.018x + 0.62$) had a stronger dependence on chlorophyll *a* than erosion thresholds of Grid B (thin solid line; $r^2 = 0.18$; $y = 0.015x + 0.72$).

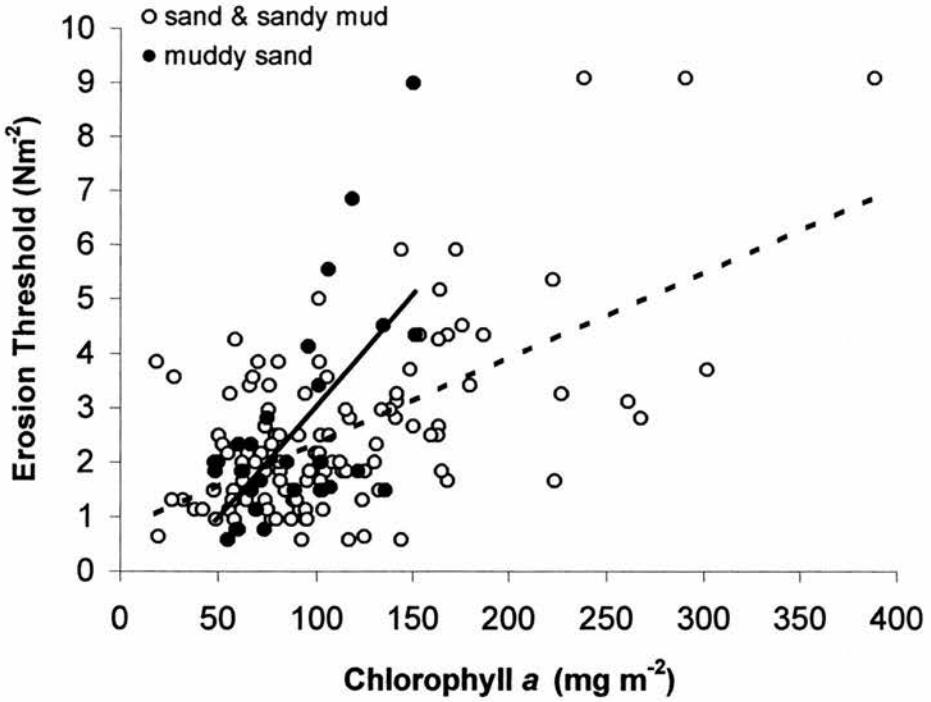


Fig 8.5 Data segregated by fine grain fraction (<63 μm). Erosion thresholds of sediments from sand and sandy-mud (dashed line; $r^2 = 0.37$; $y = 0.016x + 0.75$) and muddy-sand (solid line; $r^2 = 0.40$; $y = 0.041x - 1.044$) sites increased with increasing chlorophyll *a* concentration.

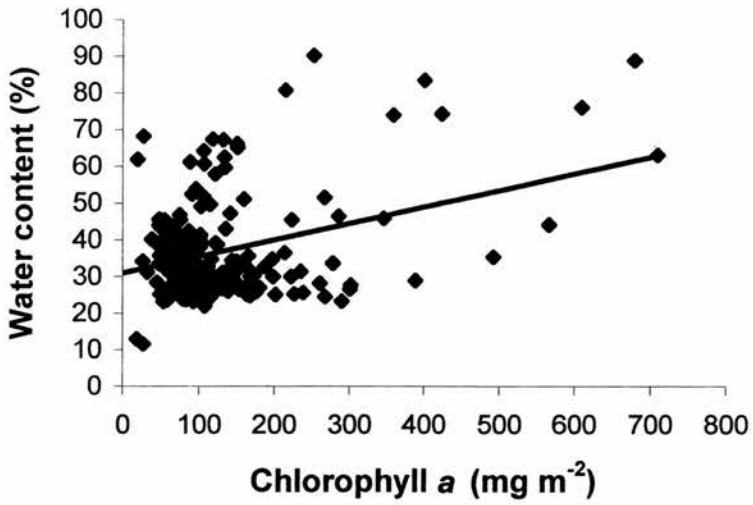
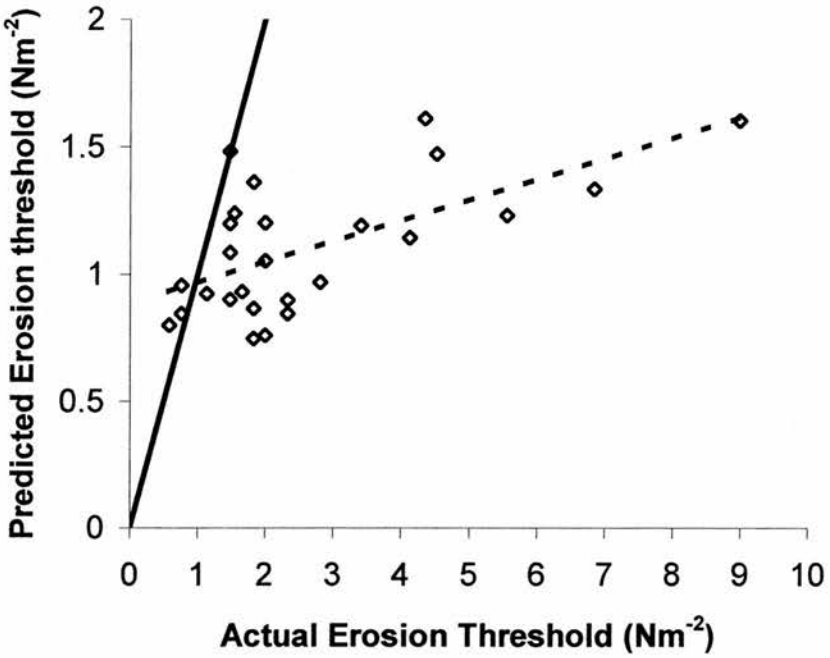


Fig 8.6 Water content plotted against chlorophyll *a* concentration ($r^2 = 0.13$).

Table 8.2 Mean ± SE and range of erosion thresholds for sand and sandy-mud, and muddy-sand sites, characterised by their water contents (low <40%; high >40%) and chl *a* concentration (low <99 mg m^{-2}; high >100 mg m^{-2}).

Sand & Sandy- mud sites	Number of sites	Mean Erosion Threshold Value	Minimum Erosion Threshold Value	Maximum Erosion Threshold Value
Low water content and high Chl *a*	37	3.42 Nm^{-2}	0.58 Nm^{-2}	9.08 Nm^{-2}
Low water content and low Chl *a*	44	1.79 Nm^{-2}	0.58 Nm^{-2}	3.84 Nm^{-2}
High water content and high Chl *a*	9	2.54 Nm^{-2}	1.48 Nm^{-2}	4.12 Nm^{-2}
High water content and low Chl *a*	11	1.38 Nm^{-2}	0.76 Nm^{-2}	2.49 Nm^{-2}
Muddy-sand sites				
Low water content and high Chl *a*	12	2.65 Nm^{-2}	0.64 Nm^{-2}	5.17 Nm^{-2}
Low water content and low Chl *a*	22	2.15 Nm^{-2}	0.76 Nm^{-2}	4.25 Nm^{-2}
High water content and high Chl *a*	10	4.05 Nm^{-2}	1.48 Nm^{-2}	8.99 Nm^{-2}
High water content and low Chl *a*	13	1.79 Nm^{-2}	0.58 Nm^{-2}	4.12 Nm^{-2}

A



B

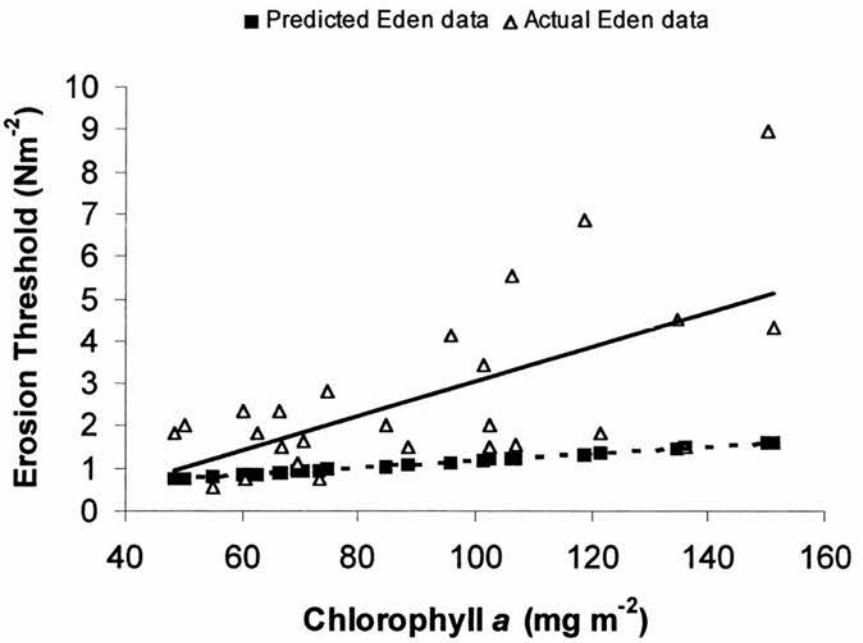


Fig 8.7: A The predicted erosion thresholds derived from the predictive model of Riethmüller *et al.* (1998) (y = 0.0084x + 0.34) plotted against the actual thresholds measured for muddy-sand sites by the CSM system. **B** Predicted erosion thresholds (derived from the predictive model of Riethmüller *et al.* (1998); y = 0.0084x + 0.34) and actual erosion thresholds (derived using the CSM) plotted against the actual chlorophyll *a* concentrations measured at the Eden muddy-sand sites.

sites ($F_{3,97} = 4.11$; $p < 0.011$), whilst low water content sites did not differ significantly between high and low chlorophyll *a* sites (Table 8.2).

8.4.4 A multivariate approach

At the 1% level, only chlorophyll *a* was found to be a significant predictor of erosion threshold ($r = 0.64$; $p < 0.001$; SE = 1.34 Nm^{-2}; $y = 0.019x + 0.536$). Water content, fine-grain fraction, organic content and the abundance of *N. diversicolor*, *C. volutator* and *H. ulvae* were all found to be insignificant at the 1% level (Table 8.3).

8.5 Testing the published model

Chlorophyll *a* concentrations for muddy-sand sites of the Eden Estuary were used to calculate erosion thresholds from the predictive function of Riethmüller *et al.* (1998). Actual erosion thresholds ranged between 0.58 Nm^{-2} and > 9.08 Nm^{-2}, whilst predicted values ranged between 0.75 Nm^{-2} and 1.61 Nm^{-2} (Fig. 8.7A). Whilst there is agreement with the model proposed by Riethmüller *et al.* (1998) at the lower end of the chlorophyll *a* scale, the relationship deteriorated when greater chlorophyll *a* concentrations (>100 mg m^{-2}) were used (Fig. 8.7B).

8.6 Discussion

Whilst the CSM still represents the most rapid deployment method for measuring the stability of estuarine sediments, proxy parameters that can be more easily mapped by field surveys or remote sensing are needed to generate maps of sediment stability that are representative of entire mudflats at a given moment in time. During the multidisciplinary project, BIOPTIS, measurements of sediment stability were made in conjunction with matched samples of chlorophyll *a*, grain size, water content, organic content, and macrofaunal densities. Thus data were available to determine whether any of these variables could provide a proxy parameter for sediment stability.

8.6.1 Chlorophyll *a* as a proxy parameter

In past studies chlorophyll *a* was found to be the most important variable correlating with sediment stability (Paterson *et al.*, 2000; Riethmüller *et al.*,

2000; Austin *et al.*, 1999; Riethmüller *et al.*, 1998; Hakvoort *et al.*, 1998). From a suite of variables obtained during this study, and stepwise multiple linear regressions, chlorophyll *a* was found to be the only significant predictive parameter of erosion threshold. Although erosion threshold increased with increasing chlorophyll *a*, chlorophyll *a* alone was still a poor predictor of sediment stability since it explained only 40% of the variation. Moreover, the surface sediment types present and the sediment water content (traditionally considered the primary controls of stability on cohesive sediments) did not explain the variation within the data set (Fig. 8.5 and Table 8.2). Interestingly, the majority of values that deviated from the published model were all from low shore sites, situated close to the channel of the River Eden on the south shore side. Friend *et al.*, (in press) found critical erosion thresholds to decrease seawards within the Ria Formosa tidal lagoon, Portugal, and this reflected a change from biostabilisation by cyanobacteria in the upper intertidal area, to biostabilisation by diatoms on the substrata of the channel edges. Future work could include the influence of assemblage structure on sediment stability. For example, Madsen et al. (1993) recorded an increase in sediment stability corresponding to an increase in the volume of Navicula, Amphora and Nitzschia species, as well as the larger sigmoid genera Gyrosigma and Pleurosigma.

The correlation between erosion threshold and chlorophyll *a* found by Riethmüller *et al.* (1998) became progressively weaker with decreasing fine-grain fraction present. This study found sediment erosion thresholds varied significantly, both within and between grids (Figs. 8.3; 8.4). The relationship between chlorophyll *a* and erosion threshold of Grid B was weaker than that of Grid A, probably because measurements from Grid A came from sediments whose fine-grain fraction (<63 μm) varied between 10% and 25%, whilst measurements from Grid B were made on sediments whose fine-grain fraction (<63 μm) varied between 10% and 50%, resulting in greater variation due to sediment type.

Chlorophyll *a* concentrations of sand and sandy-mud sites from the Eden estuary ranged from 18.5 mg m^{-2} to 388.7 mg m^{-2}, and had a weak positive correlation with erosion threshold. Paterson *et al.* (1994) found that on an intertidal sandy flat, an increase in critical erosion threshold was only observed for chlorophyll *a* concentrations greater than 500 mg m^{-2} (when a coherent mat

was formed). Sandy sediments have larger inter-particle voids that require a higher biomass before an increase in the surface cohesion of particles occurs, and consequent changes in erosion threshold are observed. This indicates that the sediment type mediates the stabilising effects of diatoms, and explains why Riethmüller *et al.* (2000) found the distinct dependencies of erosion threshold with chlorophyll *a* to be highly site specific.

8.6.2 Water content and biomass

Although high biomass may increase sediment stability due to the production of EPS, the highly hydrated nature of this substance may cause a concomitant increase in the water content of the biofilm. The stability of sediment without a biofilm would be expected to decrease with increasing water content. However, the presence of a biofilm may continue to enhance the stability of the sediment, even when the water content of the biofilm increases (Austin *et al.*, 1999). Of particular interest were the contrasting effects of water content on erosion threshold when sites were segregated by sediment type. Williamson & Ockenden (1996) found high erosion thresholds to occur at high biomass and low water content. Sites of the Eden Estuary that fall into these categories were expected to have the highest erosion thresholds on the mudflat. However, erosion thresholds of such sites ranged between 0.58 Nm^{-2} and 9.08 Nm^{-2}. This indicates that the interactions between biological and physical properties and processes are highly complex and consequently there may be unknown or unmeasured confounding factors influencing sediment stability.

8.6.3 Macrofauna did not influence stability

The presence and abundance of macrofaunal species such as *Macoma balthica* (Widdows *et al.*, 2000; Willows *et al.*, 1998), *H. ulvae* (Andersen, 2001; Austin *et al.*, 1999; Blanchard *et al.*, 1997), *Mytilus edulis* (Meadows *et al.* 1998; Widdows *et al.*, 1998a), *Cerastoderma edule* (Riethmüller *et al.*, 2000), *C. volutator* and *N. diversicolor* (Meadows *et al.*, 1990) have been shown to have a significant effect (some stabilising and some destabilising) on sediment erodibility, yet in this study no effect of benthic fauna was found. Densities of 3000 - 50000 m^{-2} (Meadows *et al.*, 1990; Möller, 1985), 7500 - 100000 m^{-2} (Gerdol & Hughes, 1993; Meadows *et al.*, 1990) and 5000 - 30000 m^{-2}

(Blanchard *et al.*, 2000; Morrisey, 1988; López-Figueroa & Niell, 1987) for *N. diversicolor*, *C. volutator* and *H. ulvae* respectively have been reported. The lack of influence on sediment stability from these species may be due to the relatively low densities found at most sites during this study (Table 8.3).

8.6.4 Measuring devices

The data from muddy sand sites of the Eden Estuary highlighted the dangers of extrapolating the predictive relationship of Riethmüller *et al.* (1998) above chlorophyll *a* concentrations of 60 mg m^{-2}. As it stands, the model is not generally applicable and is likely to be site and possibly technique specific. For example, one reason for the underestimation of erosion thresholds by the EROMES device is that it uses different criteria to the CSM in order to define the erosion threshold, although standardization between the two devices by means of an attenuation threshold has been shown to improve the comparability of the data (Tolhurst *et al.*, 2000b). Spatial heterogeneity of the sampling sites could also affect the erosion thresholds. The EROMES device integrates its measurements over a larger area (so can include biofilm and non-biofilm sites) compared to the CSM, which has a 'footprint' of 7×10^{-4} m^2, and therefore only takes a point measurement. Thus, assuming a diatom patch was measured, this would result in higher erosion thresholds from the CSM. Furthermore, it has been hypothesized that the diatom cells themselves may act to armour the sediment surface (Tolhurst *et al.*, in review). The biofilms at the Eden sites were very thick, and were probably not migrating during CSM measurements, whilst diatoms have been observed to migrate below the surface of the sediment when the motor of the EROMES device is started up (Tolhurst pers. comm.). Considering these points together, it is not surprising that stabilities from the EROMES device predict lower erosion thresholds at higher chlorophyll *a* concentrations. It would be beneficial for future research to test if measurement techniques are a confounding factor.

8.6.5 Missing factors

The underestimation of sediment erosion thresholds using the published function of Riethmüller *et al.* (1998) and the weak dependence and large scatter in the relationships presented here (despite much clearer dependencies obtained

from laboratory studies e.g. Tolhurst *et al.*, in press), suggest that the natural system exhibits non-linear dynamics; that factors have not been included in the regression analysis; or that a more advanced model is required. For example, Ruddy *et al.* (1998) propose that a highly interdependent community of benthic algae, bacteria and macro-heterotrophs, acts to regulate sediment dynamics via small-scale nitrogen cycling. He suggests that the algal exudation of excess carbon, which acts as a sediment-binding agent, is critical and controlled by nutrient availability. It has also been suggested that larger diatom species may stabilise the sediment more effectively than smaller species, since they are likely to extrude more extracellular mucopolysaccharides (Riethmüller *et al.*, 2000; Underwood & Smith, 1998; Madsen *et al.*, 1993; Holland *et al.*, 1974). Initial studies have shown that different diatom species do affect sediment stability to different degrees (CLIMEROD final report, 2002). Large species such as *Gyrosigma fasciola*, *Gyrosigma balticum*, *Pleurosigma angulatum*, *Nitzschia recta* and *Surirella gemma* are found in assemblages of the Eden Estuary, although diatom assemblage composition was not assessed with each measurement of sediment stability in this study. The actual proportions these species represent within an assemblage, together with the amount of EPS produced by each species, have yet to be established.

8.7 Conclusions

Biological and physical properties and processes act together and, depending on the conditions, their effects may be complimentary, synergistic, antagonistic or dominant. After several decades of attempts to find a simple predictive parameter for sediment stability, it must now be recognised that due to the high complexity of the system success is unlikely. It is only where a single mechanism of biostabilisation is dominant that a single parameter for a biological effect is likely to be the best predictor of sediment behaviour (Paterson, 1994). Whilst some efforts have been made to elucidate the effects of some components of the system, there has yet to be a study that investigates all of the possible biological and physical factors that may be influencing the system.

In the past, the approach to the problem was often a reductionist method, using controlled laboratory studies in order to elucidate the effects of individual biological and physical components of the system. Next, two or more of these

components should be combined to elucidate interactions between the different components. What is evident from this study, is that a holistic approach is needed, where large-scale detailed investigations of the whole system and multivariate analysis are used to determine the effect of complex interactions between biological and physical properties and processes on sediment stability. It is clear that we are missing some very important interactions. At present, it may be possible to offer a 'suite' of site-specific biological and physical parameters that could be used to estimate critical erosion thresholds. For example, using a data set that covers large areas of an estuary, it may be possible to categorise sites according to their biological status and physical characteristics. According to such groupings, a range of potential erosion thresholds could be presented. With regards to establishing which areas of coastline and mudflats will be susceptible to erosion as a result of sea level rise, we are yet to have a rapid predictive tool and are presently still reliant on the more time- consuming field measurements.

Chapter 9

Chapter 9: Site-specific features influence sediment stability of intertidal mudflats

Abstract

The factors that influence sediment stability and transport in estuarine areas are not yet fully understood, but this knowledge is essential for coastal engineering applications and pollution ecology studies. It is suggested that variation in predictive models of sediment stability might be due to site-specific characteristics, and this investigation explores this by using data from four estuarine mudflats (Eden Estuary, Scotland, and three mudflats in the Netherlands; Biezelingse Ham, Zandkreek and the Molenplaat). These estuaries differ in their environmental conditions, macrofaunal species composition and local features (e.g. Enteromorpha mats, migratory biofilms). Stable and unstable sediments were compared, and mean chlorophyll a and granulometry of the sediments were significantly different between the two groups. Stepwise multiple linear regressions were applied to the sediment stability data of all sites in order to establish the influences of microphytobenthic biomass, water content, granulometry, organic carbon content and the abundance of dominant macrofaunal species on erosion threshold. The stability of each site was influenced by different factors. An Enteromorpha bloom affected sediment stability of the Eden Estuary; Biezelingse Ham was influenced by the highly migratory nature of the diatom biofilms and the abundance of Corophium volutator; the polychaete worm Arenicola marina had a net negative effect on sediment stability of the Zandkreek; and the Molenplaat was influenced by microphytobenthic biomass. This research highlights the need for site-specific calibration of models, and suggests that a universal proxy parameter for sediment stability is unlikely to be obtained. In terms of sediment stability and with regard to studies investigating the effect of sea level rise on estuaries and coastal erosion, this investigation highlights that local features will indeed have a large role to play, and will have to be considered.

Chapter 9: Site-specific features influence sediment stability of intertidal mudflats

9.1 Introduction

The interactions between physical and biological processes in mediating sediment erosion are, for the most part, poorly quantified and understood (Widdows *et al.*, 2000). This makes it difficult for predictive models of sediment stability to be robust. In past studies, chlorophyll *a* was found to be the most important variable correlating with sediment stability (Paterson *et al.*, 2000; Riethmüller *et al.*, 2000; Austin *et al.*, 1999; Hakvoort *et al.*, 1998; Riethmüller *et al.*, 1998; Defew *et al.*, in press). However, the previous chapter and these other studies have collected information from a number of varied sites, and it has become clear that there are still large amounts of scatter in the data sets. This suggests that the factors affecting sediment stability are not simply linear relationships, but are instead much more complex. Riethmüller *et al.* (2000) suggested that variation in data sets might be due to site-specific features, and this chapter investigates this possibility by using data from four estuarine mudflats, covering a broad range of conditions, species composition and local features (e.g. an *Enteromorpha* mat and a highly migratory biofilm).

The experimental aims were to:

- Investigate further some of the factors that influence the stability of intertidal surface sediment.
- Determine how site-specific features influence sediment erodibility, and whether they contribute to variation in data sets.

Measurements of sediment stability, matched with other biological and physical variables, were taken from four estuarine mudflats that differed in their site-specific features. Two sites were used on the Eden Estuary, Scotland, where a local feature of the estuary was the formation of an extensive *Enteromorpha* spp. bloom (Fig. 9.1). Three sites in the Netherlands were used: namely the Biezelingse Ham mudflat whose local feature was the presence of a highly migratory biofilm (Fig 9.1); the Zandkreek mudflat, which to date is relatively understudied; and the Molenplaat mudflat, which is known for the seasonal

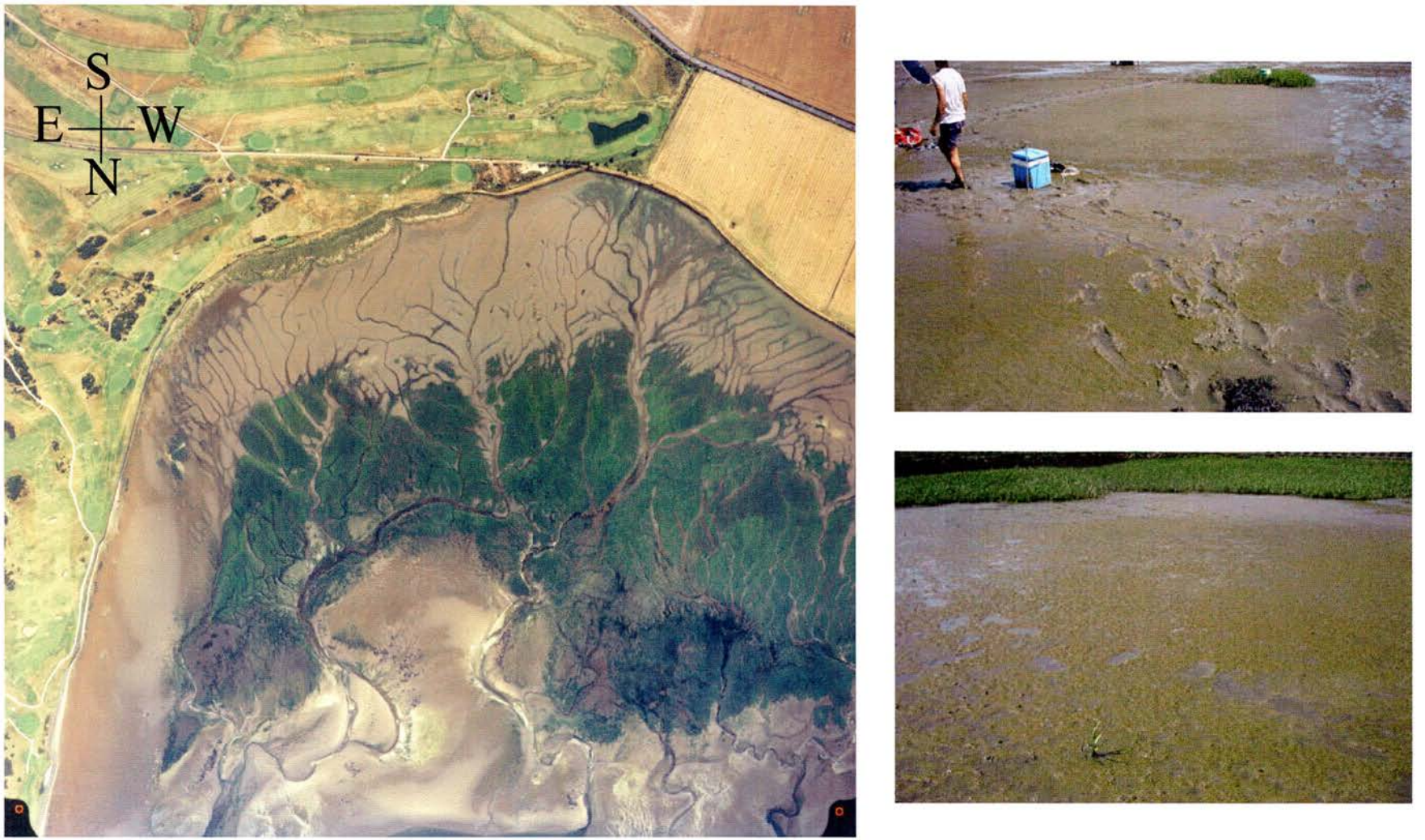


Fig. 9.1 Eden Grid A whose site specific feature was a large bloom of *Enteromorpha* (Aerial Photograph courtesy of BIOPTIS), and the thick migratory biofilm at Biezelingse Ham (Photographs courtesy of Dr Richard Ford).

appearance and disappearance of a centralised area where silt and diatoms interact and accumulate (Herman *et al.*, 2001; Lucas & Holligan, 1999).

9.2 Methods

See Chapter 2 for detailed descriptions of the sites and macrofaunal populations present. Each measurement of erosion threshold (made using the CSM) was paired with a contact core, which was used for measurement of chlorophyll *a*, sediment grain size, water content and organic content. Sediments whose erosion thresholds were >2 Nm^{-2} were considered to be '*stable*'. At *Enteromorpha*-dominated sites, this macrophyte was carefully moved off the sediment surface in order for CSM measurements to be made. For convenience, grain size distribution was split between five groups: gravel (>2 mm), coarse sand (1-2 mm), medium sand (0.250-1 mm), fine sand (0.063-0.250 mm) and silt-clay fraction (<0.063 mm). Macrofaunal densities at each grid node and F_o^{15} (fluorescence proxy for microphytobenthic biomass) were also measured.

In April 2001, sediment stability and biomass (F_o^{15}) at the sediment surface was mapped (i.e. coupled measurements were taken on the same patch of sediment at each time period) for the duration of a tidal exposure period, on a thick confluent biofilm situated at a high shore site on the Biezelingse Ham mudflat. Water content and colloidal carbohydrate concentration were determined from contact cores taken as close as possible to the CSM measurement.

9.2.1 Statistics

Using SPSS (version 10.05), stepwise multiple linear regressions (Zar, 1999) were applied to the sediment stability data to establish the influences of chlorophyll *a*, F_o^{15}, water content, sediment composition, organic content and the abundance of dominant macrofaunal species on erosion threshold. Prior to analysis, all necessary transformations were applied to the data to conform to normality of distribution and homogeneity of variances. In the analysis of the entire data set, a total of 321 measurements were considered. Significant differences in mean values between stable and unstable sediments, and between sites in the presence and absence of *Enteromorpha* spp., were examined using the T-test and one-way ANOVA.

9.3 Results

9.3.1 Eden Estuary Mudflats

For both of the grids, all measured variables were compared between sites in the presence and absence of the *Enteromorpha* bloom (Table 9.1a). Water content of the sediments was significantly higher under *Enteromorpha* on both grids ($F_{3,245} = 101.02$; $p = \leq 0.001$). Chlorophyll *a* was significantly higher at *Enteromorpha* sites on Grid A, but not on Grid B ($F_{3,245} = 34.94$; $p = \leq 0.001$). The percentage of medium sand was greater on *Enteromorpha* sites at Grid B, but not significantly different on Grid A ($F_{3,245} = 21.46$; $p = \leq 0.001$). The percentage of silt-clay and organic carbon, and minimum fluorescence was greater on *Enteromorpha* sites at Grid A, but not significantly different on Grid B ($F_{3,245} = 43.15$; $p = \leq 0.001$; $F_{3,245} = 87.17$; $p = \leq 0.001$; $F_{3,245} = 31.02$; $p = \leq 0.001$ respectively).

On Grid A, the abundances of *Scrobicularia plana*, *Hydrobia ulvae* and Tubificoides spp. were significantly greater at *Enteromorpha* sites, whilst the abundance of *Nereis diversicolor* was significantly lower ($F_{3,245} = 9.01$; $p = \leq 0.001$; $F_{3,245} = 18.24$; $p = \leq 0.001$; $F_{3,245} = 30.95$; $p = \leq 0.001$; $F_{3,245} = 6.97$; $p = \leq 0.001$ respectively). On Grid B, the abundances of *Spio filiformis* and Oligochaete spp. were significantly greater at *Enteromorpha* sites ($F_{3,245} = 6.37$; $p = \leq 0.001$; $F_{3,245} = 66.13$; $p = \leq 0.001$ respectively) (Table 9.1b).

Stepwise multiple linear regressions were carried out on all data from the Eden Estuary. Three variables were found to be significant predictors of erosion threshold at the 1% level: percentage gravel (a), abundance of *N. diversicolor* (b) and percentage silt-clay fraction (c) ($r^2 = 0.12$; $p < 0.001$; SE = 1.50 Nm^{-2}; $y = 0.069a + 0.026b - 0.018c + 2.37$). Since this was a poor predictive model, data were split according to grid, and whether *Enteromorpha* was present or absent (Table 9.2). The predictive model explaining the greatest amount of variation was for Grid B in the absence of *Enteromorpha*, with water content, chlorophyll *a* concentration and abundance of *Macoma balthica* explaining 77% of the variation. Mean erosion thresholds were significantly higher for sediments without *Enteromorpha* when Eden data was grouped together (Fig. 9.2; $t = 2.51$; $p = 0.013$; $df = 184$). However, this difference was also grid-specific. Erosion thresholds of *Enteromorpha* sites were significantly lower than non-

Table 9.1a: Descriptive statistics of erosion threshold, water content, algal biomass, granulometry and organic carbon for Eden Estuary sites, with and without *Enteromorpha*. Mean ± Standard Error, with minimum and maximum values in parentheses.

	Grid A without *Enteromorpha*	**Grid A with *Enteromorpha***	**Grid B without *Enteromorpha***	**Grid B with *Enteromorpha***
Number of samples	106	53	65	25
Erosion Threshold (Nm^{-2})	2.9 ± 0.2 (0.6 – 9.1)	2.3 ± 0.2 (0.1 – 5.9)	2.0 ± 0.2 (0.6 – 9.0)	2.1 ± 0.3 (0.6 – 6.2)
Water Content (%)	34.2 ± 1.7 (11.5 – 93.5)	80.7 ± 2.4 (30.8 – 95.0)	42.2 ± 1.2 (25.4 – 67.3)	53.9 ± 4.1 (25.1 – 94.7)
Chlorophyll *a* ($mg\ m^{-2}$)	172.8 ± 17.4 (18.5 -1303.0)	456.5 ± 53.4 (19.5 - 2091.4)	79.8 ± 3.6 (27.0 – 151.0)	83.1 ± 10.2 (27.0 – 207.0)
Gravel (%)	3.0 ± 0.6 (0.0 – 31.5)	4.4 ± 1.1 (0.0 – 31.5)	0.8 ± 0.2 (0.0 – 8.2)	1.5 ± 0.4 (0.1 – 5.2)
Coarse Sand (%)	1.2 ± 0.2 (0.0 – 8.6)	1.2 ± 0.2 (0.0 – 4.7)	0.2 ± 0.02 (0.0 – 1.0)	0.7 ± 0.2 (0.2 – 3.2)
Medium Sand (%)	7.2 ± 0.5 (0.1 – 18.6)	6.6 ± 0.6 (0.1 – 18.2)	3.9 ± 0.4 (0.9 – 16.4)	12.3 ± 1.1 (4.0 – 23.2)
Fine Sand (%)	78.4 ± 1.7 (0.2 – 98.3)	72.5 ± 2.2 (0.2 – 93.6)	69.1 ± 1.4 (41.8 – 83.5)	61.6 ± 3.6 (23.1 – 82.1)
Silt-Clay (%)	8.4 ± 0.7 (0.1 – 37.3)	13.4 ± 1.0 (0.8 – 37.3)	26.0 ± 1.5 (12.9 – 55.8)	24.0 ± 4.2 (10.2 – 72.5)
Organic Carbon (%)	5.7 ± 0.9 (0.4 – 65.7)	24.5 ± 1.5 (2.8 – 53.6)	3.6 ± 0.1 (1.9 – 5.4)	3.5 ± 0.3 (1.6 – 6.9)
Minimum Fluorescence	187 ± 28 (7 – 2166)	636 ± 67 (12 – 1818)	140 ± 24 (7 – 960)	158 ± 31 (14 – 628)

Table 9.1b: Descriptive statistics of dominant macrofaunal species for Eden Estuary sites, with and without *Enteromorpha*. Mean ± Standard Error, with minimum and maximum values in parentheses (individuals 0.028 m^{-2}).

	Grid A without *Enteromorpha*	**Grid A with *Enteromorpha***	**Grid B without *Enteromorpha***	**Grid B with *Enteromorpha***
Macoma balthica	4.0 ± 0.4 (0 – 16)	5.0 ± 0.6 (0 – 15)	2.0 ± 0.2 (0 – 6)	3.0 ± 0.6 (0 – 10)
Cerastoderma edule	7.0 ± 1.1 (0 – 48)	8.0 ± 1.2 (0 – 38)	0.3 ± 1.07 (0 – 2)	0.6 ± 0.3 (0 – 4)
Scrobicularia plana	2.0 ± 0.4 (0 - 16)	5.0 ± 0.5 (0 - 16)	3.0 ± 0.5 (0 – 13)	2.0 ± 0.5 (0 – 8)
Eteone longa	1.0 ± 0.2 (0 – 6)	0.2 ± 0.08 (0 – 2)	3.0 ± 0.8 (0 – 29)	3.0 ± 1.1 (0 – 17)
Spio filiformis	13.0 ± 2.7 (0 – 188)	15.0 ± 5.0 (0 – 188)	6.0 ± 1.4 (0 – 41)	54.0 ± 26.2 (0 – 401)
***Oligochaete* spp.**	3.0 ± 0.6 (0 – 35)	15.0 ± 5.3 (0 – 160)	132.0 ± 13.0 (24 – 444)	200.0 ± 36.5 (11 – 518)
Corophium volutator	0.2 ± 0.04 (0 – 1)	0.0 ± 0.0 (0 – 0)	104.0 ± 10.8 (0 – 230)	140.0 ± 29.0 (1 – 473)
Hydrobia ulvae	139.0 ± 20.8 (0 – 752)	227.0 ± 30.6 (0 – 840)	13.0 ± 3.3 (0 – 114)	11.0 ± 4.3 (0 – 64)
Nereis diversicolor	10.0 ± 1.4 (0 – 88)	2.0 ± 0.8 (0 – 28)	10.0 ± 1.3 (0 – 50)	9.0 ± 2.5 (0 – 40)
Mytilus edulis	5.0 ± 3.6 (0 – 383)	18.0 ± 10.1 (0 – 383)	0.0 ± 0.0 (0 – 0)	0.0 ± 0.0 (0 – 0)
***Tubificoides* spp.**	63.0 ± 12.1 (0 – 606)	799.0 ± 151.0 (0 – 4000)	0.0 ± 0.0 (0 – 0)	0.0 ± 0.0 (0 – 0)

Table 9.2: Results of stepwise multiple linear regressions on Eden Estuary data.

	Variance explained	Significance	Standard Error (Nm^{-2})	Significant Predictors
Eden Estuary Grid A (All sites)	$r^2 = 0.20$	$p = 0.022$	1.46	$Y = -0.09a + 0.065b + 0.029c - 0.0052d + 2.59$ Where a = *Scrobicularia plana* abundance, b = % gravel, c = *Nereis diversicolor* abundance and d = *Mytilus edulis* abundance
Eden Estuary Grid A (*Enteromorpha* sites excluded)	$r^2 = 0.12$	$p = 0.006$	1.60	$Y = 0.09a + 0.031b + 2.40$ Where a = % gravel and b = *Nereis diversicolor* abundance
Eden Estuary Grid A (*Enteromorpha* sites only)	$r^2 = 0.45$	$p = 0.048$	1.01	$Y = 0.11a - 0.59b - 0.85c - 0.026d + 0.0007e + 4.33$ Where a = % gravel, b = % coarse sand, c = *Eteone longa* abundance, d = water content and e = minimum fluorescence
Eden Estuary Grid B (All sites)	$r^2 = 0.40$	$p = 0.041$	1.13	$Y = 0.014a + 0.0014b + 0.14c + 0.022d - 0.58$ Where a = chlorophyll *a* concentration, b = minimum fluorescence, c = *Macoma balthica* abundance and d = water content
Eden Estuary Grid B (*Enteromorpha* sites excluded)	$r^2 = 0.59$	$p = 0.001$	0.94	$Y = 0.081a + 0.017b + 0.22c - 3.20$ Where a = water content, b = chlorophyll *a* concentration and c = *Macoma balthica* abundance
Eden Estuary Grid B (*Enteromorpha* sites only)	$r^2 = 0.29$	$p = 0.005$	1.21	$Y = 0.133a + 0.47$ Where a = % medium sand

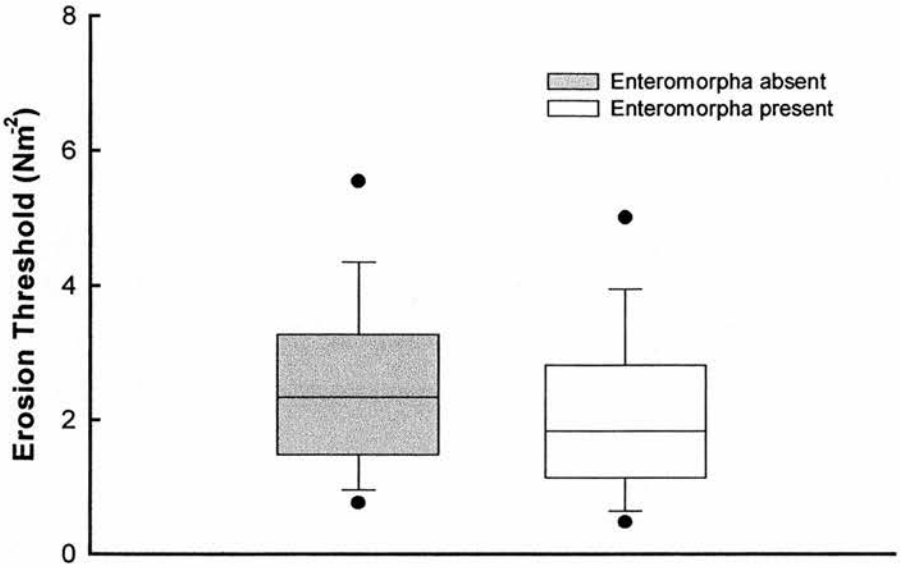


Fig. 9.2: Erosion thresholds (Nm^{-2}) for sediments in the presence and absence of an *Enteromorpha* spp. bloom. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. Black circles represent the 5^{th} and 95^{th} percentiles.

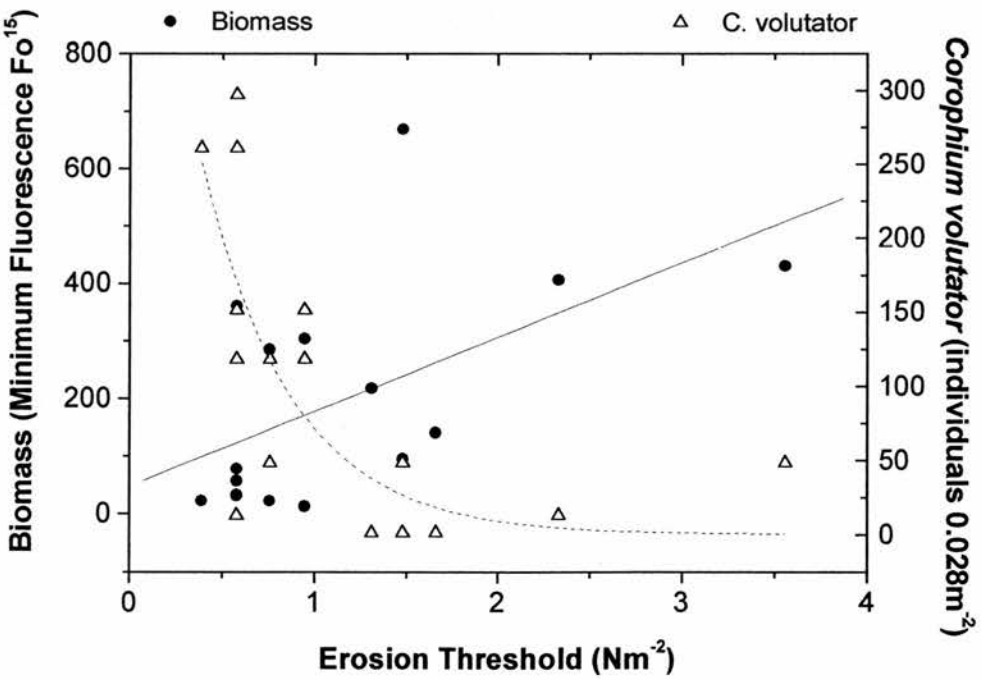


Fig. 9.3: Diatom biomass and *Corophium volutator* density at the Biezelingse Ham site were inversely correlated at high erosion thresholds, with opposing effects on sediment stability. Solid line represents minimum fluorescence ($r^2 = 0.31$; $y = 129.07x + 47.96$) and dashed line represents *C. volutator* density ($r^2 = 0.30$; $y = 251.14e^{\wedge((x-0.39)/0.49)}$).

Enteromorpha sites on Grid A, but were not significantly different on Grid B ($F_{3,245} = 7.49$; $p < 0.001$).

9.3.2 Biezelingse Ham Mudflat

Stepwise multiple linear regressions were carried out on the data (excluding the dominant macrofaunal species), and minimum fluorescence was found to be a significant predictor of erosion threshold at the 1% level ($r^2 = 0.34$; $p < 0.014$; SE = 0.71 Nm^{-2}; $y = 0.0026x + 0.61$). When dominant macrofauna were included in the analysis, the abundance of *Corophium volutator* was a significant predictor of erosion threshold at the 1% level ($r^2 = 0.35$; $p < 0.013$; SE = 0.71 Nm^{-2}; $y = -0.0048x + 1.63$). Erosion threshold increased with increasing diatom biomass (F_o^{15}), whilst stability was high below a threshold of 50 *Corophium volutator* individuals 0.028m^{-2} (Fig. 9.3).

Over a tidal exposure period, both biological and physical processes controlled sediment stability of a thick biofilm. At the beginning of the exposure period, surface stability was '*unstable*' (<2 Nm^{-2}) and surface biomass was low. For 1.5 h after the tidal emersion, surface stability and biomass increased at similar rates. This was followed by a decrease in stability and biomass until the end of the exposure period, although the rate of decrease for stability was less than the rate of decrease for surface biomass (Fig. 9.4a). During the exposure period, water content gradually decreased whilst colloidal carbohydrate increased (Fig. 9.4b).

9.3.3 Zandkreek Mudflat

Stepwise multiple linear regressions were applied to the Zandkreek data set. In the absence of macrofaunal abundance data, erosion threshold was not significantly predicted by grain size, algal biomass or water content. When macrofaunal abundance was included, the abundance of *Arenicola marina* was a significant predictor of erosion threshold at the 1% level ($r^2 = 0.14$; $p = 0.039$; SE = 0.88 Nm^{-2}; $y = -0.14x + 1.33$). Increasing abundance of *A. marina* had a negative effect on sediment stability, although all stabilities were below 2 Nm^{-2} indicating that the sediments were '*unstable*' according to the criteria used in this study (Fig. 9.5).

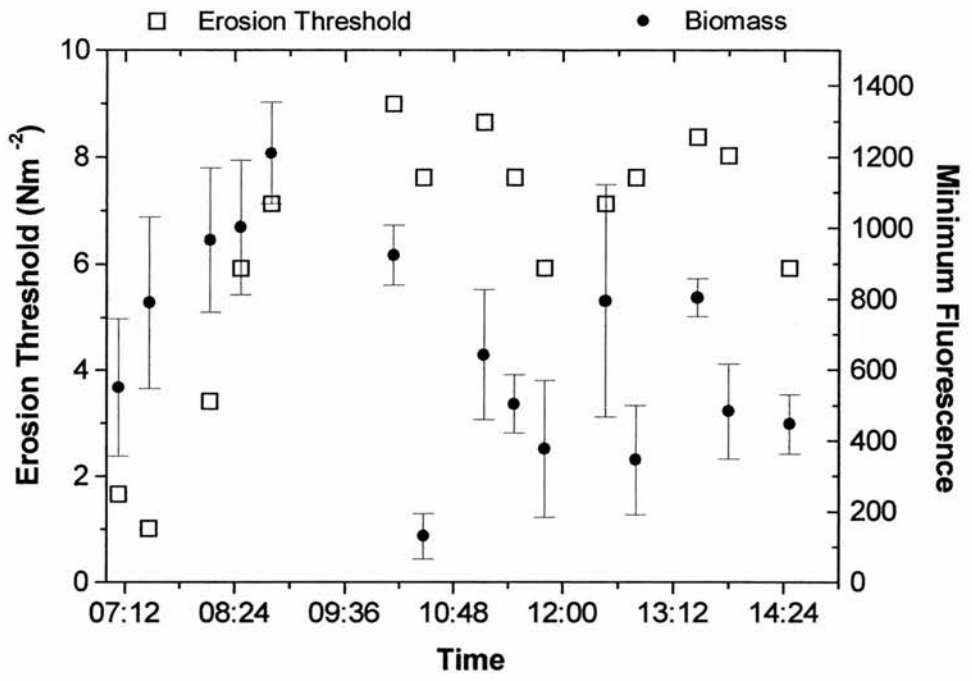


Fig. 9.4a: Erosion threshold and sediment surface biomass measured at regular intervals during a tidal emersion period on the Biezelingse Ham mudflat. Erosion threshold (n = 1) at each time interval matched with biomass measurements (n = 3).

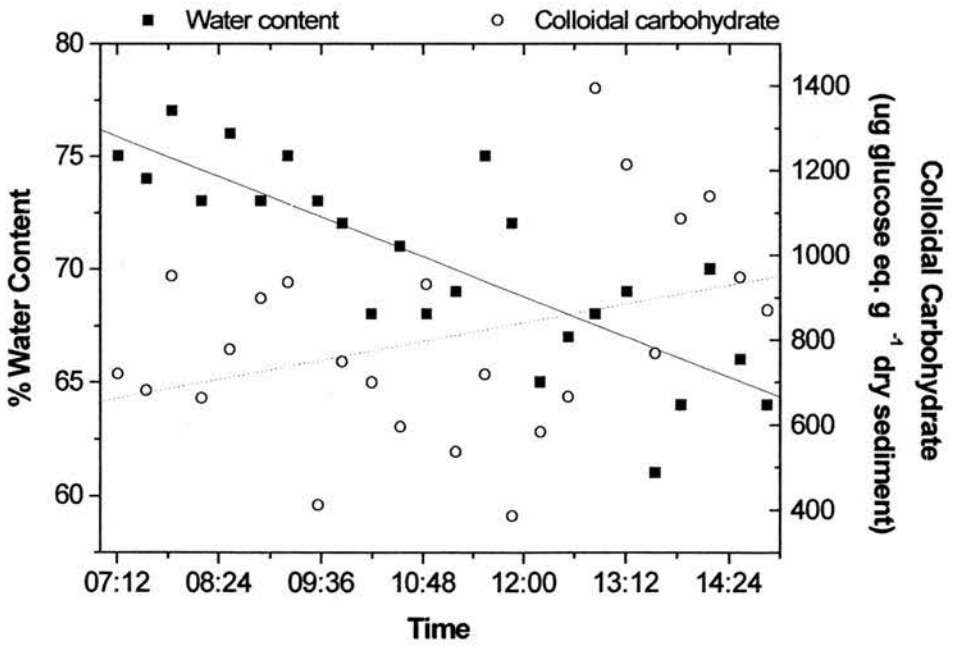


Fig. 9.4b: Water content (%) and colloidal carbohydrate (µg glucose eq. g^{-1} dry sediment) measured at regular intervals during a tidal emersion period on the Biezelingse Ham mudflat (n = 1 at each time interval). Solid line represents water content ($r^2 = 0.65$; $y = -35.49x + 86.53$) and dashed line represents colloidal carbohydrate ($r^2 = 0.13$; $y = 886.57x + 396.75$).

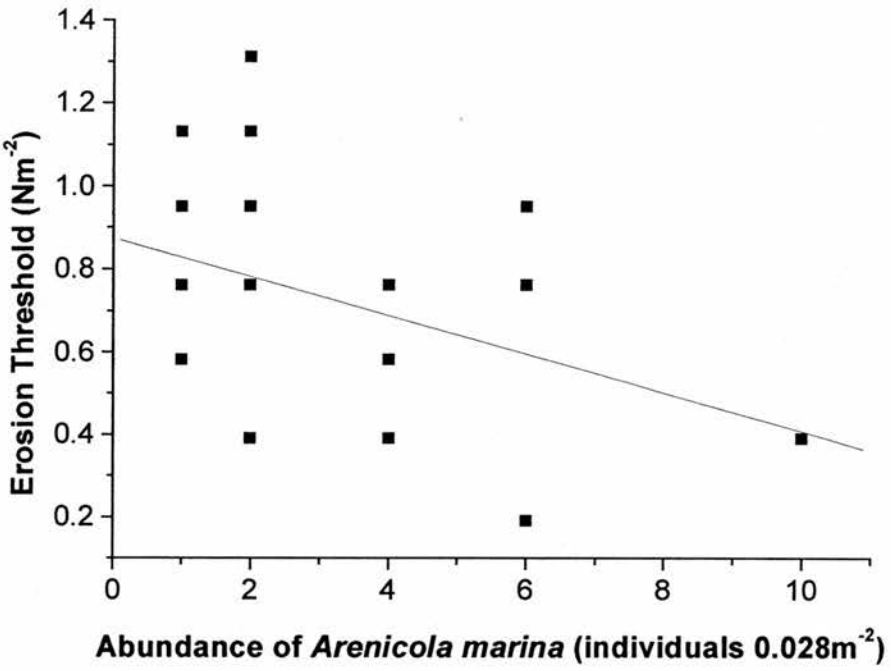


Fig. 9.5 Erosion threshold (Nm^{-2}) decreases with increasing abundance of *Arenicola marina* (individuals $0.028m^{-2}$) on the Zandkreek mudflat ($r^2 = 0.16$; $y = 0.047x + 0.88$).

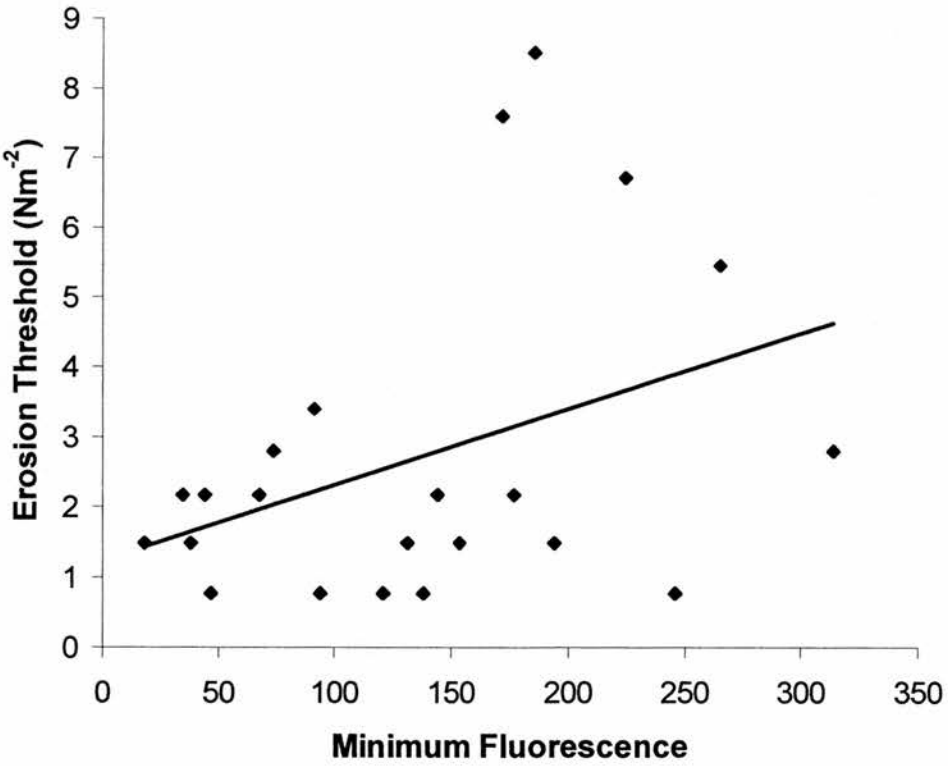


Fig. 9.6 Erosion threshold (Nm^{-2}) increases with increasing sediment surface diatom biomass described by minimum fluorescence on the Molenplaat ($r^2 = 0.15$; $y = 0.011x + 1.23$).

Table 9.3: Descriptive statistics for the combined data set (i.e. all sites) of water content, microalgal biomass, granulometry and organic carbon of "*stable*" (>2 Nm^{-2}; n = 158) and "*unstable*" (<2 Nm^{-2}; n = 163) sediments. Mean values compared using the t-test; n.s. = not significant.

		Significance	Mean	Median	Std. Deviation	Std. Error	Minimum	Maximum
Water content (%)	*Unstable*	n.s.	46.7	39.1	22.6	1.8	21.9	95.0
	Stable		43.6	32.1	22.9	1.8	11.5	94.7
Minimum Fluorescence (F_o^{15})	*Unstable*	n.s.	210	114	276	22	3	1768
	Stable		274	121	388	31	7	2166
Chlorophyll *a* ($mg\ m^{-2}$)	*Unstable*	$p \leq 0.002$	135.3	81.5	189.5	14.8	14.0	1524.9
	Stable		214.5	124.6	268.7	21.4	18.5	2091.4
% Gravel (>2 mm)	*Unstable*	$p \leq 0.001$	1.2	0.2	2.4	0.2	0.0	20.3
	Stable		3.0	0.5	6.5	0.5	0.0	31.5
% Coarse sand (1-2 mm)	*Unstable*	$p < 0.001$	0.4	0.2	0.8	0.06	0.0	4.7
	Stable		1.0	0.3	1.6	0.1	0.0	8.6
% Medium sand (0.250-1 mm)	*Unstable*	$p < 0.001$	29.7	7.0	37.6	3.0	0.05	97.5
	Stable		17.3	7.2	27.0	2.2	0.8	99.0
% Fine sand (0.063-0.250 mm)	*Unstable*	$p < 0.001$	50.7	68.0	33.2	2.6	0.16	97.9
	Stable		67.0	76.2	25.6	2.0	0.6	98.3
% Silt-clay (<0.063 mm)	*Unstable*	$p \leq 0.003$	16.2	12.3	16.6	1.3	0.1	72.5
	Stable		11.7	9.5	9.1	0.7	0.1	41.1
Organic carbon (%)	*Unstable*	n.s.	7.1	3.3	9.7	0.8	0.4	53.6
	Stable		7.8	3.2	11.1	0.9	0.9	65.7

9.3.4 Molenplaat Mudflat

Stepwise multiple linear regressions were applied to the Zandkreek data set. Minimum fluorescence was a significant predictor of erosion threshold at the 1% level ($r^2 = 0.27$; $p = 0.011$; SE = 2.15 Nm^{-2}; $y = 0.0063x + 1.81$) (Fig. 9.6).

9.3.5 Complete data set

Stepwise multiple linear regressions were applied to the complete data set. Seven variables were found to be significant predictors of erosion threshold at the 1% level; percentage fine sand (a), percentage gravel (b), *N. diversicolor* (c), *M. balthica* (d), minimum fluorescence (e), water content (f) and *C. edule* (g) ($r^2 = 0.19$; $p = < 0.001$; SE = 1.51 Nm^{-2}; $y = 0.013a + 0.072b + 0.027c + 0.009d + 0.001e - 0.013f - 0.022g + 1.48$). However, these seven variables described less than half the total variation in the data, highlighting that certain site-specific features (such as *Enteromorpha* beds, migratory biofilms and dominant macrofauna) may be having an influential role, and should be investigated more closely. Data from all the sites were pooled, and '*stable*' (erosion threshold > 2 Nm^{-2}) and '*unstable*' sediments (erosion threshold < 2 Nm^{-2}) were compared (Table 9.3). Mean chlorophyll *a* was significantly higher for stable sediments. Mean percent of gravel, coarse sand and fine sand were significantly higher, whilst the mean percent of medium sand and silt-clay fraction were significantly lower in '*stable*' sediments.

9.4 Discussion

9.4.1 *Enteromorpha* influences sediment stability

Results of this study, in which multiple variables were measured in conjunction with sediment stability, highlight the complexity of the factors controlling the stability of intertidal sediments. The presence of an *Enteromorpha* bloom had a major influence on both the biological (species present) and physical (water content) properties of the sediment bed. However, few studies have investigated these effects (Bolam *et al.*, 2000). Sediment on Eden Grid A was significantly less stable under the *Enteromorpha* bloom. Since the mat on Grid A was much thicker, and covered a larger area than the mat found on Grid B, it may have different ecological effects to the smaller, spatially heterogeneous mat (Bolam *et al.*, 2000). For example, in agreement with the

results of Bolam *et al.* (2000), water content, organic carbon content and silt-clay fraction significantly increased in the sediments under weed mats on Grid A (Table 9.1a). The stability of sediment without a biofilm would be expected to decrease with increasing water content, and when mats of *Enteromorpha* were lifted away from the sediment surface, pools of surface water were found (Defew, pers. obs). Visual observations suggested that the sediment below the mat had a highly mobile unconsolidated surface floc layer, which was disturbed when the mat was lifted away for sediment stability measurements. This undoubtedly caused a reduction in the measured erosion threshold, and may have accounted for the observed differences. The overall effect of an *Enteromorpha* bloom on sediment transport is complex since *Enteromorpha* will act to retard water flow, reduce near bed shear stress and thus will trap fine-grained material (Paterson & Black, 2000). The protection afforded by the mat means that these areas are more likely to be depositional than erosional. This highlights the importance of considering data within the context of their setting.

9.4.2 The effect of macrofauna

Different macrofaunal assemblages are known to affect sediment stability in different ways (Andersen, 2001; de Deckere *et al.*, 2001; Riethmüller *et al.*, 2000; Widdows *et al.*, 2000; 1998b; Austin *et al.*, 1999; Meadows *et al.*, 1998; Willows *et al.*, 1998; Blanchard *et al.*, 1997; Meadows *et al.*, 1990). For example, on Eden Grid A, *N. diversicolor* had a net positive association with sediment stability, possibly due to the secretions produced during burrow formation and increased drainage of the sediment, yet on the Zandkreek mudflat, *A. marina* (also a polychaete worm) had a net negative effect on sediment stability. This may have been due to bioturbation effects such as a change in the particle size composition and an increase in the water content, or grazing pressure reducing the number of diatoms and consequently the amount of EPS produced (de Deckere *et al.*, 2001). The presence of macroalgal mats impacts significantly on the invertebrate assemblages, causing declines in some species and increases in others (Bolam *et al.*, 2000; Raffaelli, 2000; Raffaelli *et al.*, 1998; 1991; Everett, 1994; Hull, 1987). A change in macrofaunal assemblage structure may affect sediment stability, due to a change in bioturbatory/feeding behavior and/or a change in the mode of living. For example, on Eden Grid A, the

abundance of *N. diversicolor* decreased under *Enteromorpha* mats, whilst the abundance of *H. ulvae* increased (Table 9.1b). *H. ulvae* can reduce stability by reducing cohesiveness through faecal pelletisation, and by moving through the surface sediment (Austin *et al.*, 1999; Blanchard *et al.*, 1997). Sediment stability may also be affected by a change in the macrofaunal assemblage if this impacts upon the microphytobenthic assemblage. A change in either the biomass or the species composition of the microphytobenthic assemblage may result in either an increased or decreased amount of EPS within the sediments, or even a change in the type of EPS present (Taylor, 1998).

Whilst *C. volutator* may enhance stability due to the mucus-lined burrows and increased drainage (Meadows *et al.*, 1990), at Biezelingse Ham, *C. volutator* is likely to have reduced sediment stability by grazing on diatom biomass and via bioturbation. This is in agreement with the results of de Deckere *et al.* (2001) who found the concentration of suspended solids in the overlying water increased with the density of *C. volutator* individuals. Diatom biomass and *C. volutator* density are inversely related, and it is therefore likely that sediment stability of a *Corophium*-dominated site will lie along a continuum, with areas of high stability having high diatom biomass and a low density of *C. volutator*, and easily eroded areas having low diatom biomass and a high density of *C. volutator* (Fig. 9.4). Site-specific features such as migratory bird populations may influence sediment stability by altering the stable state of the system. For example, Daborn *et al.* (1993) documented an ecological cascade effect, where migratory birds predated on *C. volutator*, reducing their density and altering their behavior, which allowed a diatom biofilm to develop and sediment stability to significantly increase. The Eden Estuary is a Site of Special Scientific Interest (SSSI) due in part to its migrating bird populations, and as such, may experience a similar cascade effect, although this has not been specifically investigated.

9.4.3 Diatom migration affects sediment stability

Matched measurements taken on a diatom biofilm at Biezelingse Ham provided an excellent example of the complex interactions between physical and biological processes. Initially upon exposure, biological processes dominantly influenced the sediment stability, with stability increasing with increasing surface diatom biomass. Paterson (1989) previously described this phenomenon,

although a greater time lag between diatoms appearing at the sediment surface and increased sediment stability was found in the laboratory. After diatoms migrated away from the sediment surface (probably as a result of a tidal rhythm; Serôdio *et al.*, 2001) and for the remainder of the emersion period, sediment stability decreased slightly, but remained high. This was probably due to the additive effects of the sediment becoming more consolidated and compacted as the water content of the sediment decreased, and due to the residual EPS pool on the sediment surface. A reduction in water content during the exposure period could have a number of consequences. For example, it may result in an increased secretion of EPS by the diatoms to prevent desiccation, or if the biofilm dries out, consequently trapping the diatoms within the EPS matrix, then migration may be prevented. Such effects may alter relationships (i.e. from linear to non-linear), and increase the scatter in the data.

The presence of a biofilm can have implications for studies that employ a unified erosion formula which predicts increasing sediment stability with depth, related to increased sediment density (e.g. Sanford & Maa, 2001). These studies still fail to recognise the importance of the spatial scale of biological effects. For example, biofilms result in an inversion in the stability/density relationship, with highly stable low-density sediments at the surface and denser less stable sediments below the surface. Even over a tidal cycle, diatom migration could significantly alter the density-depth stability relationship of the surface few millimetres (e.g. Hay *et al.*, 1993). If a site has a highly migratory biofilm (such as Biezelingse Ham mudflat), then variations in stability found along a grid or transect could be the result of migratory behaviour linked to the time of day and/or tidal cycle. Surface diatom biomass was found to be a significant predictor of erosion threshold for the Molenplaat, although the relationship was weak. This could have been due to the different mud and sand sedimentation patterns across the flat (Herman *et al.*, 2001).

9.4.4 Can we model sediment stability?

Traditionally, microphytobenthic biomass, the physical composition of the sediment, and the sediment water content have all been considered the primary controls of stability on cohesive sediments. All were all found to be significant predictors in the model that incorporated all of the data. The fact that

a significant predictive relationship, able to be used on all study sites, was not possible, could be due to the complex interaction of factors, particularly local features such as migratory biofilms and *Enteromorpha* blooms, together with temporal and spatial variation.

It has become obvious that there is large scatter in the data of the factors that control sediment stability. However, effective models still require a 'minimum' parameter sub-set of the factors responsible for controlling sediment stability (EstProc, 2002). Table 9.3 provides a preliminary attempt at this, comparing '*stable*' and '*unstable*' sediments. Whilst the mean values of sediment granulometry and chlorophyll *a* were significantly different between the two groups, the large range of values presented in the data increases the difficulty of producing a definitive model.

The results presented here reinforce those of Mitchener & Torfs (1996) who highlighted that there are major gaps in our understanding of the stability and thus sediment transport of mixed cohesive and non-cohesive sediments. Seasonal deposition cycles on intertidal flats are likely to be site-specific and may be a significant factor in the mud balance of an estuary (Herman *et al.*, 2001). This is likely to be a factor on the Molenplaat, and future models will need to incorporate a seasonality factor.

Experimental studies only provide a snapshot of the ecosystem, so that significant weather events (such as storms) and longer-term seasonal changes that influence the current state of the ecosystem are often overlooked. Spatial and temporal variation is an intrinsic part of the estuarine environment. It is important that measurements are matched and taken at the appropriate scale. If components are measured inappropriately, then relationships or effects may be weakened, masked or may even disappear. However, it is possible that the relationships that govern sediment stability are too complex to be easily modelled at our current state of knowledge.

9.5 Conclusions

Since the living and abiotic elements vary temporally and spatially, it is not surprising that the functional importance of these various factors in determining sediment stability also vary, resulting in apparently idiosyncratic relationships. The challenge now is to bridge the gap between our understanding

of the fine scale and the larger scale properties and processes to produce system wide models.

Whilst some factors will be similar between estuaries, this research highlights the need for site-specific calibration of models, and suggests that a universal proxy parameter for sediment stability is unlikely to be obtained. The sediment stability of all the sites of this investigation were controlled by different forcing variables, and appeared to be associated with seasonal influence and different local features. It has already been suggested that local circumstances will probably be very critical in determining the precise effects of sea level change on estuaries (Jones, 1994). In terms of sediment stability, and with regards to studies investigating the effect of sea level rise on estuaries and coastal erosion, this investigation highlights that local features will indeed have a large role to play, and will have to be taken into account.

9.6 Publication

Data from this study have been published in Hydrology and Earth System Science. See Defew, E.C., Tolhurst, T.J. &Paterson, D.M. (2002). Site-specific features influence sediment stability of intertidal flats. *HESS* **6**: 971-982.

Chapter 10

Chapter 10: General Discussion

10.1 Establishing a model system

In order to elucidate the effects of specific factors, such as those associated with global change scenarios, it is often easier to undertake laboratory experiments, where the number of variables can be minimised and controlled. The first stage of such work should be to establish the validity of the model system and ascertain whether the system could be used in laboratory experiments. As discussed in chapter 3, many workers (such as Smith & Underwood, 1998; Serôdio *et al.*, 1997; Peletier *et al.*, 1996; Hay *et al.*, 1993; Madsen *et al.*, 1993; Glud *et al.*, 1992; Admiraal, 1977) have set-up experimental laboratory systems to test the effects of various variables, without determining how stable the biofilm and species composition were under the laboratory irradiance conditions. I concluded that compared to phytoplankton assemblages, the estuarine microphytobenthos appeared to possess a certain degree of inertia when brought from the field into the reduced light regime of a laboratory. This meant that under the irradiance conditions normally imposed on microphytobenthic assemblages in the laboratory, species composition and assemblage metabolism changed very little. Consequently, estuarine microphytobenthic assemblages provide a useful laboratory experimental model when used over the medium term duration. The model has relevance to natural conditions and can be used to examine the influence of environmental factors (such as temperature, grazing, nutrients and CO_2 concentration). This experiment was only carried out once, and in order to strengthen the conclusions that were made, biofilms of different assemblage composition, from different estuaries, and at different times of the year (e.g. seasonally) should be examined further. It would also have been beneficial to monitor the assemblage composition of the field microphytobenthic biofilm, to see how and if assemblage composition changed. However, this limited evidence supports the use of estuarine microphytobenthic assemblages as laboratory model systems.

10.2 Response to environmental variables

Environmental variables do not act independently and can have different effects according to the influence of other variables. Unless an experiment is directed towards examining a specific single outcome, it is often unclear at the beginning of an experiment how a variable will influence a diatom cell or assemblage. For example, light had different effects on the microphytobenthic assemblage according to the temperature regime that the assemblage was exposed to (Chapter 4). At 10°C the light environment induced physiological changes in the diatom species present, at 18°C the light environment caused an alteration of the diatom assemblage composition, whilst at 26°C species dominance of the microphytobenthic assemblage passed from diatoms to cyanobacteria. At 18°C the change in species composition was due to the dominance of one species. This result could easily change according to the season and the initial assemblage, highlighting the need for this experiment to be replicated.

Similarly, the presence of a macrofaunal grazer could regulate assemblage composition (*Corophium volutator*), or not (*Hydrobia ulvae*) depending on its behaviour, despite the influence of nutrients and temperature (as shown in Chapter 5). This experiment would have benefited from gut content analyses to determine whether or not the *H. ulvae* were consuming the diatoms. Using different dominant macrofaunal grazers, such as *Nereis diversicolor*, *Mytilus edulis*, *Cerastoderma edule* and *Macoma balthica*, one could develop this work further. Also an effect measured in one season may not have the same effect when measured later on in the year due to an increase or decrease in the grazing populations.

The results of Chapter 5 focus attention on the need to use natural cores (Chapter 6) rather than sieved sediment (Chapters 3 and 4). The research presented in this thesis also highlights that it may be beneficial to measure as many variables as possible when carrying out an experiment, since it is not always obvious what the outcome will be, and how changes in environmental conditions will affect either the functioning and/or structure of the microphytobenthic assemblage.

In a recent review, Underwood & Kromkamp (1999) stated that, as yet, there is no evidence of CO_2 limiting photosynthesis in benthic estuarine diatoms,

and the research presented in this thesis (see Chapter 6) supports this statement. The experiment was replicated in two different seasons, with different diatom assemblages, and yet biomass, primary production and species composition of the assemblages did not differ between the cores grown under ambient CO_2 concentration (365 ppm) and those cores grown under enhanced CO_2 concentration (560 ppm).

The work investigating the influence of enhanced CO_2 concentration on the structure and functioning of estuarine microphytobenthic biofilms could be extended in a number of ways:

1. High biomass biofilms could be investigated since diffusion of CO_2 to deeper layers may be reduced.
2. Variability in Rubisco enzyme concentrations could be investigated.
3. It would be interesting to repeat the experiment using microphytobenthic assemblages from two estuaries that differ in their nutrient status (i.e. the Westerschelde and Oosterschelde Estuaries). Nutrient-poor ecosystems have often shown less response to elevated CO_2 than more fertile systems (Reich *et al.*, 2001).
4. The influence of enhanced CO_2 concentrations at sub-saturating and saturating light levels could be examined. For example, Orellana & Perry (1992) demonstrated that the Rubisco content of the phytoplankton diatom *Thalassiosira weissflogii* varied with growth conditions. In cultures of *Cylindrotheca closterium*, the EU project CLIMEROD (2001) found that different CO_2 levels had no effect on photosynthesis at irradiances < 100 $\mu mol\ m^{-2}\ s^{-1}$, whilst CO_2 became limiting at high irradiance.
5. Using the membrane permeable carbonic anhydrase inhibitor, 6-Ethoxyzolamide (Ez) and the membrane impermeable inhibitor Acetaxolamide (Ax) on natural estuarine benthic diatom assemblages would be beneficial, as it may be that enhanced CO_2 concentration are not having an effect because the diatoms have highly efficient carbon concentrating mechanisms (CCM's) that mean they are not carbon limited at ambient CO_2 concentration.
6. Experiments could also be run at a range of temperatures, although experimental studies looking at net primary production must be treated

with caution because of complex interactions with temperature and the inadequacies of the fluorescence technique which still need to be resolved (Chapter 7 and see below).

10.3 Methodologies

10.3.1 Chlorophyll *a* sampling

Most photosynthetically active biomass biomass is found in the top 400 μm, so any sampling technique that examines depths greater than this will be diluting the chlorophyll *a* content, since coarse core sediment sampling will also incorporate photosynthetically inactive biomass (e.g. chlorophyll *a* below the photic zone) (Consalvey, 2002; Honeywill, 2001). Compaction of the sediments due to a reduction in water content (as seen in chapter 4) can incorporate a significant quantity of inactive biomass into the cores (Honeywill, 2001; Perkins *et al.*, in press). In future studies it may be helpful to use a finer-scale method of sediment collection such as the cryolander (Wiltshire *et al.*, 1997). However, when using small cores (such as those used in chapters 5 and 6), the sediment sampling equipment needs to be able to fit into the core.

10.3.2 Fluorescence measurements

Fluorescence is a highly useful technique due to the rapid, *in situ*, non-destructive deployment. However, it must be used with a good understanding of the ecology and physiology of the microphytobenthos, and this thesis illustrates that further work in this area is still needed.

Laboratory and field-based measurements have shown a linear relationship to exist between minimum fluorescence and microphytobenthic chlorophyll *a* biomass (Honeywill, 2001; Serôdio et al., 2001; 1997). Although chlorophyll *a per se* cannot be inferred from this technique (Consalvey, 2002), it is sufficient to be able to follow proportional variation in assemblage biomass in the upper surface layers (Serôdio *et al.*, 2002) as was demonstrated in Chapter 4. However, the studies carried out in this thesis have highlighted a number of limitations associated with the technique, which must be taken into account.

When two cores have identical biomass, the distance of cells from the sediment surface and their orientation within the biofilm may be different, consequently altering the relationship between F_o^{15} and biomass. The

fluorescence yield is likely to be affected when particularly thick biofilms are allowed to develop (such as those seen in Chapter 4), due to cells at the sediment surface exhibiting NPQ and also shading cells beneath the surface layer.

In order to get a true measurement of minimum fluorescence, NPQ must be fully reversed and the Q_A pool must be completely oxidised. The 15 min dark adaptation period aimed to achieve NPQ reversal and Q_A oxidation, whilst minimising the effects of downward vertical migration. However, this study (see Chapter 7) clearly demonstrates that cells do migrate away from the sediment surface during the dark-adaptation period and during the light response curves. Further investigations are needed to determine whether this response is species specific or not.

By considering the theoretical distance that epipelic diatoms could migrate vertically within the sediment in a 15 min period (which has been shown to range between 153-252 μm; Hay *et al.*, 1993; Harper, 1977), it is easily possible for the cells to move in and out of the photic zone and consequently the depth penetration of the fluorometer measuring beam, or to cycle their position within the biofilm (Consalvey, 2002). As Chapter 7 demonstrates, 'sub-surface' cells are in a lower light level than cells at the sediment surface, so that values of F_q'/F_m' can be falsely inflated above the true efficiency of the cell, resulting in the over-estimation of rETR.

Carbon fluxes in estuarine systems will need to be accurately determined if the effects of global environmental change are to be established. Therefore, I believe it is worthwhile to investigate whether we can obtain more accurate light response curves. Potentially light curves could be modelled by using fibre optics to measure light attenuation within the sediments, coupled with low temperature scanning electron micrographs showing the depth to which the diatoms have migrated. This would allow accurate determination of the light level to which the cells are exposed. A drawback to this methodology would be the destructive sampling of the biofilm under study.

The light history of the cell or biofilm is very likely to affect the state of the xanthophyll pigment cycle. Since diatoms have been shown to maintain a proton gradient in the dark (Schreiber *et al.*, 1995; Ting & Owens, 1993; Caron *et al.*, 1987), it is very likely that NPQ will remain switched on. Consequently, the presence of diatoxanthin (DT) will affect the fluorescence yield because not

all of the measuring beam will strike the reaction centres. If DT is not fully converted back to diadinoxanthin (DD) during the dark-adaptation period, a decrease in fluorescence yield could be attributed to both downward vertical migration of the diatoms and residual NPQ. Further work could be performed in order to determine the importance of the xanthophyll cycle for individual estuarine benthic diatom species. The DD and DT cellular contents could be monitored using HPLC, with and without addition of dithiothreitol (DTT), which blocks the de-epoxydation of DD. Consalvey (2002) found that exposure to far-red light could be used as an alternative to, or in addition to, dark-adaptation. Far-red light drives electron transport through to PSI, but does not drive photochemistry at PSII, therefore adaptation with far-red light should result in the dissipation of the proton gradient, thus reversing non-photochemical quenching (NPQ) and resulting in the complete oxidation of Q_A. However, this process may take as long as 24 h to achieve a true F_o and does not keep the cells at the sediment surface.

Changes in the fluorescence yield associated with different taxa could be examined, although there are disadvantages to the techniques that would have to be employed. For example, changes in taxa at the sediment surface during a light response curve could be examined by LTSEM, although this destructively samples the biofilm. Mono- and mixed-cultures could be used, but they are not representative of the natural system and, once in culture, diatoms often lose their ability to migrate (one of the fundamental aspects of the diatoms ecology). The technique of High Resolution Fluorescence Imagery (Oxborough *et al.*, 2000; Oxborough & Baker, 1997) could be used which allows yields of individual cells to be examined, except that this does not allow the cells to be maintained in their ambient or experimental conditions.

10.3.3 Assemblage analysis

One factor that was not taken into account when assemblage composition was assessed was the importance of diatom cell size in community analysis. The diatom assemblage analysis presented in this thesis (Chapters 3, 4, 5 and 6) used multivariate statistics based on counts of a fixed number of diatom valves with species scores irrespective of cell size. Large differences in size among species in an ecological community pose size-related problems for synecological analysis.

For example, small diatoms (biovolume <1000 μm^3) and larger ones (biovolume >1000 μm^3) were found to respond differently to environmental factors (Busse & Snoeijs, 2002). It may be worthwhile measuring the variety in shape among the diatom species of an assemblage since this affects the surface area to volume ratio, consequently affecting the cells relative exposure to the environment (Lewis, 1976), although the environment may also affect cell size by ecotypic differentiation.

When using abundances the importance of the larger species is underestimated, and when using biomass that of the smaller species is underestimated. Snoeijs *et al.* (2002) recommend counts and analysis of relative abundances of large and small diatoms separately. Practically speaking, a greater number of slides would have to be prepared and counted in order to increase the probability of encountering large species when recording species composition. In a mixed diatom assemblage, larger species are likely to be less abundant than smaller ones in ecological assemblages because the maximum specific growth rates in algae decrease with increasing cell size (Raven & Geider, 1988). Therefore, their contribution to the response of the whole assemblage is also likely to be reduced. I am of the opinion that the extra effort involved in counting both small and large cells may not be worthwhile.

The word biodiversity is derived from the Greek word for life 'bios' and 'diversity' or variety. In determining biodiversity, one is supposed to record the 'living' component of an assemblage, but in routine diatom work, the sample is oxidised and the protoplast destroyed, in order that the fine details of the frustule can be observed and a reliable identification made. The valves of diatoms are very resistant and may be transported from elsewhere by wind, water and even birds. They also fossilise easily so that a sample may contain species that have not lived in this environment for years. Hein de Wolf (pers. com.) concluded that there is a risk of serious errors being made, unless distortion of the living diatom flora by preparation effects is taken into account. Whilst this may be true for sediment scrape samples, the majority of data presented in this thesis relate to the biodiversity of estuarine epipelic benthic diatoms that have actively migrated into the top layer of lens tissue (although there is a minimal contribution of cells due to capillary action; Consalvey, pers. comm.).

10.4 Estuarine benthic diatoms and sediment stability

We know that the response of natural sediments to hydrodynamic stress cannot be derived solely from sedimentological parameters due to the presence of the micro- and macrobenthos (Tolhurst *et al.*, 2000; Paterson & Black, 1999; Defew *et al.*, in press). Consequently, the prediction of sediment transport is now an area of interdisciplinary research with a strong motivation to correlate organismal biomass or activity with erosional parameters (such as critical erosion thresholds) (Tolhurst *et al.*, 2000a,b; Paterson & Black, 1999; Tolhurst *et al.*, 1999).

Studies have shown that concentrations of colloidal carbohydrates in sediments are closely related to the content of chlorophyll *a,* taken as a biomass proxy for diatoms, although the variability is large (Staats *et al.*, 2001; Underwood & Smith, 1998; Underwood *et al.*, 1995). However, using chlorophyll *a*, sediment granulometry and macrofaunal data, collected *in situ*, did not provide a single easily measurable sediment variable, which could be used to predict cohesive sediment stability. Erosion threshold had a weak dependence with chlorophyll *a*, which only explained 40% of the variation in the data (Chapter 8), and sediment stability at large spatial scales is more likely to be influenced by site specific features such as macrofaunal species and patches of *Enteromorpha* (Chapter 9).

In terms of depositional environments, microphytobenthos have an important role to play in stabilising the sediment surface. Several authors have concluded that the most subtle and pervasive biological effects are derived through the production and secretion of polymeric materials from microbial assemblages (comprising both prokaryotes and eukaryotes) (Paterson & Black, 1999; Underwood & Kromkamp, 1999; Paterson, 1994) but these effects seem neither consistent nor predictable. One of the main reasons for carrying out the research of this thesis was to determine if environmental factors could influence the species composition of assemblages which in turn may affect the amount of EPS produced, since this may have important consequences for transitional coastal zones in light of predicted climate change and human impacts. The natural result of a biofilm's presence is an alteration of the dynamic equilibrium between the erosion, transport and deposition of sediments. However, results

presented in this thesis would suggest that the amount of EPS produced does not differ between experimental treatments (Chapters 3, 5 and 6).

Diatoms can be regarded as ecosystem engineers (Jones & Lawton, 1995), and the form of EPS produced by the microphytobenthos varies between species, growth phase and nutrient status (Black *et al.*, 2001; Staats *et al.*, 2000 Underwood & Smith, 1998; Buzzelli *et al.*, 1997). Recent research in the Sediment Ecology Research Group (using LTSEM and measurements of sediment cohesive strength using the CSM) has shown that polymer extracted from sediment samples or diatom EPS analogues such as the bacterial EPS and Xanthum gum (Black *et al.*, 2001), differ in their sediment binding capacity and contribution to sediment stability. This may be due to the type of extraction method and sample preparation used (e.g. solvent extraction/rotary evaporation/freeze-drying; Perkins, unpublished), or it may be due to the nature and conformation of the EPS produced. It has been suggested that the soluble colloidal polymer fraction should not be operationally divided into EPS and lower molecular polymers until the method has been refined.

Previous analyses (e.g. GC-MS; Taylor *et al.*, 1999) are insufficient in providing information beyond the composition of the polymer. One pathway of future research could be to employ a technique such as ElectroSpray Ionization Mass Spectrometry (ESI-MS). ESI-MS employs the formation of a cone and jet of charged drops from a solution of the sample, using an applied electric field. The jet emits small droplets, charged due to an excess of electrolyte ions. This leads to the formation of gas-phase ions due to solvent evaporation from droplets of < 10 nm diameter, which are then analysed by conventional MS. ESI-MS has been successfully used for analysing the conformation and structure of large molecules, such as oligosaccharides - including analysis of non-covalent bonding in sugar chains (Ohasi, 1997), and may be able to determine the nature of polymeric fractions excreted from different diatom species grown under different environmental conditions.

10.5 Global change

The response of estuarine microphytobenthic assemblages to global environmental change will differ according to the variable of concern. This thesis suggests that the least effect will be due to increased atmospheric carbon dioxide

concentrations. Estuarine microphytobenthos are unlikely to provide an alternative carbon sink that is thought to be missing from carbon cycling models. The greatest effect of global climate change is likely to be due to increased temperatures altering the dominance of microphytobenthic assemblages from diatoms to cyanobacteria. This will have important consequences since cyanobacterial biofilms provide less biostabilisation on estuarine mudflats (Chapter 4). However, rapid colonisation and development of the biofilms from low biomass samples demonstrates the potential for recovery from, and resilience to, predicted climate change and human impacts. Of course, sea-level rise and storm frequency are also likely to have an impact on natural estuarine microphytobenthic assemblages, but these factors were not examined within this thesis.

10.6 Conclusion

Previous studies of estuarine diatoms have often involved using either monocultures or using assemblages without actually determining the species present within the biofilm, and whether observed differences in the measurements taken could be attributed to changes in the assemblage composition. Therefore, this thesis has made a significant step forward by examining the effects of environmental factors on both the functional (i.e. health, biomass, primary productivity and EPS production) and structural (i.e. species composition) responses of estuarine microphytobenthic biofilms. Having completed these experiments, it has confirmed my belief that some of the most interesting observations and results come from examining species change and species-specific responses. Therefore, if I were to be able to continue with this line of work, the most interesting direction would be species-specific photophysiology responses, and trying to improve the methodology behind our estimations of rETR.

10.7 Summary

- The use of estuarine benthic diatom biofilms as laboratory model systems for the study of environmental variables and change has been established.

- Light can influence a microphytobenthic assemblage via physiological changes and via changes in assemblage composition. This effect is temperature-dependent.
- Grazing effects are macrofaunal-species specific. Grazing from *Hydrobia ulvae* had no significant effect on assemblage composition. Top-down control from *Corophium volutator* can significantly affect the assemblage composition of a diatom biofilm, overriding any potential effects of temperature and nutrient conditions. These effects will vary over an entire mudflat area, and are likely to be density dependent.
- Natural estuarine diatom biofilms are not carbon limited.
- Measurements of rETR are over-estimated on migratory diatom biofilms. Phytotrays have been established as systems that allow for the calculation of a 'true' rETR. By preventing migration, the diatom cells are exposed to a known light environment. Species-specific responses to high light (i.e. migration and/or NPQ) need to be addressed further.
- Sediment stability cannot be predicted from proxy parameters. Models need to be site-specific due to the occurrence of features, such as *Enteromorpha* mats, that are typical to that Estuary.

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