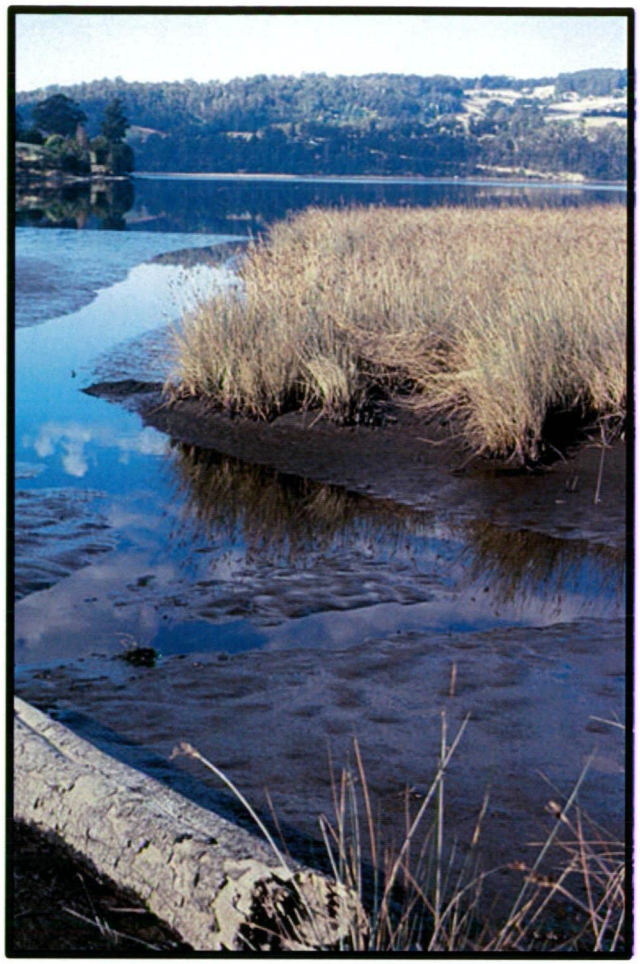

Carbon and nitrogen cycling on intertidal mudflats in a temperate Australian estuary

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Abstract

The sources of organic matter, benthic metabolism (light/dark O_2 and TCO_2 fluxes), benthic dissolved nitrogen fluxes, denitrification, nitrogen fixation and sediment NH_4^+ production were studied on the upper and lower regions of two mudflats in Huon Estuary, south east Tasmania over four seasons. One study site was located in the upper euryhaline part of the estuary and the other study site was located in a "marine" side arm of the estuary. The aim of the study was to develop a detailed conceptual understanding of sedimentary nitrogen cycling processes in relation to the activity of microphytobenthos (MPB) in this system.

The organic matter pool at both sites was generally dominated by that derived from terrestrial sources. Organic matter derived from microphytobenthos generally only comprised a small fraction of the organic matter pool. Compound-specific stable isotope ratio analysis of bacterial and algal fatty acids suggested the algal-derived fraction of organic matter was most likely the driver of bacterial respiration within the sediment. As such, this fraction of organic matter had a high turnover rate, and never built up to significant amounts. The MPB at both sites consisted of a mixed community of diatoms, chlorophytes and cyanobacteria, the relative composition of which varied with site, position on the mudflat and season.

Rates of primary production by MPB were influenced by an exposure to wave energy and an availability of light. Rates of primary production by MPB were significantly greater on the upper mudflat than the lower mudflat at the site in the upper estuary. It is proposed this arose as a consequence of light limitation across the inundation gradient caused by high concentrations of coloured dissolved organic matter in the water at this site. Benthic respiration at this site was controlled by temperature, as well as organic matter input from MPB. At the site in the marine side arm of the estuary, rates of primary production were not significant different between the upper and lower mudflat and were significantly lower than at the site in the upper estuary. A greater exposure to wave energy, as indicated by sediment grain size and aspect was the most likely cause of the lower rates of primary production at this site. As a consequence, both the upper and lower mudflats at the site in the upper estuary were

autotrophic on an annual basis, while both the upper and lower mudflats at the site in the marine side arm of the estuary were heterotrophic on an annual basis.

The balance between production and respiration was of fundamental importance in determining whether the sediments were a net source or sink for dissolved inorganic nitrogen, with autotrophic sediments showing a net uptake of nitrogen and heterotrophic sediments showing a net release. Primary production also influenced the exchange of gaseous nitrogen species. Rates of denitrification were generally very low and negatively correlated with rates of primary production, while, rates of N_2 fixation were at times high and were positively correlated with primary production. Dissolved nitrogen fluxes were dominated by dissolved organic nitrogen (DON) where and when high rates of production (uptake of DON) and respiration (release of DON) were observed.

MPB also profoundly influenced the nitrogen cycle through the production of labile, but high C:N ratio organic material. At times of high primary production, the calculated demand for nitrogen - based on simple but widely used stoichiometric models - was found to be well in excess of the measured uptake. Subsequent measurements of N_2 fixation using the acetylene reduction assay (calibrated using ^{15}N-N_2) showed that N_2 fixation could, at times, account for the observed deficit in nitrogen uptake. In general, however, N_2 fixation could not account for the deficit in dissolved nitrogen assimilation. It is suggested that a stoichiometric relationship between carbon and nitrogen assimilation reflecting the C:N ratio of algal cells will only occur during the initial development of the MPB biofilm. Once the MPB biofilm has become established the majority of carbon assimilation is directed into the production of extracellular organic carbon (EOC) such as extracellular polymeric substances (EPS), rather than cell growth. It is proposed that the input of this labile, but high C:N ratio organic material to the sediment drove bacterial respiration as well as stimulating bacterial nitrogen reassimilation. As a consequence, the ratio of TCO_2:NH_4^+ produced within the sediment was generally in excess of 15 and in some cases in excess of 60.

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Chapter 1

Introduction

Introduction

Nitrogen is generally considered to be the nutrient limiting to primary production in coastal waters (Howarth 1988). Hence, the availability of this nutrient is of fundamental importance in determining the productivity and functioning of coastal ecosystems. In many areas, increases in anthropogenic activity have resulted in increased loads of nitrogen entering the coastal zone (Nedwell et al. 1999). This nutrient enrichment can lead to nuisance algal blooms and ultimately ecosystem collapse (Paerl 1993). It is for these reasons that much attention has been directed towards the transport and cycling of nitrogen over the past 40 years. It is now widely accepted that the remineralisation of nitrogen within the sediment plays a pivotal role in nitrogen cycling in coastal systems, and much research effort has been directed at understanding sedimentary recycling processes of nitrogen (e.g. Herbert 1999; Nedwell et al. 1999).

The key role of nitrogen in biological production means that the cycling of carbon and nitrogen are inextricably linked (Paerl 1993). Indeed it is recognised that the quantity and quality of organic matter reaching the sediment are of prime importance in controlling the rates and pathways of organic matter remineralisation (Heip et al. 1995; Herbert 1999). Any studies into the cycling of nitrogen should, therefore, give due consideration to the sources and cycling of carbon to be ecologically meaningful. This is particularly pertinent in euphotic sediments where it is now recognised that both heterotrophic and autotrophic processes are important in controlling benthic nitrogen cycling (e.g. Ferguson 2002 and references therein).

Overview of sedimentary carbon and nitrogen cycling processes

Over the past 40–50 years, great progress has been made in understanding benthic nutrient cycling processes that take place. Within an ecological context though, the relative importance of each of these processes, their interaction with one another, and benthic metabolism remains to be elucidated across a range of systems. Numerous extensive reviews have already been written on these sedimentary recycling processes (Carpenter and Capone 1983; Blackburn and Sorensen 1988; Herbert 1999), and therefore, the purpose of this section is to give a broad overview of the current conceptual understanding of sediment biogeochemical processes. In the

following discussion each process is numbered and illustrated diagramatically in Figure 1.1.

Benthic production (Process 1)

Primary production may be viewed in terms of the following simplified Redfield model, where NH_4^+ is used as the source of nitrogen (Stumm and Morgan 1996).

$$106CO_2 + 16NH_4^+ + HPO_4^{2-} + 108H_2O \rightarrow \{C_{106}H_{263}O_{110}N_{16}P\} + 107\ O_2 + 14H^+$$

Simple stoichiometric models such as this are often used to make inferences about the assimilation rates of nitrogen based on measurements of primary production (Dong et al. 2000; Sundbäck and Miles 2000; Thornton et al. 2002). Essentially, it is this process that drives all of the biogeochemical cycling processes within the biosphere. Benthic primary production by microphytobenthos (MPB) is recognised as contributing significantly to ecosystem productivity in a range of coastal systems (Underwood and Kromkamp 1999; Glud et al. 2002). Benthic primary production may, therefore, be an important source of organic matter driving secondary production within the sediments (Cammen 1991; Middelburg et al. 2000). The extent to which pelagic secondary production utilises benthic production has been poorly studied, however, recent studies have shown that bottom feeders, such as mullet, may be able to significantly utilise production by benthic diatoms in salt-marsh environments (Laffaille et al. 2002).

The production of oxygen by photosynthesis during the day will increase the depth of oxygen within the sediment, affecting respiratory and nitrogen cycling pathways (Process 9). The physiological and photosynthetic activity of benthic microphytes may have a significant impact on nitrogen cycling processes including denitrification, benthic nutrient fluxes and N_2 fixation (Sundbäck et al. 1991; Paerl 1993; Risgaard-Pedersen et al. 1994). The influence of MPB on these [nitrogen cycling] processes can have significant ecological implications for an estuary, for example the re-assimilation of nutrients by MPB at the sediment water interface will reduce nutrient availability for pelagic algal growth. In shallow systems where there is a low external nutrient input, this may mean that benthic production will dominate the system, with a tight recycling of nutrients between the sediment and MPB.

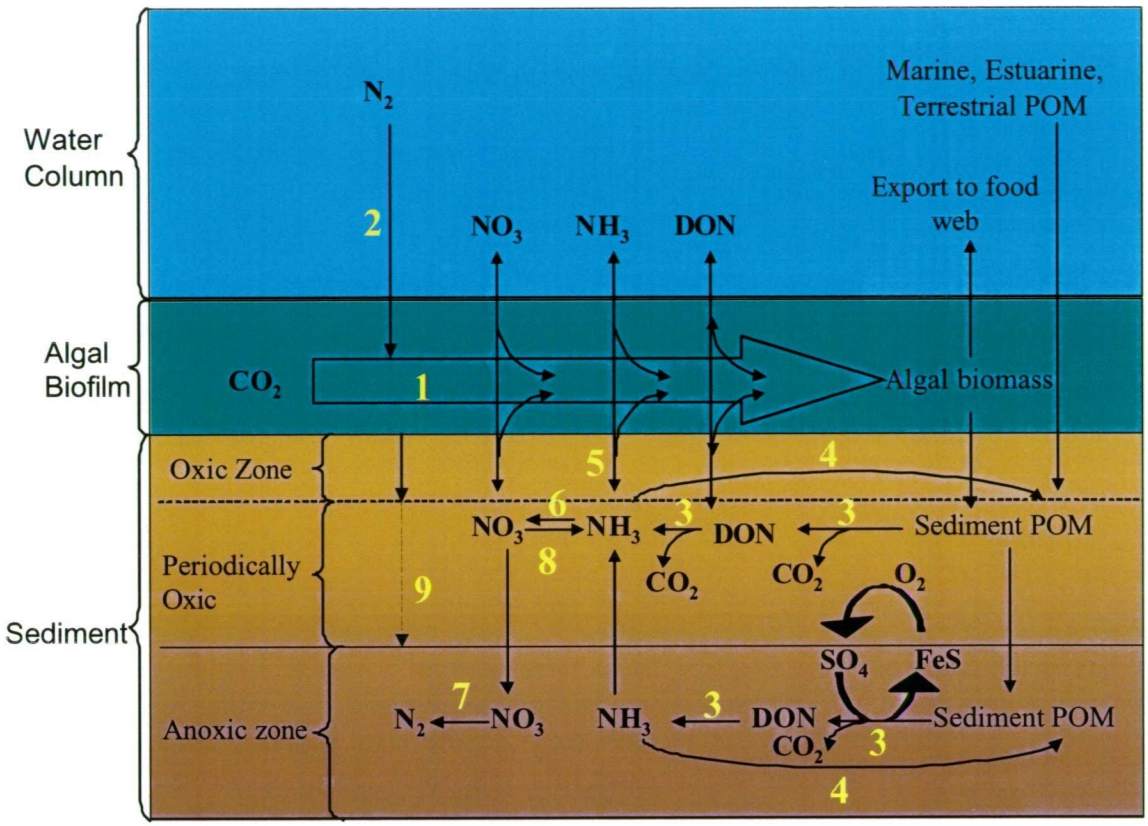


Figure 1.1. Overview of the current conceptual understanding of nitrogen cycling in euphotic coastal sediments. The yellow numbers refer to each process which is discussed in the text.

N_2 fixation (Process 2)

N_2 fixation is carried out by a range of heterotrophic bacteria as well as cyanobacteria. The highest rates of N_2 fixation are generally observed in photoautotrophs because of the high metabolic cost of N_2 fixation (Herbert 1999). Nitrogen fixation refers to the process of nitrogen reduction to ammonia, which is carried out by the nitrogenase enzyme.

$$N_2 + 8H^+ + 8Fd^- + 16ATP \rightarrow 2NH_3 + H_2 + 8Fd^+ + 16ADP + 16Pi$$

Where:

Fd = ferredoxin

ATP = adenosine triphosphate

ADP = adenosine diphosphate

The nitrogenase enzyme is extremely sensitive to oxygen so this process may only occur under anoxic conditions. In order to overcome this problem some species of cyanobacteria have developed heterocysts, which are cells that have lost the capacity for photsynthesis. Oxygen concentrations are very low within these cells due to a thick cell wall and active metabolism. By contrast, some benthic non-heterocystous cyanobacteria overcome the problem by temporally separating nitrogenase activity from nitrogen fixation (Stal 1995). Thus, the highest rates of nitrogen fixation are observed during the dark, a period which has often been ignored or under assessed when determining nitrogen fixation rates (Paerl 1993).

In coastal waters, nitrogen fixation is potentially a key process in determining ecosystem productivity, because it provides a source of nitrogen which is generally considered to be the limiting nutrient in coastal systems. The importance of benthic nitrogen fixation as a nitrogen source for shallow water ecosystems has up until now usually been considered to have only very localised importance, for instance in cyanobacterial mats (Pinckney et al. 1995, Stal 1995, Paerl et al. 1996) and coral reefs (Charpy-Roubaud et al. 2001, and references therein). Benthic nitrogen fixation in temperate benthic systems has been scarcely studied.

Remineralisation of organic matter (Process 3)

The remineralisation of organic matter can essentially be viewed as the reverse process of photosynthesis, where heterotrophic organisms derive their energy requirements from the dissimilation of organic matter to its mineral constituents (Stumm and Morgan 1996). Such a recycling of nutrients is the prerequisite for the production of organic matter by autotrophs. The following reaction represents the remineralisation of "Redfield" algal-type material under aerobic conditions.

$$\{C_{106}H_{263}O_{110}N_{16}P\} + 107O_2 + 14H^+ \rightarrow 106CO_2 + 16NH_4^+ + HPO_4^{2-} + 108H_2O$$

This simple stoichiometric model is often applied to remineralisation processes in sediment systems as a means of calculating a mass balance for nitrogen in relation to organic matter re-mineralised (Banta et al. 1995; Berelson et al. 1998). While this approach has been shown to be applicable in certain systems, (e.g. LaMontagne et al. 2002), its applicability to shallow coastal sediments is questionable. Actively growing bacteria will reassimilate a large portion of nitrogen remineralised within the sediment in order to synthesis their low C:N ratio biomass (van Duyl et al. 1993; Lomstein et al. 1998), particularly where the organic matter reaching the sediments has a high C:N ratio. Indeed, it is not only the quantity of organic matter reaching the sediment but also the quality (or lability) that is crucial in driving the rates and pathways of carbon and nitrogen remineralisation (Herbert 1999). The C:N ratio of organic matter reaching the sediment is a widely used proxy for its quality. Organic matter with a low C:N ratio is generally regarded as being labile and may originate from algal or bacterial sources. Organic matter with a high C:N ratio is generally considered more refractory and generally originates from already degraded phytodetritus as well as lignin-rich terrestrial sources (Thornton and McManus 1994) and references therein). Identifying the origin of organic matter reaching the sediment is, therefore, of crucial importance when studying benthic remineralisation processes.

In sediments where there is a significant accumulation of organic matter, oxygen will be consumed more rapidly than it is able to diffuse into the sediment. Other major oxidants of organic matter include NO_3^-, NO_2^-, Mn and Fe oxyhydroxides, SO_4^{2-} as well as O_2 in organic matter itself. The use of these oxidants occurs in the sequence

of decreasing energy yield for each mole of carbon oxidised. This results in vertically stratified zones where each of these reduction processes occurs. It is within, and at the interface of these zones, that some important nitrogen cycling processes such as nitrification and denitrification occur (Heip et al. 1995). End products of anoxic respiration such as HS^- and Fe^{2+} may also interact further with various nitrogen cycling processes (Sørensen 1987; Joye and Hollibaugh 1995).

Sedimentary nitrogen recycling pathways

The remineralisation of nitrogen can be viewed as a series of metabolic steps where nitrogen containing macromolecules are broken down into their constituent compounds. The identity of these organic compounds are still poorly characterised; but are known to include short polypeptides, amines, nucleic acids and urea (Herbert 1999). Where there is a high input of labile nitrogen to the sediment, the release of dissolved organic nitrogen (DON) may be significant (Hansen and Blackburn 1992; Enoksson 1993). There is now evidence to suggest that urea may make up a significant fraction of organic nitrogen efflux and as such, readily available to primary producers in the water column (Herbert 1999). Deamination of low molecular weight organic compounds ultimately results in the production of NH_4^+, which is generally regarded as the most bioavailable form of nitrogen. NH_4^+ may then be reassimilated by bacteria (Process 4), released from the sediment (Process 5), become adsorbed onto sediment particles or oxidised to NO_3^- (Process 6) (Blackburn and Henriksen 1983; Klump and Martens 1983).

Nitrification (Process 6)

Nitrification is the term used to describe the oxidation of NH_4^+ to NO_3^- by chemoautotrophic bacteria. This process has received much interest primarily as it is a precursor to denitrification (see below). The process is generally considered obligately aerobic, although recent evidence suggests nitrification may be coupled to manganese IV reduction (Hulth et al. 1999). The process is regulated by a number of factors including the availability of O_2 and NH_4^+, as well as salinity and pH. These, in turn, are often controlled by bacteria, benthic photoautrophs and macrofauna. It has been found that the activity of benthic diatoms significantly inhibits nitrification possibly through competition for NH_4^+ and CO_2 as well as limitation by high pH, light and O_2 (Henriksen and Kemp 1988). It has also been recently found that competition for NH_4^+ with heterotrophic bacteria may limit nitrification (Strauss and

Lamberti 2000). Rates of nitrification are generally highest around burrows of macrofauna, where there is a high availability of NH_4^+ within an oxic environment (Henriksen and Kemp 1988).

Nitrate reduction

Denitrification (Process 7)

Much interest has been focussed on the process of denitrification in coastal sediments, as it potentially represents a significant sink for anthropogenic inputs of nitrogen. Denitrification is generally carried out by facultatively anaerobic bacteria of the genus *Pseudomonas* (Herbert 1999). The factors controlling denitrification are complex and it is only with recent methodological advances that these factors are beginning to be understood (Cornwell et al. 1999). The process of denitrification requires an availability of NO_3^- (or NO_2^-), organic carbon and anoxic conditions. NO_3^- consumed in the denitrification process may be derived either from the water column (uncoupled denitrification) or nitrification within the sediments (coupled denitrification). Denitrification is, therefore, controlled by nitrification rates as well as an availability of NO_3^- from the water column, which in turn depends upon the NO_3^- concentration in the water column and the depth of the sediment oxic layer (Rysgaard et al. 1995). In systems with high NO_3^- concentrations in the water column, extremely high rates of denitrification may be observed (Trimmer et al. 2000a), while in systems with low concentrations of NO_3^- in the water column, denitrification will depend exclusively on nitrification within the sediment.

The ecological significance of denitrification is that this process can attenuate excess loads of nitrogen entering the coastal zone by up to 25% (Dong et al. 2000, Trimmer et al. 2000). Denitrification may play a vital role in maintaining the ecological health of shallow enclosed systems receiving high nutrient loads. A specific example is Port Phillip Bay, where it has been suggested denitrification is a vital process in removing high nitrogen loads derived from the city of Melbourne (Berelson et al. 1998).

Dissimilatory nitrate reduction to ammonium (DNRA) (Process 8)

As well as being reduced to N_2 gas, NO_3^- may also be reduced to NH_4^+ by fermentative bacteria. Few systematic studies of this process have been conducted, but it is apparent that this process is favoured under conditions of low NO_3^- and in

organic-rich environments. As a result, the ratio of NO_3^- to organic carbon is likely to be the major controlling influence on this process, with a high NO_3^- to organic carbon ratio favouring denitrification and a low NO_3^- to organic carbon ratio favouring DNRA (Nedwell et al. 1999). It has recently been shown that in sediments affected by fish farming, the rate of DNRA was 7 times greater than denitrification, but in nearby unaffected sediments DNRA was insignificant (Christensen et al. 2000). DNRA is potentially has serious ecological implications for highly organically enriched systems because it maintains fixed nitrogen within the system rather than removing it as does denitrification. The consequences of this would be a system where ammonium (rather than N_2) is released from the sediment fuelling algal blooms, which then returns more organic matter to the sediment, perpetuating the DNRA cycle.

The annamox reaction

It has been suggested that in sediments where there are high concentrations of NO_3^- and NH_4^+, denitrification may proceed via the annamox reaction (not shown) where the oxidation of NH_4^+ is coupled to the reduction of NO_3^- to form N_2. This process is now only starting to become studied in detail and was though to occur mainly in hypernutrified estuaries (Nedwell et al. 1999). Recent evidence however, suggests this process may be more widely distributed than previously thought (Dalsgaard et al 2003, Kuypers et al 2003).

The study context

Estuaries act as major conduits between the land and the sea. As such, they concentrate waters from a very large land surface area into a relatively small zone. Rapid rates of sedimentation in estuaries mean that large amounts of terrigenous and marine organic matter are trapped within these zones (Heip et al. 1995). Within estuaries, mudflats are important zones for the accumulation of fine grain sediments and organic matter (Jickells and Rae 1997). Hence, these areas may also be important zones of organic matter remineralisation (Middelburg et al. 1996a). Organic matter deposited within these zones may come from a variety of marine and terrestrial sources, thus influencing the biogeochemical recycling pathways that take place. The presence of microphytobenthos (MPB) on mudflats (Underwood and Kromkamp 1999) will also contribute to the organic matter deposited within these zones, as well as influence benthic nutrient cycling processes.

The dynamic nature of mudflats means they have received much attention in Europe, and to a lesser extent in North America, in terms of their role in nutrient recycling and primary production (Jickells and Rae 1997; Nedwell et al. 1999; Underwood and Kromkamp 1999). As a result, much has been learned about the role that mudflats play in these coastal systems. By contrast, very few detailed biogeochemical studies of Australian mudflats have been undertaken. The few that do exist have all been undertaken in tropical systems; studies of Australian temperate mudflats are non existent. A conceptual understanding of how temperate Australian mudflats function and the biogeochemical role they play within estuaries is purely the subject of speculation based on studies of European and North American systems. In a review of coastal research in Australia, Fairweather (1990) specifically identified the need for process-based studies on mudflat systems.

The high population density and intensive agricultural land use in Europe means that their mudflat systems are heavily influenced by high loads of nutrients and labile organic carbon (Middelburg et al. 1996a; Nedwell et al. 1999). Australian systems, by contrast, have relatively low nutrient loading due to lower rates of atmospheric deposition, lower population densities and more intact catchments (Harris 2001). The applicability of European and North American conceptual models to Australian systems is questionable. Indeed, recent studies of subtropical Australian estuaries have shown that sediment biogeochemical processes are dominated by a tight internal cycling, with very little release of inorganic nutrients (Ferguson 2002). This supports a more general conceptual understanding of nutrient poor ecosystems, where tight biotic recycling mechanisms will act to conserve nutrients within biomass (Odum 1993). It may be expected, therefore, that mudflats within the Australian context would function in a very different manner to those in European and North American systems.

The overall aim of this study was to identify and understand the important biogeochemical carbon and nitrogen recycling processes in an Australian temperate mudflat, based upon the general conceptual understanding set out in Figure 1.1. Of particular interest were the interactions between the carbon and nitrogen cycles and how these interactions control the key processes of nitrogen fixation and

denitrification and sediment-water exchange of nitrogen. It was anticipated that temperature, light and the availability and quality of organic matter would be of fundamental importance. MPB was also expected to exert a significant influence on the benthic biogeochemical processes, both through the delivery of carbon to the sediment, as well as through physiological processes such as oxygen production and nutrient uptake.

Four chapters are presented here which address the following questions

Chapter 2. Sources of organic matter to intertidal mudflats.

- What are the major sources of organic carbon and how do they vary spatially and seasonally?
- What are the major classes of MPB present and how do they vary spatially and seasonally?

Chapter 3. Benthic metabolism on intertidal mudflats.

- What are the rates of respiration and primary production and how do they vary spatially and seasonally?
- What are some of the major factors controlling production and respiration?
- What is the net metabolism balance for this system?

Chapter 4. Nitrogen remineralisation on intertidal mudflats.

- What are the major recycling pathways for nitrogen within the sediment? Is denitrification an important process?
- How do these processes vary spatially and seasonally?
- How does the remineralisation of nitrogen relate to benthic metabolism?

Chapter 5. Nitrogen assimilation by microphytobenthos on intertidal mudflats.

- What is the relative importance of nitrogen derived from the water column with regard to nitrogen remineralised within the sediment to primary production?
- Do the measured rates of nitrogen assimilation by microphytobenthos match those calculated from primary production and simple stoichiometric models?

- Is nitrogen fixation a significant source of nitrogen to the microalgal community?

Experimental approach

A variety of approaches and techniques have been used to measure benthic processes and related important parameters. What follows is a discussion of these approaches and techniques and the reasons for the choice of techniques used in this study.

Denitrification

The high background levels of N_2 in the atmosphere complicate the measurement of denitrification. Stoichiometric methods have been applied to estimate rates of denitrification in a number of systems. This approach uses measured rates of organic matter remineralisation and dissolved inorganic nitrogen (DIN) fluxes to estimate the loss of nitrogen as N_2 assuming Redfield stoichiometry of the organic matter undergoing decomposition and steady state conditions (Banta et al. 1994; Joye et al. 1996; Berelson et al. 1998). This method fails to take into account reassimilation of DIN by bacteria (van Duyl et al. 1993) and often assumes there will be no assimilation by MPB in the dark, which may not be realistic (Rysgaard et al. 1993). The assumptions made about the C:N ratio of the organic matter undergoing decomposition and steady state are also unlikely to hold, and, therefore, this method may not to give a meaningful estimate of denitrification. Entire system mass balance approaches have also been employed in lakes and rivers, however, these methods are subject to considerable error and give no information on the processes controlling denitrification (Seitzinger 1988). The discovery that acetylene blocks the final reduction of N_2O to N_2, led to the widespread use of the "acetylene block" method (Oremland and Capone 1988). The major drawback of this method is that acetylene also blocks nitrification, hence this method will only measure denitrification fed by NO_3^- from the water column. This severely underestimates rates of denitrification where a significant fraction of denitrification is fed by NO_3^- produced by nitrification within the sediment (Seitzinger et al. 1993). As a consequence this technique is now rarely used to measure denitrification where ecologically meaningful estimates of denitrification are required.

A number of direct methods to measure denitrification have been employed. One method involves removing all N_2 from an intact sediment core by sparging with a

He/O_2 mixture. This method, however, requires long pre-incubation periods (up to 10 days) to allow N_2 within the sediment to diffuse out (Seitzinger 1988). A modification of this technique involves running anoxic cores in parallel, preventing denitrification. The background flux out of the sediment can then be estimated within a few days (Nowicki 1994). This method assumes both sets of cores will have the same rates of N_2 diffusion out of the sediment, which seems unlikely given that all macrofauna within the anoxic cores will be killed. This will prevent bioirrigation, and hence, reduce the rate of diffusion of N_2 out of the anoxic cores compared to the oxic cores. This method is also likely to suffer from atmospheric contamination of N_2, either from leaks into the cores or during sampling and analysis. As a consequence, this method has not been widely used. The invention of high precision (0.05%) membrane inlet mass spectrometry (MIMs) to measure N_2:Ar ratios has allowed the relatively straightforward determination of denitrification rates in cores incubated with ambient N_2 levels (Kana et al. 1998). This method does have a number of drawbacks, most significantly, the formation of bubbles within cores will allow the diffusion of N_2 out of solution. This is particularly likely to be a problem in euphotic sediments where benthic microalgal productivity can be high. It also relies on the assumption that Ar concentrations do not change throughout the incubation, however, only a small change in temperature can result in a change in Ar solubility sufficient to change the calculated N_2 concentration significantly (Eyre et al. In Press). The limit of detection for this method is ~5 $\mu mol\ m^{-2}\ h^{-1}$, which is above the rates of denitrification recorded in many sediments with low NO_3^- concentrations in the overlying water (e.g. Sundbäck et al. 2000).

The use of ^{15}N is another means of distinguishing the production of N_2 produced by denitrification from ambient background levels. ^{15}N labelling techniques have been in use since the 1970s (Seitzinger 1988), however, such techniques only became widely used after the invention of the isotope pairing technique. This method involves adding $^{15}NO_3^-$ to the water column and measuring the production of $^{30}N_2$ and $^{29}N_2$ on an isotope ratio mass spectrometer. The rate of denitrification of ^{14}N is then calculated using an isotope mixing model (Nielsen 1992). This method also allows a partitioning of denitrification into that driven by NO_3^- from the water column (D_w) and that driven by NO_3^- produced by nitrification within the sediment (D_n). A model simulation analysis of this technique has shown that isotopic

fractionation effects are negligible, however, it was argued that partitioning denitrification into D_w and D_n was meaningless (Middelburg et al. 1996b). Comparisons with the direct N_2 flux technique in a flow through system (Seitzinger et al. 1993) as well as MIMS in batch cores (Eyre et al. In Press) were found to be in good agreement with the isotope pairing technique. This indicates that the isotope pairing technique apparently gives real and reliable estimates of denitrification. One of the major disadvantages of this technique is that it can only measure denitrification in the surface sediment where ^{15}N is able to rapidly diffuse to the sites of denitrification. In sediments vegetated by macrophytes, denitrification may potentially occur within the root zone fed by nitrification driven by oxygen transported through the root system (Nielsen 1992), although (Welsh et al. 2001) found no evidence for this in a seagrass meadow. Perfusion of $^{15}NO_3^-$ into sandy sediments has been utilised to overcome this problem (Risgaard-Peterson and Jensen 1997). Another potential problem with the isotope pairing technique occurs if the system is carbon-limited (T. Dalsgaard, pers com). For the isotope pairing technique to work, there must be a sufficient amount of electron donor (organic carbon) such that the labelled nitrate added can be denitrified – the total rate of denitrification should increase linearly with the amount of nitrate added. To ensure the assumptions inherent in the isotope pairing technique are met, it is generally recommended that the isotope pairing technique be run with $^{15}NO_3^-$ added at a range of concentrations, ensuring that the calculated rates of denitrification of ^{14}N (D_{14}) remain the same over a range of $^{15}NO_3^-$ concentrations.

An attempt was made to measure denitrification using the MIMs technique for this study, however, it was deemed impractical given bubble formation within the cores. It was decided to use the isotope pairing technique, given its high sensitivity as well as its widespread use, which thus enables a meaningful comparison with other systems.

Nitrogen fixation

As for denitrification, high background levels of N_2 complicate the measurement of N_2 fixation. The acetylene reduction assay is a convenient means of measuring nitrogenase activity and has become widely used. Acetylene has a much greater affinity for the nitrogenase enzyme than N_2, and therefore, will completely displace

N_2 at a partial pressure of 0.1atm. The reduction of acetylene to ethylene requires 2 electrons compared to 6 for the complete reduction of N_2 to NH_3 so theoretically there should be a conversion factor of 3:1. However, under N_2 fixing conditions, nitrogenase evolves one mole of H_2 per mole of N_2 reduced, therefore, the conversion factor is closer to 4:1 (Oremland and Capone 1988). Even this ratio is questionable, with some authors reporting ratios in the range of 10:1 up to 100:1 in sediments where heterotrophic nitrogen fixation was likely to dominate (Seitzinger and Garber 1987). As a consequence of this uncertainty in the conversion ratio, many authors report nitrogenase activity in terms of ethylene produced rather than converting the data to N_2 reduced (e.g. Paerl et al. 1996; Pelegri and Twilley 1998). Ideally, the acetylene reduction assay should be calibrated using ^{15}N-N_2, especially if any specific budgetary inferences about the rate of N_2 fixation are to be made. This is rarely undertaken due to the expense of both the $^{15}N_2$ substrate, as well as the cost and difficulty of analysis by mass spectrometry (Oremland and Capone 1988). The growing availability of high precision isotope ratio mass spectrometers in line with elemental analysers has meant that the process of determining ^{15}N enrichment within biomass has been greatly simplified. A relatively simple method for determining direct rates of $^{15}N^{-}N_2$ fixation has now been described for water column samples (Montoya et al. 1996). In this study the acetylene reduction assay was employed, but was calibrated with ^{15}N-N_2 to ensure the correct acetylene:N_2 ratio was used.

Nitrogen remineralisation rates

The rate of nitrogen remineralisation is generally inferred as the rate of production of NH_4^+. This is often achieved by measuring the exchange of NH_4^+ across the sediment-water interface (see below). This neglects NH_4^+ consuming processes that may be occurring at the sediment-water interface such as nitrification and consumption by MPB. Production rates of NH_4^+ within the sediment have been estimated in a number of ways. Sediment samples may be incubated for various periods of time, and the rate of NH_4^+ accumulation is measured. The danger with this method is that changes in the sediment environment associated with core handling will lead to the death of microbes, which may contribute to sediment NH_4^+ production rates. There are also problems associated with extracting NH_4^+ from the adsorbed sediment pool. A variation on this method is the $^{15}NH_4^+$ tracer technique, which allows the net and gross remineralisation rates to be calculated. This is particularly useful as it allows the rate of NH_4^+ uptake into bacterial cells to be

calculated (Blackburn 1988). The use of this technique requires access to a mass spectrometer set up to measure the ^{15}N enrichment in NH_4^+.

Another approach is the use of gradient models. The production of solutes within the sediment and diffusion rates across the sediment-water interface can be modelled from concentration gradients within the sediment and at the sediment-water interface (Berner 1980). More complex models have also been proposed which take into account the effects of bioirrigation and bioturbation (Boudreau 1984). Recently a sophisticated porewater modelling routine has been developed, which enables the calculation of sediment production and consumption rates as a function of depth as well as calculating the flux across the sediment-water interface (Berg et al. 1998). Of key importance, this model includes the non-diffusive transport mechanisms of bioturbation and bioirrigation, which are particularly important in coastal sediments. The free availability of the executable code for this routine allows this relatively complex modelling to be undertaken rapidly and easily.

Porewater profiles of solutes in soft sediments are generally obtained by sectioning of sediment cores and subsequent centrifugation (e.g. Blackburn et al. 1996), or by squeezing (e.g. Rysgaard et al. 2000) of the porewater out of the sediment. Such techniques are generally capable of a resolution of up to two mm. One of the major obstacles to accurately predicting diffusive fluxes of solutes is obtaining a profile with a high enough resolution to define zones of production and consumption which may occur on a mm to sub mm scale. The advent of microelectrodes has allowed the measurement spatial gradients of a number of solutes at the sub-mm scale (Revsbech and Jørgensen 1986). Unfortunately the range of solutes that can be routinely measured using microelectrodes in saline environments is still limited to O_2, N_2O, S^{2-} and pH. The use of gel probes to determine high resolution profiles of porewater solutes is a recent advancement which allows the determination of a wide range of solutes at a resolution of up to 1 mm (Mortimer et al. 1998). One of the major limitations of this technique is that is requires specialised equipment for the analysis of the small volumes of sample. This technology is still relatively new and the use and production these probes is not widespread.

In this study, the core slicing and centrifuging technique was utilised to obtain powater profiles. Due to the fine nature of the sediment, a resolution of 0.5 cm was obtained. This method could also be easily set up using existing equipment. Rates of NH_4^+ production within the sediments were calculated using the porewater modelling routine of Berg et al. (1998).

Sediment-water exchange of solutes

Direct flux measurements are usually made on intact sediment cores, or in a benthic chamber placed over the sediment. The concentration of a particular solute is then measured over time, and the exchange rate between the sediment and the water column calculated as a function of time and sediment surface area. The advantage of this methodology is that it integrates all solute transport processes, without any assumptions inherent in modelling processes (Blackburn 1986). It however gives very little information on processes occurring at the sediment-water interface though. Both core and chamber incubations suffer from a number of problems inherent with isolating a body of water. As the solute concentration changes over time, so too will the diffusional gradient between the sediment and the water interface, which will then change sediment flux rates (Miller-Way et al. 1994). In order to minimise such artefacts, solute concentrations should not change by more than 20% over the incubation period (Blackburn 1986). The pumping of solutes from the sediment as a consequence of natural water movement is also is also excluded, which may mean that fluxes are underestimated using these techniques (Asmus et al. 1998; Jahnke et al. 2000).

There is ongoing debate about the relative merits of cores and benthic chambers. Benthic chambers have the advantage of minimising disturbance to the community and sediment structure, other factors such as in-site temperature and light regimes are also maintained. Benthic chambers also generally encompass a larger surface area than cores, thus better integrating community processes, including the activity of large macrofauna, which are generally excluded from cores. Chambers are, however, large and cumbersome to deploy, often requiring divers for emplacement, which makes replication difficult. Where current velocities are high, the movement of water over the chamber may lead to a pressure wave behind the chamber resulting in artificial pumping of water through the sediment (Huettel and Gust 1992). Cores have the advantage of being relatively easy to sample and handle, hence making

spatial replication easier. Removing cores from the field to a controlled environment also removes the effects of stochastic events confounding data interpretation (e.g. changes in temperature and light). It is also easier to establish relationships between sediment properties and metabolism using cores.

Comparative studies of core and benthic chamber studies have generally shown a good agreement between the two methods (Miller-Way et al. 1994; Nielsen and Glud 1996; Asmus et al. 1998; Holcombe et al. 2001). The choice of methodology then, largely comes down to the particular aims of the study. In intertidal sediments, the use of benthic chambers is less practical due to the periodic exposure of the sediments. The use of flumes in intertidal sediments allows the determination of the sediment-water exchange of dissolved solutes without excluding the effects of currents and turbulence. It has been found that sediment-water exchange of solutes such as NH_4^+ are much greater when measured in flume systems compared to cores and bell jars (Asmus et al. 1998). The use of open flumes, however, precludes the measurement of the exchange of gases. Asmus et al. (1998) concluded that where the aim of the experiment was to evaluate the controlling influences on benthic microbial metabolism and nutrient fluxes, the use of cores and bell jars was a suitable technique, however, caution should be exercised when scaling up measurements of nutrient fluxes to in-situ rates.

Much attention has recently been focused on nitrogen cycling on intertidal flats and shallow water sediments in European systems under the "NICE" project (Dalsgaard 2000). One of the major aims of that project was to investigate the role of benthic primary producers in the sedimentary nitrogen cycle. The consensus amongst leading European researchers involved in this project was that ex situ core incubations were the best approach to use. As one of the major aims of my study was also to investigate the role of benthic primary producers in the sedimentary nitrogen cycle, it was considered prudent to adopt a similar approach to allow meaningful comparisons between this study and the results from European systems. The ex-situ core approach was also considered the most practical due to climatic factors. The regular passage of cold fronts across Tasmania from the Southern Ocean meant that in-situ field studies were considered impractical, particularly during winter. Therefore, the protocols described by Dalsgaard (2000) were

followed as closely as possible for the determination of the fluxes of dissolved solutes.

Benthic metabolism

Primary production

A number of methods are currently used for the measurement of primary production by MPB, the following discussion is based on the three methods outlined by Underwood and Kromkamp (1999).

1) The bell jar technique: this essentially measures the flux of oxygen under light and dark conditions in an enclosed system as described above, in the direct flux measurement section. Gross sediment primary production is calculated by subtracting the dark flux of O_2 (respiration) from the light flux (net production). This assumes that sediment oxygen demand does not increase in the light. This, however, may not be the case, due to increases in respiration and the reoxidation of reduced sulfides caused by increased oxygen penetration into the sediments (Glud et al. 1992; Epping and Jorgensen 1996; Fenchel and Glud 2000). The measurement of TCO_2 fluxes over a light/dark cycle will give more accurate estimates of primary production because the TCO_2 flux will not be affected by the oxidation of reduced sulfides during the light period (Fenchel and Glud 2000).

2) ^{14}C uptake method: for this method, the uptake of $^{14}CO_2$ over a specified time period is measured. One of the major limitations of this method in sediments, is the uncertainty in the $^{14}CO_2$ activity at the sediment-water interface due to the production of CO_2 within the sediment. One means to overcome this is to slurry sediment samples, however, this will destroy sediment gradients resulting in a greater availability of nutrients and CO_2, potentially changing the rates of photosynthesis.

3) Oxygen microelectrode techniques: two approaches have been used to measure primary production using this technique. The light/dark shift technique involves measuring the decrease in oxygen concentration within a discreet surface sediment layer upon darkening the sediment. The decrease in oxygen concentration in the first seconds immediately after darkening is equivalent to the gross rate of oxygen production within that layer. The gross rate of oxygen production within the

sediment can then be calculated by integrating the rate of production within each sediment layer (Revsbech and Jørgensen 1986). It has been shown that this method can provide accurate estimates of gross production (Glud et al. 1992). Alternatively the net rate of photosynthesis may be calculated from a concentration profile of O_2 using Fick's law and diffusion coefficients to calculate the upward and downward flux of O_2 (Dalsgaard 2000). The major drawback of this method is that it can be time consuming and scaling up such microscale measurements is contentious (Underwood and Kromkamp 1999).

Respiration

Benthic respiration may be measured using the consumption of oxidants such as O_2, and the production of the end products of respiration such as TCO_2. The production and consumption of these species may be measured as described above, using either diffusive modelling techniques, or directly measured fluxes as described in the sediment-water exchange of solutes section. In coastal marine sediments, oxygen will generally only penetrate a few mm into the sediment (e.g. Revsbech and Jørgensen 1986) and references therein). Therefore, the bulk of sedimentary respiration processes will take place via anoxic pathways. Oxygen consumption within the sediment will occur primarily through the reoxidation of reduced solutes diffusing up into the oxic zone of the sediment (e.g. Rysgaard et al. 2000). Where respiration rates are high, a build up of reduced sulphur species may occur which will result in O_2 fluxes being lower than true carbon remineralisation rates (e.g. Hargrave and Phillips 1981). Conversely, the reoxidation of reduced sulphur species may mean that sediment oxygen consumption rates are in excess of carbon remineralisation rates (e.g. Alongi et al. 1999). As a consequence, the measurement of TCO_2 fluxes will give a better estimate of sediment remineralisation rates than O_2 fluxes. In freshwater sediments, a significant fraction of respiration may proceed via methanogenesis. Where there are significant concentrations of sulphate (such as in marine waters), sulphate reduction will be the predominant oxidation pathway compared to methanogenesis (Capone and Kiene 1988).

TCO_2 fluxes are generally measured in two ways. Firstly, TCO_2 may be measured directly via acidification and subsequent trapping of the CO_2 in a basic solution, e.g. via coulometry (Rysgaard et al. 2000) or flow injection analysis (Hall and Aller 1992). Secondly, the change in TCO_2 concentration may be followed by measuring

pH and alkalinity and calculating TCO_2 concentrations (Millero and Sohn 1992). The alkalinity changes determined using this method can also be used as a means of estimating the rates of solute reduction and oxidation within the sediment (Anderson et al. 1986; Eyre and Ferguson 2002). The measurement of both TCO_2 and alkalinity fluxes are complicated by the precipitation and dissolution of $CaCO_3$ within the sediment. Previous studies have shown that the dissolution varies from being of minor importance (Anderson et al. 1986; Mackin and Swider 1989) to making up >30% of TCO_2 flux where Foraminifera are present (Green et al. 1993).

In order to make measurements of benthic respiration and nutrient fluxes from the same core it was decided to measure benthic respiration and productivity by measuring the light and dark fluxes of O_2 and TCO_2 (pH-Alkalinity).

Organic matter pools and sources

Of fundamental importance in establishing the role mudflats play within a system is the identification of the various sources of organic matter to the sediments. This includes living biomass produced within the sediments, as well as that deposited from terrestrial and marine sources. Methods used to identify sources of organic matter can be based on microscopic, elemental, isotopic and molecular biomarker parameters. What follows is a brief overview of the commonly used methods for identifying sources of organic matter.

Stable isotope ratios

Stable isotope ratios are generally expressed in δ notation, which is defined as follows:

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ ‰$$

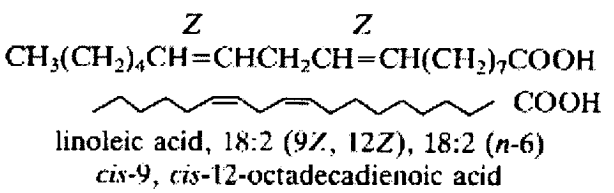
where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon is PDB limestone and the standard for nitrogen is atmospheric N_2. The stable carbon isotope ratio of organic matter will depend on the $\delta^{13}C$ of the carbon utilised and the photosynthetic cycle used in its production. Terrestrial plants generally use the C_3 (Calvin-Benson photosynthetic cycle) pathway and usually have $\delta^{13}C$ values in the range of -30 to -

23‰, whereas plants that use the C_4 (Hatch-Slack cycle) pathway (e.g. seagrasses) will have $\delta^{13}C$ values in the range -14 to -10‰ (Heip et al. 1995). The stable isotope ratios of $^{12}C/^{13}C$ in particular, can be particularly useful for distinguishing the relative contribution of organic matter from two well defined end-members, such as in estuaries (Heip et al. 1995). The advantage of this technique is that all of the organic carbon in the sample is analysed and will, therefore, provide a good estimated of the relative composition of total carbon in the sample.

Molecular markers

Molecular biomarkers such as fatty acids and sterols are one of the most powerful means of distinguishing sources of organic matter from various sources, as well as studying microbial communities in-situ (Killops and Killops 1993; Heip et al. 1995). They have the advantage of providing specific information on the sources of organic matter. For the sake of clarity, a rudimentary introduction to the structure of these biomarkers and their nomenclature is given here.

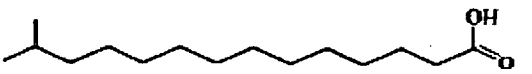
Fatty acids fulfil a variety of roles within living organisms including cellular membrane components (e.g. phospholipids), energy stores (e.g. triglycerides) and protective coatings (e.g. wax esters) (Killops and Killops 1993). Fatty acids are analysed in their free form after sample saponification. Fatty acids are normally referred to by their carbon chain length and degree of unsaturation. In the following example, the fatty acid would be commonly referred to as 18:2(n-6). The 18 refers to the number of carbon atoms, the 2 the number of double bonds and the 6 the position of the first double bond counting from the terminal methyl group.



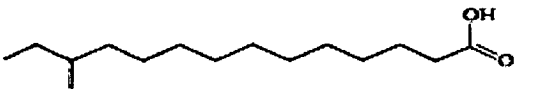
linoleic acid, 18:2 (9*Z*, 12*Z*), 18:2 (*n*-6)
cis-9, *cis*-12-octadecadienoic acid

Fatty acids with more than one double bond are referred to as polyunsaturated fatty acids (PUFAs) and are biosynthesised from saturated fatty acids by the action of desaturase enzymes. PUFAs are often useful for distinguishing source organisms. Branched fatty acids may be formed via the incorporation of branched amino acids into the biosynthetic pathway and are usually particularly abundant in bacteria

(Killops and Killops 1993). The following diagram illustrates the nomenclature of the two commonly occurring types of branched fatty acids. When the branch occurs, one carbon away from the terminal methyl group the fatty acid is given the prefix iso and when the branch occurs two carbon atoms away from the terminal methyl group, the prefix anteiso is used.

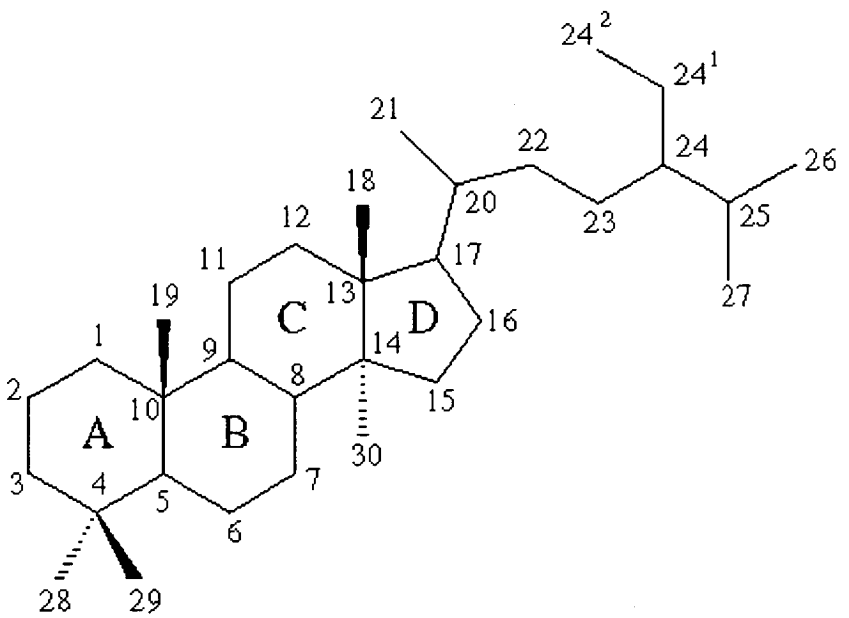


iso-15:0 Fatty acid



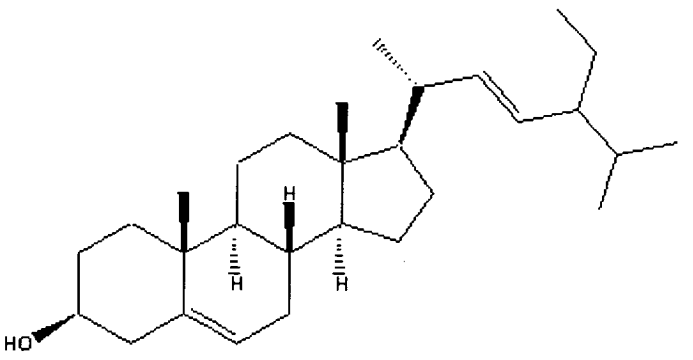
anteiso-15:0 Fatty acid

The term sterol refers to steroidal alcohols, which may be saturated (stanols) or unsaturated (stenols). Most sterols are found in cell membranes and lipoproteins. In cell membranes, sterols act as rigidifiers, lipoproteins are the means by which hydrophobic sterols are transported around organisms (Killops and Killops 1993). Nomenclature of sterols is based on a systematic steroidal numbering system as shown below for the cholestane structure (Moss 1989).



Generally, the sterols of geochemical interest are the C27 to C30 compounds with a β hydroxy group at C3, and a degree of unsaturation at the 5–6 position within the ring and a side chain at C17 (Killops and Killops 1993). In the case of a sterol such

as stigmasterol (24-ethylcholesta-5,22E-dien-3β-ol), as shown below, the 24 ethyl refers to the ethyl group at position 24, the 5,22diene refers to the two double bonds starting at positions 5 and 22 and the 3β-ol refers to the hydroxy group at position three.



In order to elucidate the sources of organic matter, as well as provide information on the microbial community present, fatty acid and sterol biomarkers as well as stable isotopes of carbon and nitrogen were analysed.

Microbial community parameters

It was expected that the benthic microbial community including photoautotrophs and bacteria were likely to exert an important controlling influence on carbon and nitrogen cycling within the mudflat system studied. It was also expected that there would be a significant interaction between the autotrophic and heterotrophic components of the community and that it was, therefore, imperative to have some means of quantifying the various components of each community.

Microphytobenthos

Because of the effort and expense of counting microalgal cells, as well as problems with scaling up cell counts to microalgal biomass, this technique is generally not used (MacIntyre et al. 1996). Traditionally chlorophyll *a* has been used as an index of MPB biomass. This is usually determined in three ways; fluorescence, spectrophotometry and HPLC. Spectrophotometry and fluorescence have the disadvantage of suffering interference from pigment pigment degradation products and chlorophyll *b*. While good agreement is generally achieved between these three methods, HPLC is generally considered the benchmark, and, therefore, the use of this technique is preferable (MacIntyre et al. 1996). The HPLC technique also has the

added advantage of providing information on accessory pigments and chlorophyll *a* degradation productions (Jeffrey et al. 1997). Accessory pigments can provide invaluable information on the community composition of microalgae (Jeffrey and Vesk 1997), and the presence and type of degradation products can give information on possible algal degradation pathways (Brotas and Plante-Cuny 1998; Lucas and Holligan 1999) and references therein. A summary of the accessory pigment biomarkers and the algal classes that produce them as well as the degradation products and their likely sources is shown in Table 1.1. In this study, HPLC was used to determine chlorophyll *a* as well as accessory pigments which could be used to obtain an estimate of the microalgal community composition of the sediment.

Table 1.1. A summary of carotenoid pigments, chlorophylls, chlorophyll degradation products and their likely sources that have previously been reported in intertidal sediments, (modified from Brotas and Plante-Cuny (1998)). Algal classes marked in *italic* produce the particular carotenoid in question as their major accessory pigment (Jeffrey and Vesk 1997).

Pigment	Algal type, material or process
Chlorophylls and breakdown products	
Chlorophyll a	Cyanobacteria, bacillariophytes, euglenophytes, chlorophytes cryptophytes macrophytic debris
Chlorophyllide a	Chlorophyllase containing cells, senescent cells
Pheophytins a	Microbial degradation processes
Pheophorbides a	Plant detritic material, grazed material
Chlorophyll b	Euglenophytes, chlorophytes, phanerogam debris
Pheophytins b	Detrital material from euglenophytes, chlorophytes phanerogams
Chlorophylls $c_1 + c_2$	Bacillariophytes
Chlorophyll c_3	Cryptophytes
Carotenoids	
Alloxanthin	Cryptophytes
β,β-Carotene	Cyanobacteria, bacillariophytes, euglenophytes, chlorophytes, macrophyte debris
Diadinoxanthin	Eugleneophytes, bacillariophytes
Diatoxanthin	Eugleneophytes, bacillariophytes
Fucoxanthin	*Bacillariophytes*, phaeophyte debris
Lutein	*Chlorophytes* phanerogam debris
Neofucoxanthin	Bacillariophytes
Neoxanthin	Cyanobacteria, euglenophytes, *chlorophytes*, bacillariophytes phanerogam debris
Violaxanthin	Bacillariophytes, *chlorophytes*, phaeophyte debris, *xanthophytes*
Zeaxanthin	*Cyanobacteria*, chlorophytes, *Rhodophytes* phanerogam debris

Microbially associated nitrogen pool

Intracellular nitrogen is likely to be the major nitrogen pool in surface sediments (Lomstein et al. 1990; Aller 1994). This pool can be estimated indirectly from measures of cell biomass such as direct counts, or the measurement of biochemical components such as fatty acids or ATP. The factor used to convert this back to the intracellular nitrogen pool is however, highly uncertain (Joergensen and Mueller 1995). Freezing the sediment samples will lyse microbial cells releasing intracellular nitrogen species such as NO_3^- and NH_4^+, thus giving an estimate of the intracellular nitrogen present within the sediment (Lomstein et al. 1990; Aller 1994). The degree of cell destruction by freezing is unknown. Soil scientists have long used chloroform fumigation as a selective means of releasing intracellular nitrogen. The nitrogen released by this fumigation is then extracted and total nitrogen determined, after suitable digestion of the sample (Brookes et al. 1985; Joergensen 1995). This method has also been applied to intertidal sediments in order to study the relationship between microbial-biomass associated nitrogen and sediment properties (Joergensen and Mueller 1995). While this approach is unlikely to provide an exact measurement of microbially-associated nitrogen, it will give a useful empirical measure of how the microbial biomass nitrogen varies spatially, temporally and in relation to biogeochemical processes. In this study, the fumigation extraction technique described by Joergensen (1995) was used to obtain an estimate of microbially bound nitrogen in the surface sediments.

Fatty acids are also a useful means by which to identify the genus of bacteria present, and also to provide a proxy for microbial biomass (Findlay and Dobbs 1993). The use of compound-specific isotope ratio mass spectrometry also offers a powerful means of identify the substrate being consumed by bacteria (Boschker and Middelburg 2002).

Study area, design and structure

The Huon Estuary is a drowned river valley, and begins just above Ranelagh, south of Hobart, stretching for 39 km southeast to Huon Island (Figure. 1.2). The CSIRO Marine Laboratories in Hobart recently completed a three-year interdisciplinary environmental study of the Huon Estuary (Butler et al. 2000). The estuary has an estimated mean flushing time of 7 days, with a riverine input averaging ~90 cumecs. It is a microtidal (mean tidal amplitude ~1 m), salt-wedge system, with a tendency to

become partially mixed near the mouth. The waters of the estuary are generally close to oxygen saturation apart for oxygen-depleted subhalocline waters at the top of the Egg Islands (Figure 1.2), influenced by anoxic sediments. The system could be classed as mesotrophic, and has a low nutrient input from the catchment, which is mostly intact wilderness. Nutrient budgets show that the estuary imports nutrients from coastal waters and that loads of inorganic nutrients from the catchment and aquaculture are small by comparison (Butler et al. 2000). In this respect, the Huon Estuary is still likely to function in much the same way as it did in a pristine state. The levels of dissolved inorganic nitrogen (DIN) in the surface layer of the estuary are usually severely drawn down (<1μM) between October and April as a consequence of biological uptake. Between April and October DIN concentrations increase and are dominated by NO_3^- derived from coastal waters which have a NO_3^- concentration of ~3–5 μM. Concentrations of DIN in the Huon River are negligible (<1μM). The waters of the Huon River are highly coloured due to elevated concentrations of coloured dissolved organic matter (CDOM) which are derived from a range of subalpine and "buttongrass" (*Gymnoschoenus sphaerocephalus*) moorlands as well as ti-tree stands (*Leptospermum* spp.) in the upper catchment. Dissolved organic nitrogen (DON) associated with this CDOM is the dominant form of dissolved nitrogen in the Huon Estuary with DON concentrations generally between 9–16 μM being observed in the Huon River (Butler et al. 2000).

Intertidal areas do not represent a large portion of the estuary as a whole, however, a number of large mudflats exist in depositional zones such as upper Port Cygnet and around the Egg Islands which is currently the fluvial delta region of the Huon River (Figure 1.2). The mudflats chosen for this study were located at Port Cygnet (Site PC) and Castle Forbes Bay (Site CF). Port Cygnet is a somewhat isolated side-arm of the estuary, with small and sporadic discharge from two rivulets. It has a southerly aspect and is, therefore, more exposed to the prevailing SW winds than Site CF, which has an easterly aspect. These two mudflats were selected as they represented a gradient across salinity and exposure to wave energy, which were both thought to be potentially important factors controlling the activity of MPB and carbon and nitrogen cycling. It was also considered likely that there would be gradients across the intertidal zone in terms of the type of organic matter deposited, light availability and also in terms of the rates and importance of the various biogeochemical cycling

processes. A hierarchical sampling design was, therefore, employed so that the cycling of carbon and nitrogen could be investigated across two spatial scales likely to influence the processes of interest, namely, site within the estuary and position on the mudflat

There is anecdotal evidence to suggest that the mudflat at Site PC mudflat has only accreted over the past 100 years due to land clearing in the lower catchment. In spring and summer this mudflat had an approximately 50% cover of the macroalgae *Gracillaria* sp. During autumn and winter this was greatly reduced to ~10%. Port Cygnet is considered to be part of the marine end of the estuary (Butler et al. 2000). The mudflat at Castle Forbes Bay was unvegetated at all times of the year. This site was located at the transition between the fluvial and the mixing zones of the estuary (Butler et al. 2000). The elevation of each of the sampling locations above lowest astronomical tide (LAT) was estimated by measuring the water depth at high tide. The exposure time for the upper and lower zones of each mudflat were calculated using the elevation of each position and hourly tidal data (National Tidal Facility Unpublished Tidal Data – Hobart Tide Gauge). These data are presented in Table 1.2.

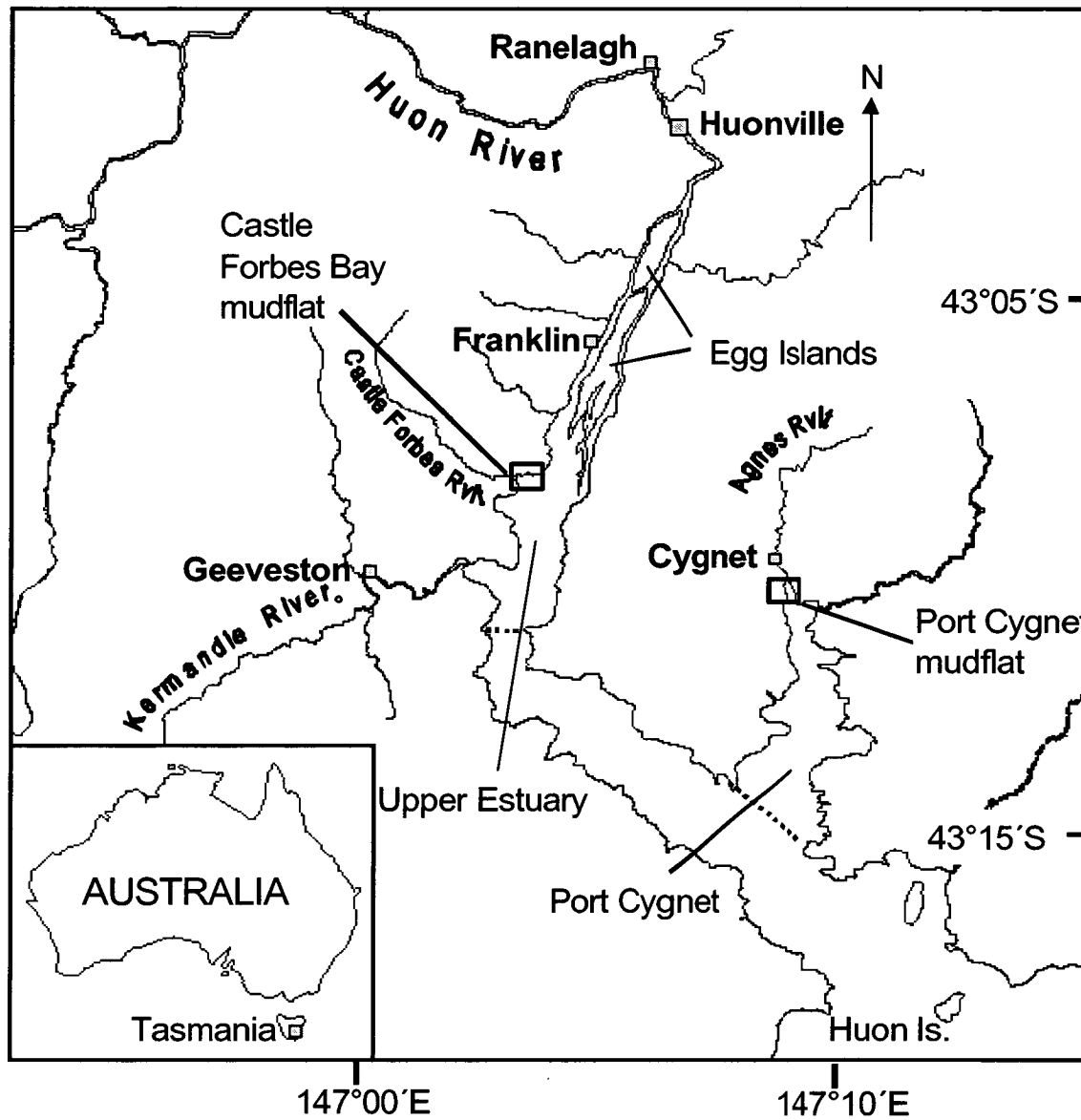


Figure 1.2. A map of the Huon Estuary in southeast Tasmania, showing the location of the study sites at Castle Forbes Bay and Port Cygnet, as well as the area defined as the upper estuary and Port Cygnet for the purposes of this study.

Table 1.2. Summary of the elevations of each sample site – meters above lowest astronomical tide (LAT), the percent of time the mudflat was exposed and the distance of each sample site from the edge of the mudflat (defined as where rooted macrophytic marsh vegetation began).

Site	Elevation (m above LAT)	% of time exposed	Distance from edge of mudflat
Castle Forbes Upper	0.5	28	12
Castle Forbes Lower	0.1	0.28	60
Port Cygnet Upper	0.7	43	5
Port Cygnet Lower	0.2	0.76	54

At Site CF, the mud was thick and unstable which made it difficult to move about on the mudflat. In order to overcome this problem, a small board walk was constructed on the mudflat during the sampling campaign to allow easy access to the sample sites on the upper and lower mudflat. At Site PC, the mud was not so deep and it was possible to move around on the mudflat relatively easily. At Site PC, the sample site on the upper mudflat was marked with a small peg, and the lower mudflat was located within 5m of a small (<1m high and <1m wide) rock outcrop. Samples were taken at Site PC, avoiding any areas vegetated by *Gracilaria* sp because one of the major aims of this study was to investigate the cycling of nitrogen with respect to MPB. At Site CF, the upper and lower positions were located at a marked position on, and, at the end of the board walk respectively. Samples on the upper and lower mudflat were collected haphazardly within several meters of each other. Samples at each site were collected at lower tide when the water on the lower mudflat was <1m deep.

The study was conducted in two campaigns. The first campaign was conducted to measure:

- Sources of organic matter (lipid biomarkers, stable isotopes) and benthic microalgal community composition (pigment biomarkers) – Chapter 2
- Rates of benthic metabolism (light and dark O_2 and TCO_2 fluxes) – Chapter 3
- Nitrogen cycling processes (denitrification, benthic nutrient fluxes, porewater profiles) – Chapter 4.

Samples for this campaign were collected during autumn, winter, spring and summer 2001. A preliminary sampling was also undertaken in spring 2000 at Site CF upper, for dissolved nitrogen fluxes and benthic metabolism for which the data have been presented. In order to minimise environmental variability masking any interactions between the biogeochemical processes and environmental parameters measured, the metabolic and nitrogen cycling processes were measured concurrently within the same cores. Sediment samples for biomarker, pigment, stable isotope, %C, %N and microbial nitrogen analysis were subsequently taken from these same cores. Porewater profiles were taken separately on subsequent field trips, due to logistical constraints with sample processing. The second campaign was conducted in order to measure nitrogen fixation at both study sites – Chapter 5. Samples were collected

during summer, autumn, winter and spring 2002. A synthesis of the findings from entire data set is presented in Chapter 6.

Chapter 2

Sources of organic matter to intertidal mudflats in a temperate Australian estuary

Abstract

The sources and cycling of organic matter on two mudflats in the Huon Estuary SE, Tasmania, were investigated over four seasons using pigment and lipid biomarkers as well as stable isotopes. One site was in the upper estuary, and the other one was in a marine side-arm of the estuary. The organic matter pool within the sediments at both sites was dominated by terrestrial sources. This was most likely refractory and not remineralised to a significant extent. Organic matter derived from microphytobenthos (MPB) only comprised a minor fraction of the sediment organic matter pool. Compound specific stable isotope analysis of bacterial biomarkers suggested the bacteria within the sediment were consuming microalgal derived material. The taxonomic composition of MPB was estimated using pigment biomarkers and a mathematical analysis routine (CHEMTAX). It was found that the MPB at the site in the upper estuary consisted of a mixed community of diatoms, cyanobacteria and chlorophytes. At the more marine site, chlorophytes and diatoms generally dominated. Separation of MPB from the sediment at the site in the upper estuary using the lens tissue technique was found to significantly fractionate the algal community with cyanobacteria being enriched in the separated fraction. Nitrogen fixation was likely to contribute significantly to the growth requirements of cyanobacteria at the site in the upper estuary, as indicated by the low $\delta^{15}N$ of the cyanobacteria separated from the sediment.

Introduction

The unique position of mudflats at the interface between the land and sea means that organic matter originating from a wide range of terrestrial and marine sources may be deposited within these environments. This may include organic matter exported from adjacent communities including mangroves and saltmarshes (Meziane et al. 1997; Meziane and Tsuchiya 2000), terrestrially eroded material such as peats (Volkman et al. 2000), as well as the discharge of organic matter from anthropogenic sources (Meziane and Tsuchiya 2002). Detritus from marine sources, such as seagrasses, as well as phytodetritus from pelagic algal blooms have also been identified as contributing to organic matter on mudflats (Volkman et al. 1980; Rohjans et al. 1998). Autochthonous production of organic matter by microphytobenthos (MPB), macroalgae and bacteria may contribute signficantly to the organic matter pool within mudflat sediments (Volkman et al. 1980; Meziane et al. 1997; Meziane and Tsuchiya 2000; Meziane and Tsuchiya 2002).

The quality (C:N ratio) of organic matter is of great importance in terms of the rates and recycling pathways of carbon and nitrogen (Herbert 1999). Where organic matter undergoing decomposition has a high C:N ratio (such as that of a terrestrial origin), much of the nitrogen remineralised may be assimilated into microbial biomass (Schlesinger 1997). More labile organic matter with a lower C:N ratio (such as that derived from algal material) will stimulate rapid remineralisation rates and a release of nitrogen from the sediment (Hansen and Blackburn 1992). Identifying these sources of organic matter to mudflats will give an insight into how organic matter is likely to be processed.

The classes of algae present on the mudflat may also have significant implications for the ecological functioning of the system. Most studies to date have shown that MPB on mudflats are dominated by diatoms (Cariou-Le Gall and Blanchard 1995; Barranguet et al. 1997; Brotas and Plante-Cuny 1998; Lucas and Holligan 1999; Cahoon and Safi 2002), but this may shift seasonally to chlorophytes and cyanobacteria (Barranguet et al. 1997). In some areas intertidal sediments may be dominated by cyanobacteria, where high rates of nitrogen fixation may supply a significant amount of nitrogen to the benthic community (Stal et al. 1984; Bautista and Paerl 1985; Stal 1995).

A number of approaches have been used to estimate the contributions of organic matter (OM) from various sources to sediments. Isotope ratios of $^{12}C/^{13}C$ have been used to estimate the relative contribution of terrestrial and marine sources to sedimentary OM (Fry and Sherr 1984). This is based on the fact that terrestrial OM will generally have a $\delta^{13}C$ of -26 to -30‰ and organic matter with a marine origin will generally have a $\delta^{13}C$ of -19‰ to -23‰ (Heip et al. 1995). The relative proportion of marine and terrestrial carbon in a sample can then be estimated using these end-members. While this approach is relatively simple and gives an integrated estimate of sources for the total carbon in the sample, it will only provide useful information when there are well-defined end-members. Furthermore, this technique gives no information about the type of marine or terrestrial organic matter in question. In a similar manner, C:N ratios have been used to give estimates of the relative importance of terrestrial or marine sources of OM (Thornton and McManus 1994 and references therein). A C:N ratio close to that of the Redfield ratio (6.7) is indicative of organic mater derived from phytoplankton while organic matter derived from terrestrial sources generally have C:N ratios greater than 12 (Bordovskiy 1965). This approach is, however, very approximate as degradation may significantly alter these ratios (Thornton and McManus 1994).

The use of lipid and pigment biomarkers can allow the various sources of various sub-fractions of organic matter to be identified much more specifically than any of the above approaches. Fatty acids provide a range of useful markers for microalgae, macroalgae, bacteria, seagrasses and terrestrial plants (Volkman et al. 1980; Meziane et al. 1997; Volkman et al. 1998; Kharlamenko et al. 2001). Sterols have also been used to identify sources of organic matter including that derived from faeces, diatoms and terrestrial sources (Volkman 1986; Barrett et al. 1995). Triterpenoid alcohols have been used as relatively unambiguous markers for higher plants (Volkman et al. 1987), and hopanoid alcohols have recently been shown to be markers for cyanobacteria (Summons et al. 1999). Unambiguously assigning sources to various other biomarkers is difficult, as they are rarely produced by a single class of organism. The advent of compound-specific stable isotope analysis has provided a powerful new tool for the differentiation between two sources of the same biomarker, provided the isotopic signatures of the two sources are different. The use of compound-specific stable isotope analysis also allows a greater understanding of the

flows of carbon through the microbial food web, because, in general, the isotopic signature of heterotrophic organisms will be similar to their food source. Thus, the measurement of the isotopic ratios of bacterial biomarkers can help to identify their carbon sources in environments where primary producers have a range of isotopic compositions as occurs across estuarine gradients (Boschker and Middelburg 2002).

A number of approaches exist to identify the presence, and relative importance of microalgal classes. Simple visual methods have commonly been used, however, they are laborious, and microscopic cells are difficult to count on a large scale (Brotas and Plante-Cuny 1998; Cabrita and Brotas 2000). The sediment may often interfere with a range of measurements including cell counting, algal C:N ratios and stable isotope measurements. Therefore, it is often necessary to separate the MPB from the sediment for these purposes (Couch 1989; Cabrita and Brotas 2000). A common method of separating MPB from intertidal sediments is the use of the lens tissue method (Eaton and Moss 1966). However, differences in the mobility of algae within the MPB may lead to a fractionation of the community, resulting in a bias against less mobile taxa. I am not aware of any studies which have investigated this possibility.

Another means of distinguishing algal classes is through the use of carotenoid accessory pigments, some of which are biomarkers for specific algal classes (Jeffrey and Vesk 1997). Fatty acids also offer the potential to distinguish various algal classes (Volkman et al. 1989; Volkman et al. 1998), although these may be less specific than pigments. Using these methods to estimate the relative importance of algal classes requires an estimate to be made of the ratios of pigments and fatty acids to a measure of biomass such as chlorophyll a (chl *a*). These may not necessarily be accurate as the ratios of pigments and fatty acids will change depending upon environmental conditions (Thompson et al. 1990; Porra et al. 1997).

Most studies of mudflats to date have generally only used one or two of the above techniques to study the ecological processes occurring in these systems. In this study I have combined a range of modern methods to gain a detailed understanding of the sources, and cycling of organic matter on two mudflats in a temperate Australian estuary.

Methods

Sampling

Sediments

A description of the study sites and the sample collection procedure from the upper and lower mudflat at Castle Forbes Bay (Site CF) and Port Cygnet (Site PC) is given in Chapter 1. Immediately after sediment incubations were performed as outlined in Chapters 3 and 4, the top 0.5 cm of sediment was collected with a spatula from half of each core and a 4 ml sub-sample was placed in cryo vials and frozen in liquid nitrogen (-176 °C) for later pigment analysis. For the fatty acid, sterol, stable isotope, %C and %N analysis, a second 10 ml subsample was frozen at -20°C. Because fatty acid analysis requires more biomass, four replicate samples from each site were pooled in equal proportions prior to extraction and analysis.

Separation of microphytobenthos

MPB were separated from the sediment using the lens tissue method as described by Eaton and Moss (1966). Briefly, four cores of sediment (id = 6.5 cm) were taken from the two study sites and returned to the laboratory. Two layers of lens tissue (Kimwipes^{R}, Kimberly Clark, Milsons Point, Australia) were then placed on the sediment surface. The cores were then left in the dark at ambient laboratory temperature (20 °C) overnight and the top layer of lens tissue removed the following day. Prior to the analysis of pigments, the lens tissue was frozen under liquid nitrogen. Lens tissue collected samples for analysis of stable isotopes, sterols and fatty acids, were placed in a vial of NaCl solution (15 g l^{-1}) and agitated to resuspend the algae back into solution. The algal suspension was taken up in a syringe and filtered through a pre-combusted 25 mm Whatman GF/F glass fibre filter. The filter containing the microalgae was frozen at -20°C until analyses. Lens tissue samples with no algae present (blanks) were treated in an identical fashion to ensure that carbon derived from the lens tissue did not interfere with the analysis of the separated MPB

In order to investigate the sampling bias of the MPB community caused by the lens tissue method, an experiment was undertaken using pigment biomarkers. Six sediment cores were taken from the upper mudflat at Site CF. The surface sediment of three cores was sampled immediately by extruding the cores and slicing off the

top 0.5 cm with a spatula, these samples were frozen under liquid nitrogen for later pigment analysis. The MPB in the remaining three cores were separated from the sediment using the lens tissue method as described above. MPB separated in this manner were subsequently frozen for pigment analysis. After the separation of MPB from the sediment, the top 0.5 cm of sediment was taken from these cores also. The lens tissue and sediment samples were then stored under liquid nitrogen until used for pigment analysis.

Analytical Methods

Microscopy

Visual observations of algae separated from the sediments were made using a Zeiss Axioplan compound microscope. Selectected samples were photographed using a Zeiss Axiocam mounted on a Leica DMIRB inverted microscope. Images were captured using Axiovision software.

Lipid biomarkers

Sediment samples were extracted three times by a one-phase chloroform-methanol-water mixture (1:2:0.8 v/v/v) according to a modified version of the Bligh and Dyer method (Bligh and Dyer 1959). Samples were ultrasonicated for 10 min during each extraction, then centrifuged and the supernatant extracts combined. After phase separation, the lipids were recovered in the lower chloroform layer (solvent removed *in vacuo*) and were made up to a known volume and stored sealed under nitrogen at 4°C. An aliquot of the total extract was taken for alkaline saponification with 3 ml of 5% KOH in methanol:water (80:20). The test tubes were vortex mixed and heated at 80°C for 2 hours. The neutral fraction was extracted 3 times into hexane:chloroform (4:1). The fatty acid fraction was subsequently extracted in the same manner after the saponified total extract had been acidified to pH < 3 with HCl. Fatty acid methyl esters (FAMEs) were formed by treating the fatty acid fraction with MeOH:HCl at 80°C for 2 h and extracted into hexane: chloroform (4:1). The neutral fractions were treated with *bis*(trimethylsilyl)trifluoroacetamide (BSTFA, 100 µL, 60°C, 60 min) to convert hydroxylated compounds such as sterols and alcohols to their TMSi-ethers.

Gas chromatography (GC) was initially performed using a Varian CP 3800, interfaced with Waters Millenium chromatography software. For the analysis of the

neutral fraction, the gas chromatograph was equipped with a 50 m × 0.32 mm i.d. cross-linked 5% phenyl-methyl silicone (HP5, Hewlett Packard) fused-silica capillary column; hydrogen was the carrier gas. Samples were injected through a hot injector. The initial oven temperature was 45°C with a 30°C min^{-1} ramp rate to 140°C and then a 3°C min^{-1} ramp rate to 310°C which was held for 5 min. The fatty acid fraction was analysed on the same instrument, except that the samples were injected through a SPI injector (ramped at 200°C min^{-1}) onto a 50 m × 0.32 mm i.d. cross-linked 1% phenyl-methyl silicone (HP1, Hewlett Packard) fused-silica capillary column, with hydrogen as the carrier gas. The initial oven temperature was 45°C with a 25°C min^{-1} ramp rate to 180°C, then a 2°C min^{-1} ramp rate to 290°C followed by a 10°C min^{-1} ramp rate to 310°C, which was held for 10 min. Sterol and fatty acid fractions were analysed using a flame ionisation detector, with 5β(H)-cholestan-24-ol as the internal standard for sterols and the methyl ester of tricosanoic acid as the internal standard for fatty acids. Peak identifications were based on retention times relative to authentic and laboratory standards and subsequent GC-MS analysis. Data acquisition was by Millenium chromatography software and personal computers operating Windows NT. The detection limit for individual sterols and fatty acids was approximately 0.2 mg m^{-2} of 0.5 cm deep sediment.

Verification of the identity of individual sterols and fatty acids by GC-MS analyses was performed on a Thermoquest / Finnigan GCQ-Plus benchtop mass spectrometer fitted with a direct capillary inlet and an automated on-column injector. Data were acquired in scan acquisition or selective ion monitoring and processed using Xcalibur software supplied with the instrument. The nonpolar column (HP5) and operating conditions were similar to that described above for GC-FID analyses, but helium was used as the carrier gas.

Pigment biomarkers

Sediments were extracted in 100% acetone with in an ice-filled ultrasonication bath for 20 min. The sediment-acetone mixtures were left overnight at 4°C before being centrifuged and the acetone extract decanted into a volumetric flask which was stored at 4°C. A second extraction was then performed and the samples again left for several hours in a refrigerator. The second extract was combined with the first extract, made up to 25 ml with water and acetone such that the final mixture was

90:10 acetone:water by volume, and filtered (0.2-μm membrane filter, Whatman, Anatope) for immediate analysis. Pigment analysis used a Waters high-performance liquid chromatograph, comprising a 600 controller, 717 plus refrigerated autosampler, and a 996 photodiode array detector. Pigments were separated using a stainless steel 25 cm × 4.6 mm ID column packed with ODS 2 of 5-μm particle size (SGE) with gradient elution as described by Wright et al. (1991). The separated pigments were detected at 436 nm and identified against standard spectra using Waters Millenium software. Concentrations of chlorophyll *a* (chl *a*), chlorophyll *b* (chl *b*), β,β-carotene, and β,ε-carotene in sample chromatograms were determined from standards, and all other pigment concentrations were determined from standards of purified pigments isolated from algal cultures.

Stable isotopes and % organic carbon and nitrogen

Algal samples on glass-fibre filters were fumed over HCl to remove any carbonates present and dried at 60°C for several hours, before being divided using a scalpel and tweezers and packed into tin cups for analysis (Elemental Microanalysis Ltd., Okehampton, UK). Sediment samples for stable isotope analysis were dried in an oven overnight at 60°C, before being ground with a mortar and pestle. Sediment samples were weighed into tin cups for nitrogen analysis. For the analysis of carbon, samples were weighed into aluminium cups and were subsequently acidified using sulphurous acid to remove any mineral carbonates. Samples were then analysed for nitrogen and carbon contents, $\delta^{15}N$ and $\delta^{13}C$ using a Carlo Erba NA1500 CNS analyser interfaced *via* a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1090°C and reduction at 650°C. Where necessary, due to high carbon contents, the carbon signal was quantitatively diluted with helium. Samples were analysed at least in duplicate. Results are presented in standard δ notation:

$$\delta^{15}N \text{ or } \delta^{13}C\ (‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ ‰$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon is PDB limestone and the standard for nitrogen is atmospheric N_2. The reproducibility of the stable isotope measurements was ~0.5‰ for C and 1‰ for N

The proportions of different algal classes present were estimated using fatty acid and pigment data. These derived results are presented in the discussion.

Compound specific stable isotope ratio analysis

Compound specific isotope ratio mass spectrometry was performed on selected sediment extracts using a Hewlett Packard 5890 series II gas chromatograph, which was coupled via a Finnigan MAT GC combustion interface to the isotope ratio mass spectrometer described above. The gas chromatograph was equipped with a 60m J&W DB-1, 0.32 mm id column with He as the carrier gas. Samples were injected on-column via a "duck bill" (Hewlett Packard) cold on-column injector. The initial oven temperature of 40°C was maintained for 1 min followed by a 30°C min^{-1} ramp rate up to 120°C followed by a 4°C min^{-1} ramp rate up 315°C, which was held for 15 min. Samples were co-injected twice with C_{16} and C_{24} deuterated n-alkanes of known isotopic composition, the average of the two injections are reported here. To obtain the ratio of the biomarker compounds, the carbon isotope ratio of the derivatised samples were corrected for the number of carbon atoms that had been added during derivatisation (3 for TMSi ethers and 1 for FAMEs) as described by Boschker et al. (1999). Results are presented in standard δ notation as described above.

Results

Stable Isotopes, organic matter content and C:N ratios

Figure 2.1 shows the organic carbon content and stable isotopic ratios recorded for carbon and nitrogen at Sites CF and PC during the study period. The organic carbon content of the sediment was always greater on the mudflat at Site CF compared to that at Site PC. At Site CF, organic carbon was generally 5% of the sediment, whereas at Site PC, organic matter always comprised less than 3% of the sediment. At Site CF, the organic carbon content of the sediments was not significantly different between the upper and lower mudflats (1-way ANOVA). At Site PC the organic carbon content of the sediment was significantly greater on the lower mudflat (1-way ANOVA, $p<0.01$). The bulk C:N ratio of the organic matter was generally higher at Site CF than at Site PC.

The $\delta^{13}C$ values at all sites were between -26 and -23‰ over the course of the year. At Site CF, the most enriched $\delta^{13}C$ values were observed during spring, coinciding with the highest chl *a* values and greatest productivity on the upper mudflat (Chapter 3). At Site PC upper, the carbon isotopic signature of the mudflat remained relatively constant throughout the year, whereas on the lower mudflat it became more depleted over the course of the year. The $\delta^{15}N$ at Site CF ranged between 1–3‰ on the upper mudflat and 0–2‰ on the lower mudflat. At Site PC, the $\delta^{15}N$ ranged 2.5–5‰ and was generally greater than at Site CF.

The C:N ratios and isotopic signatures of a range of possible sources of organic matter are shown in Table 1. At Site CF, the isotopic signature of MPB was consistently in the range of -14 to -17‰ for $\delta^{13}C$ and 1.5–2.8‰ for $\delta^{15}N$. Snails (*Salinator solida*) taken from the mudflat at Site CF had a $\delta^{13}C$ of -14.5 and a $\delta^{15}N$ of ~ 3, similar to that of the local MPB. At Site PC, the MPB was isotopically more depleted for carbon with a $\delta^{13}C$ of -20.5‰ and isotopically enriched for nitrogen with a $\delta^{15}N$ of 7.1‰. Macroalgae (*Gracilaria* sp.) taken from Site PC had a $\delta^{13}C$ of -19‰ and a $\delta^{15}N$ of 8.4. Terrestrial sources of organic matter including that from creek sediments and plant detritus consistently had a $\delta^{13}C$ more depleted than -27.5‰ and a $\delta^{15}N$ more depleted than 2‰. The marsh reed *Juncus kraussi* had an intermediate signature of -26‰ for $\delta^{13}C$ and 3.5‰ for $\delta^{15}N$.

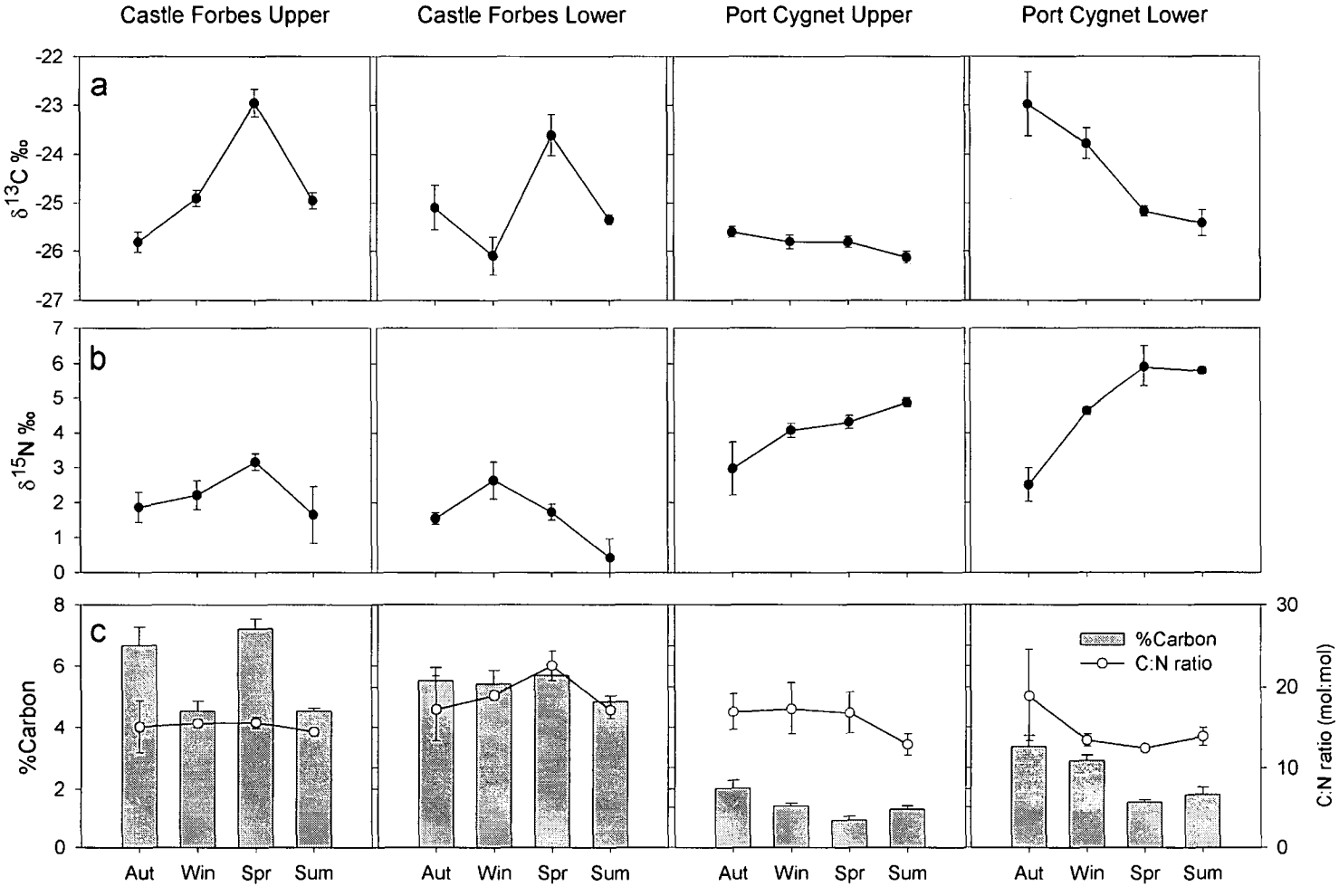


Figure 2.1. Summary of the $\delta^{13}C$ (a), $\delta^{15}N$ (b), % carbon and C:N ratios (c) measured in the top 0.5 cm of sediment in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) at the two study sites during the year 2001. Error bars represent the standard error of the mean (n=3–4, except for the $\delta^{15}N$ of Port Cygnet lower during summer n=1).

Table 2.1. Summary of the $\delta^{13}C$, $\delta^{15}N$ and C:N ratios of likely sources of organic matter at Sites CF (Castle Forbes Bay) and PC (Port Cygnet) including microphytobenthos (MPB), and sediments collected from adjacent rivulets (Rvlt). Sampling date denotes month/year. Huon "marine" and "terrestrial" denote the average isotopic signature of sediments taken from the marine and terrestrial end–members of the Huon Estuary as part of the Huon Estuary study (Butler et al. 2000). Standard errors are shown in brackets (n=3–4). Snails were analysed as a composite of ~10 individuals.

Sample	Date sampled	$d^{13}C$ (‰)	$d^{15}N$ (‰)	C:N (mol:mol)
Snails (*Salinator solida*) CF	3/01	-14.5	2.7	4.7
Snails (*S. solida*) CF	2/02	-14.5	3	4.7
MPB CF upper	5/01	-17	1.5	15.05
MPB CF upper	7/01	-15.6	-	-
MPB CF upper	1/02	-16.7 (0.75)	1.6 (0.2)	6.2 (0.28)
MPB CF lower	5/01	-15	2.8	14.7
MPB CF lower	2/02	-14 (0.26)	2.1 (0.67)	4.8 (0.25)
MPB range CF		**-14 to -16.7**	**1.5 to 2.8**	**4.8-14.7**
MPB PC lower	2/02	-20.5 (0.2)	7.1 (1.1)	11.2 (0.61)
Castle Forbes Rvlt sediment	2/02	-29.1 (0.13)	1.9 (0.47)	34 (7)
Agnes Rvlt sediment	2/02	-27.5 (0.6)		
Huon "Marine"	Huon Estuary Study	-24.5 (0.3)	7 (0.08)	8.4 (2)
Huon "Terrestrial"	Huon Estuary Study	-28	2	17.5
Macroalgae *Gracilaria* spp. PC	2/02	-19.2 (0.27)	8.4 (0.39)	27 (2.9)
Marsh Reed *Juncus* CF	3/01	-26	3.5	35
Eucalyptus Detritus CF	3/01	-29	-2.14	97

Biomarkers

Sterols

The concentrations of total sterols found at the two sample sites throughout the year can be seen in Figure 2.2. Total sterol concentrations varied between 77 and 550 mg m^{-2} and were highest on the upper mudflat at Site CF during spring. The percent composition of the sterols are shown in Table 2. The relative composition of the sterol biomarkers were generally constant throughout the year suggesting a relatively consistent input of organic matter from the various sources. Sterol composition was consistent between the upper and lower mudflats and between the two sites. Total sterols were highly correlated with chl *a* ($r=0.93$, $p<0.01$) and phytol ($r=0.97$, $p<0.01$). The sterol fraction was dominated by 24-ethylcholest-5-en-3β-ol (sitosterol / clionosterol) at all sites throughout the year, comprising between 17 and 38% of total sterols. 24-ethylcholest-5-en-3β-ol concentrations were highly and significantly correlated with chl *a* ($r=0.9$, $p<0.01$), phytol ($r=0.96$, $p<0.01$) and 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol) ($r=0.92$, $p<0.01$), as well as the

accessory pigments fucoxanthin ($r=0.85$, $p<0.01$) and zeaxanthin ($r=0.73$, $p<0.01$). Cholest-5-en-3β-ol (cholesterol) comprised approximately 10% of total sterols throughout the year at Site CF, and between 8 and 18% of sterols at Site PC. At Site CF, 24-methylcholesta-5,24(28)-dien-3β-ol was also an important component of the sterol fraction comprising between 10 and 20% of total sterols, but at Site PC this sterol was generally only present in very small quantities. 24-methylcholesta-5,22E-dien-3β-ol (brassicasterol/crinosterol) comprised between 10 and 18% of total sterols at Site CF and between 6 and 14% of total sterols at Site PC. 4,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol) was detected occasionally in the sediments at both sites, but followed no apparent seasonal pattern, and was generally less than 5% of total sterols. Other sterols detected include 24-ethylcholesta-5,22E-dien-3β-ol (stigmasterol) (<6% of total sterols), 24-methylcholest-5-en-3β-ol (campesterol) (<1.5% of total sterols), cholesta-5,24-dien-3β-ol (desmosterol) (<3.5% of total sterols), 24-ethylcholesta-5,24(28)Z-dien-3β-ol (isofucosterol) (<3% of total sterols), and 24-methylcholesta-5,7,22-trien-3β-ol (ergosterol) which was only detected at Site CF and comprised less than 3% of total sterols.

Stanols

The faecal markers 5β-cholestan-3β-ol (coprostanol), 5α-cholestan-3β-ol (cholestanol) and 24-ethyl-5β-cholestan-3β-ol were detected at Port Cygnet, with concentrations being greater on the upper mudflat than the lower mudflat throughout the year (Table 3). 24-ethyl-5α-cholestan-3β-ol (sitostanol) was also a major component of the total sterol fraction comprising between 3 to 9% of total sterols at CF and between 6 and 11% of total sterols at Site PC. 24-methyl-5α-cholestan-3β-ol (campestanol) comprised a relatively consistent 2–4% of total sterols at both sites. 24-ethyl-5α-cholest-22E-en-3β-ol (stigmastanol) was also detected at times, only comprising <1.3% of total sterols. The stanol:sterol (total stanols:sterols) ratio at Site PC (0.7–0.85) was much greater than at Site CF (0.3–0.57). The ratios of 5α-cholestan-3β-ol:cholest-5-en-3β-ol and 24-ethyl-5α-cholestan-3β-ol:24-ethylcholest-5-en-3β-ol were both significantly greater at Site PC than at Site CF on an annual basis (t-test, $p<0.01$).

Phytol

Phytol concentrations varied between 17 and 642 mg m^{-2} (Figure 2.2). Phytol was converted to chl *a* equivalents using a multiplication factor of 3 to correct for the differences in molecular mass (296 compared to 893 for chl *a*). After this correction chl *a* equivalents of phytol were always in excess of the actual chl *a* concentration by a factor of between 2.5 and 6.

Fatty Acids

The total concentrations of fatty acids on the mudflats studied are shown in Figure 2.2. Concentrations of total fatty acids ranged between 57 and 822 mg m^{-2}. Fatty acid concentrations were highest on the upper mudflat at Site CF (1-way ANOVA, Fisher LSD Post hoc, $p<0.001$) where rates of primary production and MPB biomass were highest (Chapter 3). Table 3 shows the percent composition of the fatty acids measured at both sites over the study period. Total fatty acids were correlated with chl *a* ($r=0.88$, $p<0.01$) and net primary production ($r=0.58$, $p<0.05$). Fatty acids were dominated by 14:0, 16:0, 16:1(n-7), 18:1(n-7) and 20:5(n-3). Levels of 18:4 and 18:2(n-6) were elevated on the upper mudflat at Site CF during winter, spring and summer relative to autumn at this site and also all the other sites sampled over the course of the year. The uncommon fatty acids 15:0 and 17:1(n-8) comprised a significant fraction of the fatty acids on both mudflats at times, most notably during autumn at Site CF. These two fatty acids were also highly correlated ($r=0.97$) suggesting they arose from the same source.

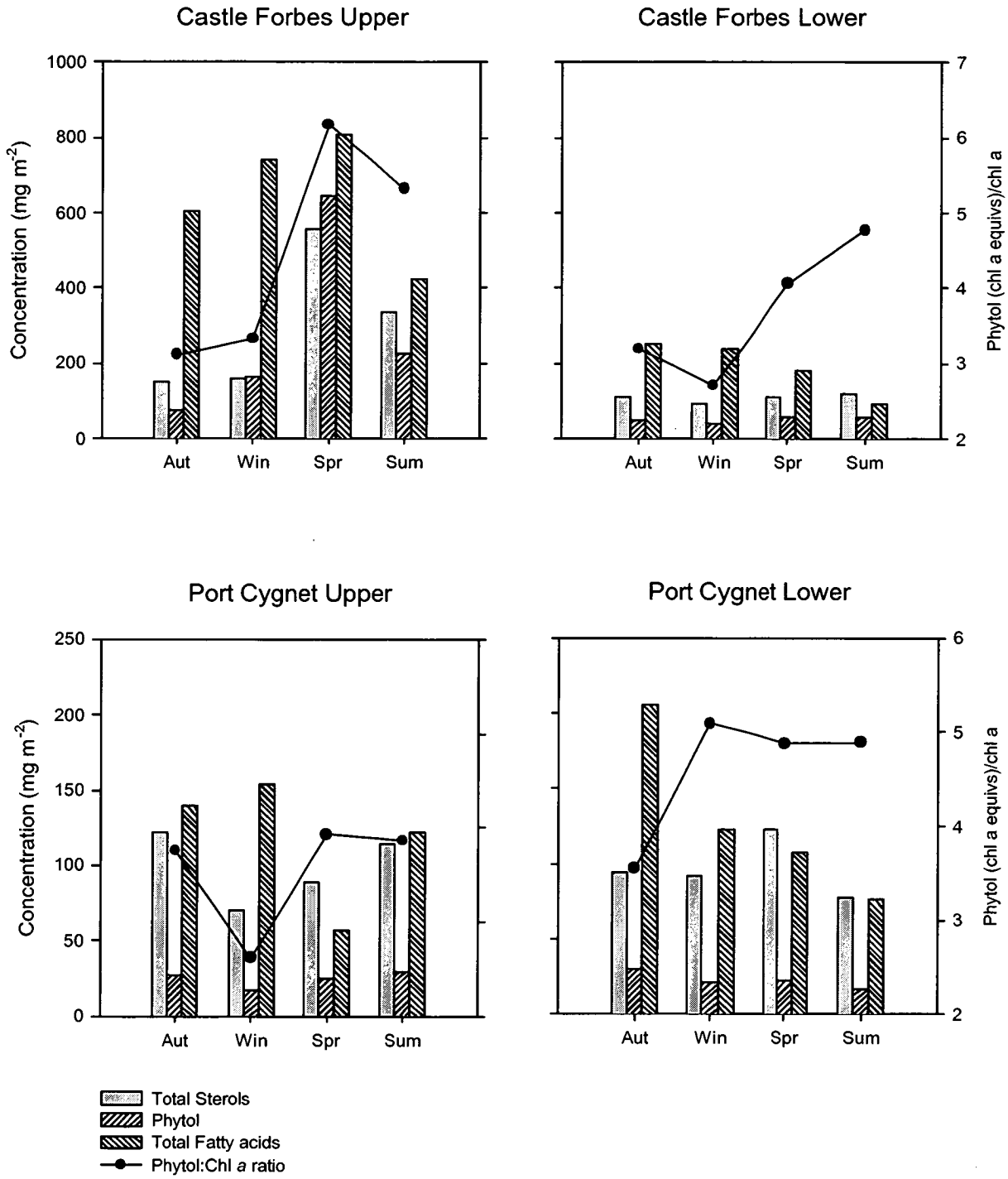


Figure 2.2. Summary of total sterols, total fatty acids and phytol and the phytol:chl *a* ratio measured in the top 0.5 cm of sediment in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) on the upper and lower mudflats of the two study sites during the year 2001 (n=1).

Table 2.2. The percent composition of sterols and stanols as a proportion of total sterols + stanols at in the top 0.5 cm of sediment at Castle Forbes and Port Cygnet during 2001. – indicates not detected.

	Castle Forbes Upper				Castle Forbes Lower				Port Cygnet Upper				Port Cygnet Lower			
	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum
Stenols																
Cholest-5-en-3β-ol	10.3	9.9	11.3	10.6	11.6	10.0	19.5	10.6	7.7	18.4	9.8	19.2	18.3	17.4	10.5	18.8
24-ethylcholest-5-en-3β-ol	22.8	29.1	24.2	33.2	28.4	34.8	26.5	30.7	37.8	21.7	38.5	17.9	27.3	21.8	38.7	25.7
24-ethylcholesta-5,22E-dien-3β-ol	3.5	4.0	4.3	4.4	2.7	4.5	3.1	4.2	5.0	4.1	5.6	3.9	4.9	4.6	4.6	4.4
24-methylcholest-5-en-3β-ol	1.0	1.5	0.9	1.2	0.5	1.0	0.6	1.1	0.9	1.0	0.9	1.1	0.6	1.1	0.9	1.1
cholesta-5,22E-dien-3β-ol	5.0	3.9	5.6	4.1	2.7	3.6	2.4	2.2	1.3	5.4	-	3.3	2.7	3.1	1.3	2.8
cholesta-5,24-dien-3β-ol	-	-	0.8	2.0	3.2	-	2.2	2.3	-	-	-	2.0	-	-	-	-
24-methylcholesta-5,22E-dien-3β-ol	13.5	10.4	18.6	10.2	10.6	12.4	12.0	11.0	6.7	10.1	6.9	9.5	14.1	9.3	7.6	8.2
24-methylcholesta-5,24(28)E-dien-3β-ol	23.2	16.0	12.0	11.4	22.1	12.8	10.6	9.9	-	-	-	6.1	-	-	-	5.4
24-ethylcholesta-5,24(28)Z-dien-3β-ol	1.1	1.3	1.6	1.4	1.9	1.8	1.0	1.8	1.0	2.8	1.2	1.5	1.7	1.5	1.8	1.8
4,23,24-trimethyl-5α-cholest-22E-en-3β-ol	-	5.0	2.7	-	4.2	-	4.2	3.3	-	5.3	-	4.8	-	5.0	-	-
24-methylcholesta-5,7,22-trien-3β-ol	2.3	-	2.9	-	1.1	-	3.0	2.8	-	-	-	-	-	-	-	-
sum%	**83**	**81**	**85**	**79**	**89**	**81**	**85**	**80**	**60**	**69**	**63**	**69**	**70**	**64**	**65**	**68**
Stanols																
24-ethyl-5α-cholestan-3β-ol	5.6	8.3	4.3	8.3	3.1	9.0	4.2	7.4	10.6	6.0	11.1	6.0	8.9	6.4	10.1	7.3
5α-cholestan-3α-ol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24-ethyl-5β-cholestan-3β-ol	0.4	0.5	0.3	0.7	0.3	0.7	0.5	0.6	14.7	3.5	12.9	3.3	7.9	3.5	10.6	3.6
24-ethyl-5β-cholestan-3α-ol	-	-	-	-	-	-	-	0.3	1.3	1.2	0.9	1.1	0.5	1.1	0.9	1.3
5α-cholestan-3β-ol	6.0	5.5	4.5	6.6	3.7	5.8	3.9	5.7	7.4	12.5	8.3	12.5	7.9	17.0	7.4	13.3
5β-cholestan-3β-ol	-	0.2	-	0.2	-	0.2	-	0.2	0.9	2.4	1.2	2.1	0.9	2.5	1.2	2.2
5β-cholestan-3α-ol	-	-	-	-	-	-	-	-	-	0.4	-	0.4	-	0.3	-	0.3
24-ethyl-5α-cholest-22E-en-3β-ol	0.6	-	0.5	-	0.3	-	0.5	0.6	1.0	1.0	-	1.3	-	-	-	-
24-methyl-5α-cholestan-3β-ol	2.7	3.5	2.5	3.7	2.2	3.4	3.7	3.0	2.1	2.4	2.5	2.3	2.9	2.4	2.7	2.4
5α-cholest-22E-en-3β-ol	-	-	1.0	-	0.5	-	0.6	0.7	-	-	-	-	-	1.4	-	-
24-methylcholest-22E-en-3β-ol	1.7	0.9	2.0	1.8	0.7	-	1.1	1.5	1.6	1.8	-	1.7	1.3	1.6	1.5	1.6
sum%	**17**	**19**	**15**	**21**	**11**	**19**	**14**	**20**	**39**	**31**	**37**	**31**	**30**	**36**	**34**	**32**
total%	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
stanol/stenol (cholest-5-en-3β-ol)	0.6	0.6	0.4	0.6	0.3	0.6	0.2	0.5	1.0	0.7	0.8	0.7	0.4	1.0	0.7	0.7
stanol/stenol (24-ethylcholest-5-en-3β-ol)	0.2	0.3	0.2	0.3	0.1	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

Table 2.3 Summary of the percent composition of fatty acids in the top 0.5 cm of sediment at Castle Forbes and Port Cygnet during 2001. – indicates not detected.

	Castle Forbes Upper				Castle Forbes Lower				Port Cygnet Upper				Port Cygnet Lower			
	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr	Sum
saturates																
12:0	2.3	1.0	1.2	1.6	0.8	0.7	-	1.3	0.8	0.4	0.9	0.9	0.8	0.9	0.8	0.7
14:0	4.1	2.8	2.9	3.1	4.7	4.4	3.9	5.5	5.2	3.8	5.6	5.1	4.3	4.8	5.8	5.6
i15:0	2.1	1.5	1.7	2.0	2.5	2.8	1.9	2.5	3.4	2.7	2.7	2.8	4.0	4.4	4.6	4.4
ai15:0	3.3	2.0	1.8	2.8	4.1	4.2	2.7	3.9	5.9	5.0	5.2	5.1	5.6	7.2	8.4	7.4
15:0	13.4	3.5	4.5	5.7	15.8	4.5	5.5	7.0	5.8	3.6	4.2	5.4	6.3	2.9	2.8	3.3
16:0	15.4	20.8	20.3	17.9	14.9	14.7	15.5	13.4	19.3	20.4	12.8	14.4	13.8	15.3	13.3	14.2
17:0	1.2	0.7	0.6	1.1	0.9	0.7	0.9	0.9	0.9	1.0	-	0.6	0.9	0.9	-	0.6
ai17:0	2.4	0.7	2.0	1.7	3.1	1.2	1.5	1.9	0.9	1.0	0.8	0.6	2.0	0.9	1.2	1.4
18:0	1.8	2.0	1.8	2.4	0.8	1.7	2.0	1.6	1.9	3.6	1.0	1.6	2.1	2.1	1.3	1.5
20:0	0.2	0.6	0.5	0.8	0.4	1.0	1.5	0.9	1.1	2.0	-	1.1	1.0	1.0	0.5	0.7
22:0	1.6	1.0	0.8	1.7	0.5	2.1	3.5	1.9	1.3	2.0	-	2.1	1.6	1.7	0.7	0.9
24:0	2.2	1.3	0.9	2.3	0.7	2.7	4.9	2.2	1.5	1.0	-	2.6	2.3	2.3	1.1	1.2
26:0	1.5	0.9	0.6	1.2	0.5	2.0	3.6	1.3	1.3	0.8	-	2.6	2.4	2.0	1.1	1.0
28:0	1.0	0.6	0.4	0.6	0.4	1.6	2.2	0.8	0.9	0.4	-	2.0	1.9	1.1	0.6	0.5
30:0	0.7	0.2	-	0.3	0.5	0.9	0.7	0.4	-	-	-	1.3	0.8	-	-	-
sum%	**53.2**	**39.8**	**40.1**	**45.3**	**50.5**	**45.2**	**50.4**	**45.5**	**50.2**	**47.6**	**33.4**	**48.2**	**49.6**	**47.5**	**42.4**	**43.3**
monounsaturates																
15:1	1.7	1.0	1.8	1.2	1.9	1.3	1.1	1.3	1.1	0.8	1.3	1.2	1.3	1.0	1.0	1.0
16:1(n-7)	11.4	15.5	17.4	15.0	13.1	17.6	17.7	15.2	22.5	18.7	29.9	21.0	18.0	22.5	30.1	24.4
17:1(n-8)	10.2	3.8	4.8	5.3	10.0	4.5	5.2	5.4	3.9	3.8	3.3	1.0	3.7	2.7	1.2	1.2
18:1(n-7)	4.3	5.2	3.5	5.8	3.1	5.9	5.0	4.3	6.7	9.2	3.9	4.9	7.8	10.8	7.1	7.4
sum%	**27.6**	**25.4**	**27.5**	**27.3**	**28.1**	**29.2**	**29.1**	**26.3**	**34.1**	**32.5**	**38.3**	**28.1**	**30.8**	**37.0**	**39.5**	**33.9**
polyunsaturates																
16PUFA	1.8	2.3	2.3	1.7	2.4	3.6	2.7	3.9	1.8	2.0	4.9	2.9	2.6	1.8	2.8	2.7
17PUFA	2.3	1.2	2.1	2.3	3.2	2.1	1.3	3.6	1.6	1.5	2.0	2.0	1.8	2.4	1.4	2.1
18:4(n-3)	1.9	7.4	7.4	4.7	2.4	2.6	1.9	3.3	1.0	1.4	2.2	1.8	1.3	1.3	1.8	1.9
18:2(n-6)	2.1	6.5	5.5	4.2	1.6	1.8	0.9	1.6	0.9	2.6	1.5	2.0	0.9	0.8	1.2	1.2
18:3(n-3)	1.3	1.5	1.4	1.8	1.0	1.7	1.7	1.5	2.4	3.3	1.4	1.7	1.9	2.5	1.8	2.4
19 PUFA	2.1	0.9	1.6	1.3	2.5	0.7	1.1	1.6	-	-	-	0.6	1.9	-	-	0.6
20:4(n-6)	0.6	-	0.3	0.7	1.2	1.6	-	2.2	2.1	1.1	1.6	3.1	0.6	1.0	1.2	1.1
20:5(n-3)	6.1	12.1	10.3	9.4	6.1	9.3	9.3	8.7	5.3	6.5	13.3	7.8	7.0	4.4	6.9	9.3
22:6	1.0	2.9	1.4	1.3	1.0	2.0	1.6	1.7	0.6	1.4	1.4	1.9	1.6	1.2	1.1	1.4
sum%	**19.2**	**34.8**	**32.2**	**27.4**	**21.4**	**25.5**	**20.5**	**28.2**	**15.6**	**19.8**	**28.3**	**23.7**	**19.7**	**15.5**	**18.1**	**22.8**
total%	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Compound specific stable isotope analysis

The results from the compound specific stable isotope analysis from selected lipid biomarkers are shown in Table 2.4. The fatty acids 22:0, 24:0 and 26:0 were the most depleted in ^{13}C, with the $\delta^{13}C$ of these fatty acids ranging between -26.0 and -31.4‰. The 19 PUFA was consistently the most enriched of the fatty acids, with the $\delta^{13}C$ of this fatty acid ranging between -12.3 and -13.9‰. The branched 15:0 and 15:1 fatty acids were not sufficiently resolved from one another to allow their individual $\delta^{13}C$ values to be measured and the value of the composite of all three is presented. The $\delta^{13}C$ of these bacterial fatty acids were heaviest at Site CF and lightest at Site PC and were 1–4‰ lighter than the bulk $\delta^{13}C$ of the separated MPB obtained at each of the respective sites.

Table 2.4. The $\delta^{13}C$ (‰) of lipid biomarkers extracted from sediments taken from Port Cygnet (PC) and Castle Forbes Bay (CF) during autumn (Aut) and spring (Spr)

Fatty acid	CF upper Aut	CF upper Spr	PC upper Aut	PC lower Aut
14:0	-21.0	-17.5	-23.4	-23.4
i/ai 15:0 + 15:1	-18.7	-16.1	-22.7	-21.1
15:0	-14.4	-13.7	-15.4	-13.4
16:1(n-7)	-18.4	-21.2	-18.8	-22.8
16:0	-18.0	-15.9	-21.6	-22.0
18:4	-16.9	-15.5		
18:1+18:2+18:3	-23.9	-19.1	-24.5	-25.6
18:0	-24.1	-19.8	-26.4	-25.0
19 PUFA	-13.9	-12.3		-12.4
20:5(n-3)	-18.9	-18.5	-17.8	-15.0
20:0			-26.8	-24.9
22:6	-19.4	-14.9	-28.7	-21.2
22:0	-26.4	-26.0	-28.2	
24:0	-29.6	-27.4	-28.4	-27.6
26:0	-31.1	-28.9	-30.6	-31.4

Algal pigments

Algal accessory pigments detected included lutein, zeaxanthin, fucoxanthin, diatoxanthin and diadinoxanthin. These algal pigments can be used as biomarkers for various algal classes (see Table 1.1 in Chapter 1, p. 25). In this study, lutein was used as a marker for chlorophytes, zeaxanthin for cyanobacteria and fucoxanthin for diatoms (Jeffrey and Vesk 1997). Chlorophyll *c* (chl *c*) was also present as well as small amounts of chlorophyll *b* on occasions. The ratios of the major accessory pigments detected to chl *a* over the year at each of the different sample sites are

shown in Figure 2.3. Fucoxanthin was detected at all sites throughout the year, and generally had a fuc:chl *a* ratio of less than 0.1. Lutein generally dominated the accessory pigments at all sites during autumn and winter with lut:chl *a* ratios of up to 0.4 being recorded on the upper and lower mudflat at Site PC. Zeaxanthin was detected at all times of the year at Site CF with ratios of zea:chl *a* generally falling within the range of 0.01–0.04. At Site PC, zeaxanthin was present in the highest proportions during spring and summer on the upper and lower mudflat with zea:chl *a* ratios of up to 0.3 being observed. The algae *Vaucheria* sp. (Xanthophyta) was observed at Site CF during spring, however, the vaucheriaxanthin ester and violaxanthin, particular to this class (Jeffrey and Vesk 1997) were not detected.

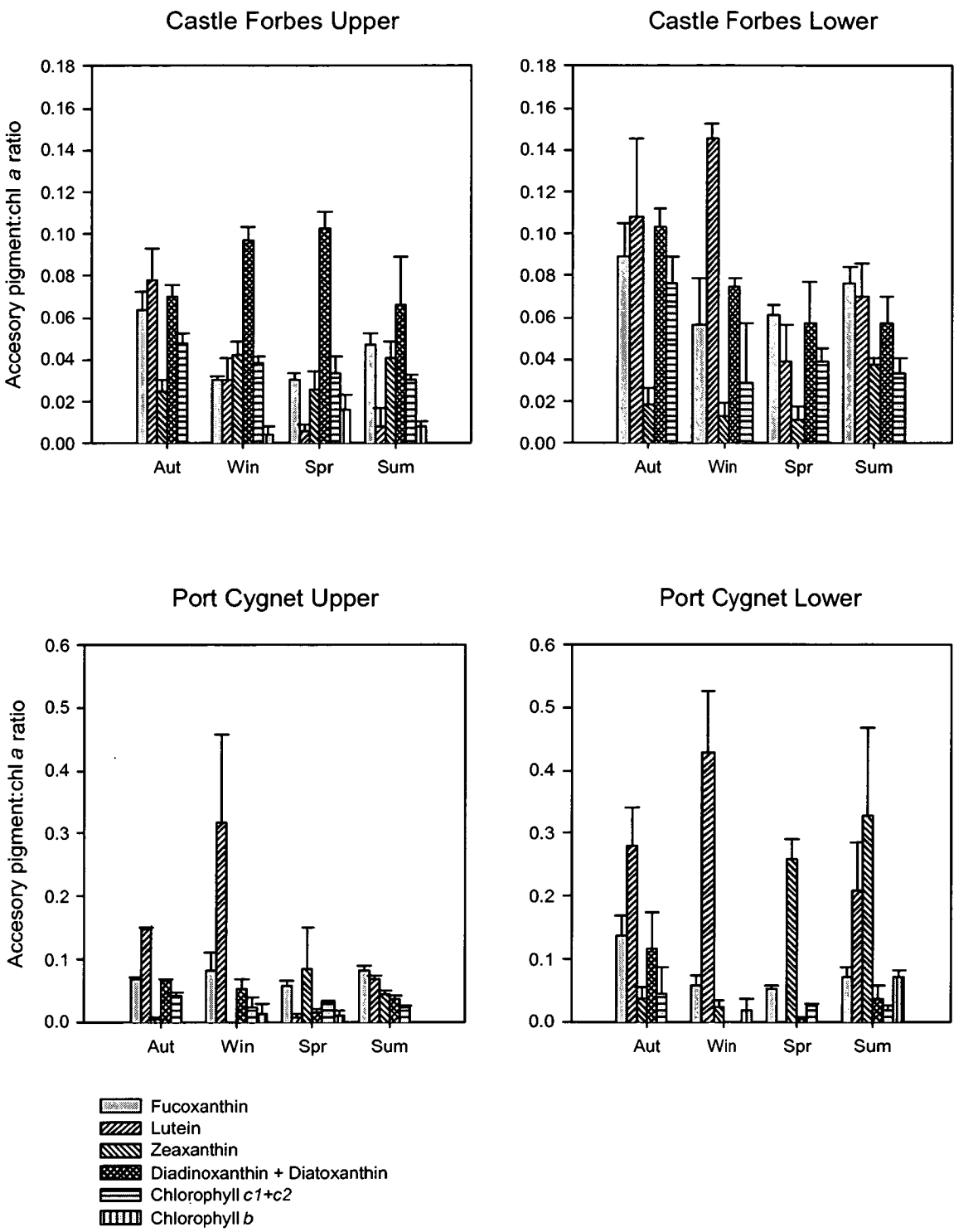


Figure 2.3. Summary of the accessory pigment:chl *a* ratios of the major pigments detected in the top 0.5 cm of sediment in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) on the upper and lower mudflats of the two study sites during the year 2001. Error bars represent the standard error of the mean (n=3–4).

Biomarkers in separated microphytobenthos

Microscopic examination of the MPB which had been separated from the sediment using the lens tissue method consistently showed that cyanobacteria were the dominant taxa present. A typical image of the MPB after separation from the sediments using the lens tissue method is shown in Figure 2.4a. The dominant cyanobacteria was identified to genus level and was found to be *Oscillatoria* sp., *Spirulina* sp. was also observed but at a much lower relative abundance. It is uncertain as to whether there was one or more species within the genus of *Oscillatoria* and it will hereafter be referred to as *Oscillatoria* spp.

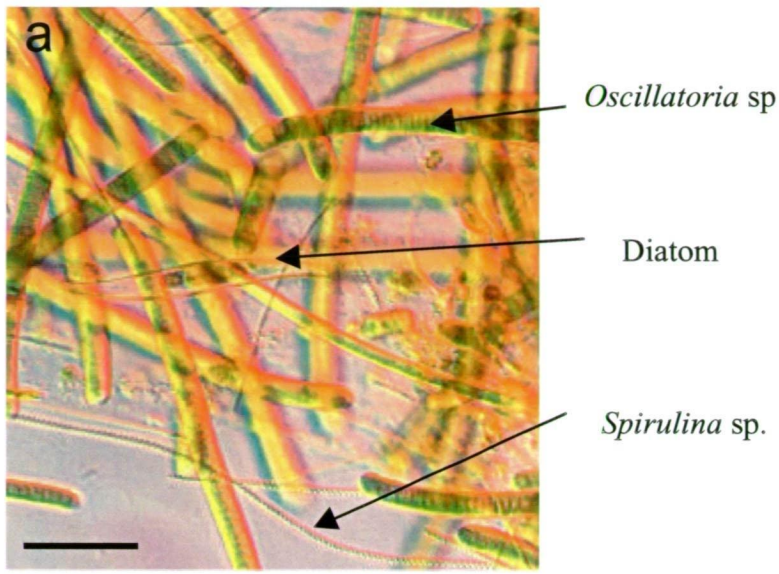
Sterols and hopanoid alcohols were below the detection levels in the separated sample of MPB. The relative proportions of the fatty acids detected in the separated fraction of MPB are shown in Table 2.5. The major fatty acid present was 16:0 which comprised 50% of the total fatty acids, the other major fatty acids found in the microalgae were 18:4 (23%) and 18:3 (15%). The carbon to chl *a* ratio in the separated MPB fraction was measured during July 2000 at Site CF upper and was found to be 19.

Table 2.5. Percent composition of fatty acids and the ratio of the fatty acid 18:4 to chlorophyll *a* equivalents of phytol in benthic microalgae separated from the sediment using the lens tissue technique. Sample was taken from the upper mudflat at Castle Forbes Bay during January 2002.

Fatty acid	**% composition**
14:0	1.6
16:1(n-7)	5.8
16:0	50.7
18:4(n-3)	23.3
18:3(n-3)	15.8
18:0	2.7
total	**100.0**
18:4/phytol (chl *a* equivs)	0.3

A summary of the relative proportion of accessory pigments (pigment:chl *a* ratio) in the MPB from intact cores, the lens tissue separated fraction and the fraction remaining after separation are shown in Figure 2.4b. The algal fraction separated using the lens tissue method showed a significant enrichment in the relative (to chl *a*) proportion of zeaxanthin compared to the algal fraction remaining within the

sediment (t-test, $p<0.01$) as well as the total algal fraction extracted from intact cores (t-test, $p<0.01$). Conversely the relative (to chl *a*) proportions of lutein, chl *c* and diadinoxanthin to chl *a* were all significantly less in the lens tissue separated fraction than the whole and the remaining fractions (t-test, $p<0.05$). The relative (to chl *a*) proportion of fucoxanthin was significantly less in the separated fraction compared to the remaining fraction (t-test, $p<0.01$), but not significantly different to that in the total algal fraction (t-test, $p>0.05$).



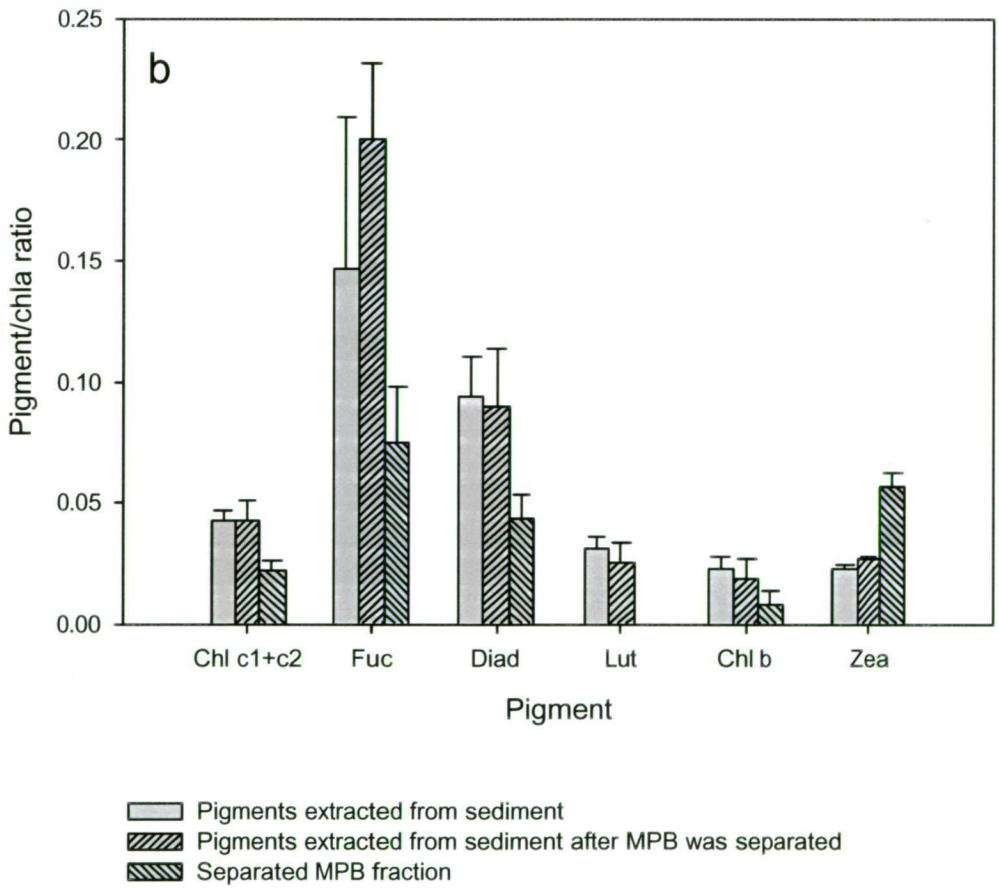


Figure 2.4. **(a)** An image of microphytobenthos (MPB) separated from the sediment using the "lens tissue" method showing organisms identified as *Oscillatoria* sp., *Spirulina* sp. and a diatom, taken in Feb 2002. Scale bar = 50 μm. **(b)** The pigment to chlorophyll *a* (chl *a*) ratios of the accessory pigments chlorophylls c_1 and c_2 (chl c_1+c_2), fucoxanthin (Fuc) diadinoxanthin (Diad), lutein (Lut), chlorophyll *b* (chl *b*) and zeaxanthin (Zea) in sediment extracts, sediment extracts after MPB had been separated and extracts of the separated MPB. Samples were taken in Aug 2001.

Discussion

Fractionation of MPB using the lens tissue method

The pigment data presented here clearly show that the lens tissue method fractionated the MPB as evidenced by the significant changes in the ratios of accessory pigments to chl *a* (Figure 2.4a). The increase in the zeaxanthin:chl *a* ratios indicates that the cyanobacterial component of the MPB became enriched (Jeffrey and Vesk 1997). The decrease in chl *c*, fucoxanthin, diadinoxanthin, lutein and chl *b*:chl *a* ratios indicates that the diatom and green algal components of the MPB were depleted (Jeffrey and Vesk 1997). Biomarker analysis of the separated algal fraction further suggested a significant fractionation of the MPB occurred using the lens tissue method. Diatom markers, including 20:5(n-3) and 24-methylcholesta-5,22E-dien-3β-ol, were not detected at all (Table 1), nor were the green algal fatty acid markers including 18:2(n-6) and 18:1(n-9) (Volkman et al. 1989; Volkman et al. 1998). These markers were all detected in the sediments throughout the year further indicating that the separated fraction of MPB was depleted in diatoms and chlorophytes compared to that remaining within the sediment. Visual observations of the sediments at Site CF were also in agreement with the molecular methods, with cyanobacteria dominating the lens tissue extracted fraction of the MPB (Figure 2.4b).

The finding that the epipelic fraction of the MPB was depleted in diatoms is somewhat surprising as diatoms are generally regarded as the most motile fraction of MPB communities, particularly in intertidal sediments (e.g. de Jonge 1980; Dalsgaard 2000). The enriched cyanobacterial fraction was dominated by *Oscillatoria* spp, the motility of which, in cyanobacterial mats is well documented (Stal 1995). In a hot spring microbial mat, *Oscillatoria terebriformis* was found to move to the surface of the sediment in the morning, move back into the sediment during the middle of the day, returning to the surface in the afternoon, before moving back into the sediment at night (Stal 1995). The motility of *Oscillatoria* spp. was also noted by Eaton and Moss (1966), where it was observed that virtually all of the trichomes migrated out of the sediment onto a coverglass. The data presented here clearly shows that the lens tissue method will fractionate the MPB in favour of the most motile algal species, which in this instance was the cyanobacteria *Oscillatoria* spp. The findings presented here show that great cautions should be excercised when separating MPB from sediments using the lens tissue technique.

The implication of this fractionation for previous studies of MPB is that less mobile taxa such as green algae may have been underestimated. Before using the technique in a particular sediment, it would be wise to perform some experiments to check the fractionation of algae by the lens tissue technique within that system.

Lipid biomarkers as indicators of sources of organic carbon within the sediments

In order to estimate the relative contributions of different organic matter sources to the sediments, fatty acid data are often broken down into algal, terrestrial plant, aquatic macrophyte and bacterial markers (Volkman et al. 1980; Canuel and Martens 1993; Shi et al. 2001; Wilson et al. 2001). Here, I have used an *a posteriori* approach to group the major sources of fatty acids. Cluster analysis of the normalised (raw data – mean/standard deviation) fatty acid and chl *a* data showed that the fatty acids could be broken down into 3 major groups (Figure 2.5). These groups of fatty acids were assigned sources as discussed below, the fatty acids in each group were then summed to obtain an estimate of total fatty acids derived from each source.

The first group consisted of chl *a* as well as established algal markers including 20:5(n-3), 16:1(n-7) as well as the PUFA 18:4 which was found to be the major fatty acid in algae separated from the sediment. This strongly suggests that this cluster represented fatty acids derived from a mixed community of MPB (see later discussion – Taxanomic composition of microphytobenthos). The fatty acid 18:1(n-7) which is often used as a bacterial marker (e.g. Volkman et al. 1980) clustered with the algal markers. This fatty acid may also be produced by microalgae (Volkman et al. 1989), however, the fact that it was clustered on the periphery of the microalgal fatty acids suggests that it is produced by both bacteria and microalgae. The fatty acid 22:6 was also clustered on the periphery of the algal markers, most likely reflecting the fact that this fatty acid is produced by both algae (Volkman et al. 1989) as well as sediment infauna (Kharlamenko et al. 2001). The second cluster of fatty acids consisted predominantly of odd chain fatty acids including the widely used bacterial markers i15:0 and ai15:0 (Findlay and Dobbs 1993), strongly suggesting that the fatty acids within the group were predominantly derived from bacterial sources. The unusual fatty acids 15:0 and 17:1(n-8) were present in high relative

proportions at times, and were closely clustered, suggesting a common source within the microbial fatty acids. The third cluster of fatty acids contained long chain fatty acids which are generally used as indicators of higher plants, suggesting this group was derived from terrestrial sources (Wannigama et al. 1981). The fatty acid 20:4(n-6) was grouped on its own and was, therefore, apparently not derived from microalgal or bacterial sources. Red and brown macroalgae are known to produce 20:4(n-6) in significant quantities, in particular the red alga *Gracilaria* sp. in which 20:4(n-6) can account for 50% of total fatty acids (Vaskovsky et al. 1996). As a *Gracilaria* sp. was observed to cover up to 50% of the mudflat at Site PC, it seems extremely likely that the 20:4(n-6) was derived from this macroalga. It was assumed that the 20:4(n-6) observed at Site CF was also derived from this source. For this study, the fatty acids 18:1(n-7) and 22:6 were regarded as being derived from mixed bacterial, algal and faunal sources.

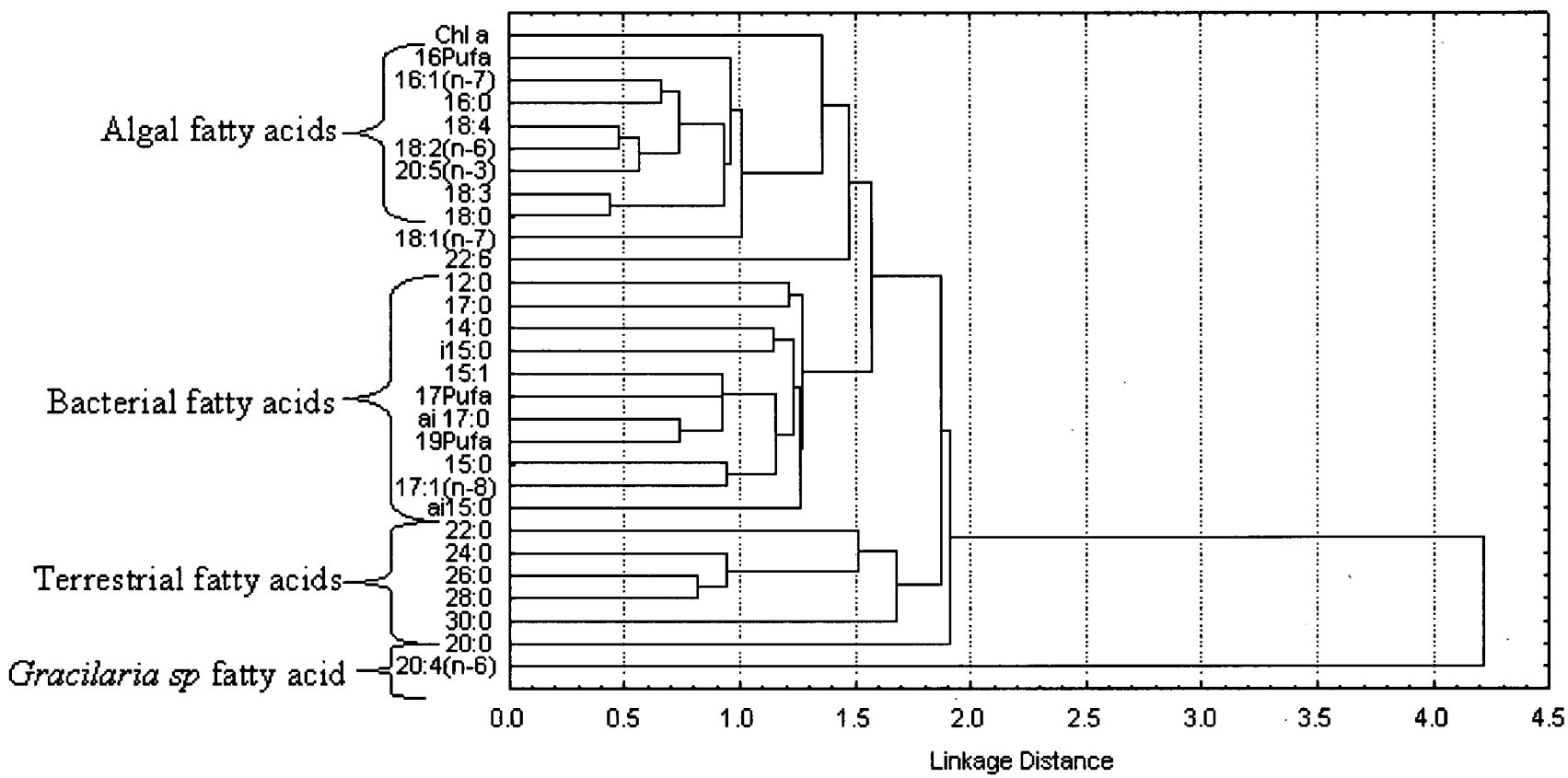


Figure 2.5. Cluster analysis (Euclidean distances) of all the normalised fatty acid data pooled from Castle Forbes Bay and Port Cygnet over the study period (n=16). The data were clustered into groups which were defined as being derived from algal, bacterial, terrestrial or macroalgal sources (see text for details).

Figure 2.6 shows the relative proportions of the fatty acids grouped according to the cluster analysis scheme. It can be seen that the proportion of fatty acids derived from microalgal sources dominated relative to other sources at both mudflats throughout the year, generally comprising around 50% of total fatty acids. The high proportion of PUFAs within the algal fatty acids (Table 4) suggests that MPB are likely to be a major source of labile organic carbon to the sediments. The relative proportions of bacterial fatty acids were greatest (up to 50% of total fatty acids) during the warmest sampling periods of summer and autumn at Site CF. A similar seasonal variation in bacterial fatty acids was not so noticeable at Site PC. Canuel and Martens (1993) also observed that the relative contribution of organic carbon from bacteria dropped during winter and peaked during the warmer summer months when sediment respiration rates (as indicated by sulphate reduction) were highest. Consistent with this, I observed a significant relationship between sediment respiration rates and total bacterial fatty acids ($r=0.7$, $p<0.01$). Algal fatty acids became relatively more abundant compared to bacterial fatty acids during the colder sampling periods in winter and spring. This realitve increase in algal fatty acids during cooler periods is consistent with previous observations that bacterial metabolism is reduced to a greater extent than that of algae during the colder months, both at this site (Chapter 3) and elsewhere (Davis and MacIntyre 1983; Grant 1986; Kristensen 1993).

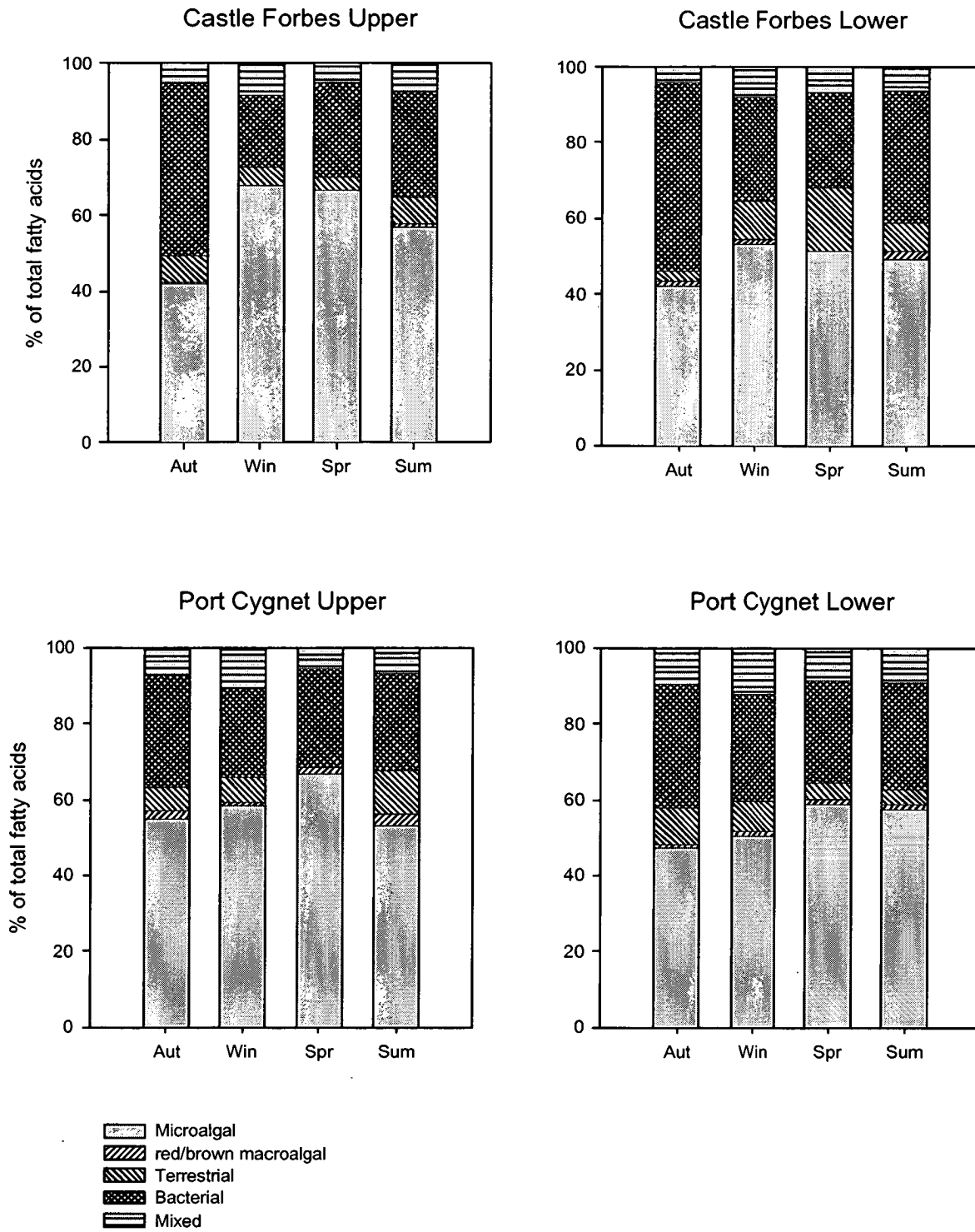


Figure 2.6. Summary of the relative proportions of fatty acids derived from algal, bacterial, terrestrial, mixed or macroalgal sources, in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) at the two study sites during the year 2001. The grouping of the sources of these fatty acids was carried out using cluster analysis as shown in Figure 2.5.

Bacterial carbon substrate sources

Given that bacterial fatty acids comprised a significant proportion of fatty acids and presumably also labile organic carbon, the question still remains as to the source of the carbon being consumed by these heterotrophs. The bulk of the organic matter pool within the sediments was derived from terrestrial sources (see later discussion), however, this organic matter was most likely to be refractory as indicated by high C:N ratios (Table 2). A more likely supply of organic carbon to bacteria within the sediment is that derived from MPB, which had a lower C:N ratio and also contained high proportions of PUFAs which are generally regarded as being labile forms of organic carbon (e.g. Budge et al. 2001). Evidence to support this comes from the significant correlations between specific bacterial and algal fatty acids such as i15:0 and 20:5(n-3) ($r=0.83$, $p<0.001$).

Compound specific stable isotope ratio data also supports this hypothesis. One would normally expect the $\delta^{13}C$ of the branched bacterial 15:0 fatty acids to be depleted by about 4–6‰ relative to the substrate they are consuming (Boschker and Middelburg 2002). If this were the case here, then this suggests that the bacteria within the sediment are consuming a substrate with a $\delta^{13}C$ of -10 to -14‰ at Site CF and -15.1 to -18.7‰ at Site PC based on the isotopic composition of the branched 15:0 and 15:1 fatty acids (Table 2.6). Based on this, it seems unlikely that terrestrial sources of organic matter provide a significant substrate for bacteria within the sediment, because terrestrially derived organic matter had $\delta^{13}C$ values generally more depleted than -26‰. If bacteria were consuming a terrestrially derived substrate to a significant extent, then one would expect to see a $\delta^{13}C$ of ~ -30‰ for the branched 15:0 fatty acids. MPB, then, are the most likely source of substrate to bacteria at Site CF, although at site PC, the contribution of material derived from *Gracilaria* sp. cannot be ruled out on the basis of stable isotope data. The suggestion that MPB, and not higher plant derived material, provides the substrate for bacterial respiration within the sediments, is also consistent with the findings of other similar studies which have used compound specific stable isotope ratios (Canuel et al. 1997; Boschker et al. 1999; Boschker et al. 2000).

If bacteria in the sediment were consuming MPB-derived material, then the question still remains as to why the isotopic signatures of the branched 15:0 and 15:1 fatty

acids suggest the substrate consumed was slightly more enriched than the measured bulk isotopic signatures of the MPB. The most likely explanation for this is that bacteria are consuming a sub-fraction of biomolecules which are isotopically more enriched than the bulk of the algal cells. Indeed it has previously been shown that compounds such as carbohydrates may be selectively assimilated by zooplankton consuming microalgae (Cowie and Hedges 1996). Sugars are generally isotopically enriched in primary producers compared to the bulk cellular material (Degens et al. 1968; van Dongen et al. 2002), it has also been reported that sugars and carbohydrates are enriched compared to the bulk cellular material in cyanobacterial mats (Moers et al. 1993, and references therein). MPB are known to secrete large amounts of extracellular organic carbon (EOC), consisting predominantly of sugars and carbohydrates (Underwood et al. 1995; Goto et al. 1999). EOC has been found to be highly labile and readily consumed by bacteria (Goto et al. 2001). While there are no reports of the $\delta^{13}C$ of EOC produced by MPB, it seems highly likely that this would be enriched in ^{13}C compared to the bulk cellular material given the fact that it consists predominantly of sugars and carbohydrates. Thus, the consumption of EOC by bacteria seems a plausible explanation for the estimated $\delta^{13}C$ of the carbon consumed by bacteria being more enriched than the bulk $\delta^{13}C$ of the MPB present. The hypothesis that EOC drives a significant fraction of respiration within the sediment will be developed further in the following chapters.

Microbial lipids

Of particular interest were the unusual fatty acids 15:0, 17:1(n-8) and the 19 PUFA, which at times showed elevated concentrations, especially during autumn at Site CF. The compound specific stable isotope ratio analysis of the 15:0 and the 19 PUFA fatty acids (the $\delta^{13}C$ of the 17:1(n-8) fatty acid could not be determined due to poor resolution) showed that they were consistently more enriched in ^{13}C than the commonly occurring ai and i15:0 bacterial fatty acids, suggesting they arose from a separate source. There was also a significant correlation between these two fatty acids and the ratio of dissolved nitrogen to "microbial biomass nitrogen" (data presented in Chapter 4) within the sediments, (for 15:0, $r=0.74$, $P<0.01$). As the amount of these fatty acids increased, so did the amount of nitrogen sequestered in microbial biomass. It is suggested in Chapter 4 that an uptake of nitrogen by microorganisms within the sediment was taking place at these sites. Therefore, the

organisms responsible for this fatty acid profile are likely to play an important role in the nitrogen cycle within this system.

High proportions of 15:0 (up to 9% of total fatty acids) have been observed in temperate intertidal sediments in France (Meziane et al. 1997), however, 17:1(n-8) was not detected in their study. Volkman et al. (1980) also observed slightly elevated (4%) proportions of 15:0 in a temperate intertidal sediment. Elevated concentrations of 15:0 and 17:1(n-8) (up to 18% of total fatty acid for 17:1(n-8)) have been reported in the pteropod *Clione limacina* in the polar oceans (Kattner et al. 1998). It seems unlikely that the unusual lipid profile originates from gastropods in my study because studies of the lipid profiles of gastropods from seagrass communities and intertidal flats have not reported high proportions of 15:0 or 17:1(n-8) (Meziane and Tsuchiya 2000; Kharlamenko et al. 2001; Meziane and Tsuchiya 2002). Elevated concentrations of the fatty acids 15:0 (up to 8.5%) and 17:1(n-8) (up to 19%) have also been reported in marine bacteria of the genera *Alteromonas* and *Pseudoalteromonas* (Ivanova et al. 2000). Recent studies of the fatty acid profiles of thraustochytrids (marine protists) have shown elevated concentrations of 15:0 and 17:1(n-8) as well as 17:0 and 17:1 (P. Mansour unpublished data 2002). It has been suggested that thraustochytrids may play an important role in the microbial food web, a role which has until recently been overlooked (Santangelo et al. 2000).

Further insight into the possible origins of these unusual fatty acids can be derived from the sterol profiles measured within these sediments. Lewis et al. (2001) analysed the sterol profiles of thraustochytrids isolated from coastal waters in southeastern Tasmania, and found that cholest-5-en-3β-ol was generally the dominant sterol with 24-methylcholesta-5,22E-dien-3β-ol, 24-ethylcholesta-5,22E-dien-3β-ol and 24-ethylcholesta-5,7,22-trien-3β-ol also comprising the major sterols. Low ($r<0.6$), and generally insignificant ($p>0.05$) correlations were observed between the sterols cholest-5-en-3β-ol, 24-methylcholesta-5,22E-dien-3β-ol and 24-ethylcholesta-5,22E-dien-3β-ol, and the fatty acids 15:0 and 17:1(n-8). The sterol ethylcholesta-5,7,22-trien-3β-ol was not detected. This strongly suggests the unusual fatty acid profile observed here does not originate from thraustochytrids. Furthermore, there was a generally low ($r<0.55$) or insignificant ($p>0.05$) correlation

between all of the sterols and the fatty acids 15:0 and 17:1(n-8). The absence of a correlation should be interpreted with caution as it may simply mean that the sterols are being produced by more than one source, hence, obscuring any relationship between the organisms producing the 15:0 and 17:1(n-8). However, in this instance, given the, at times high, contribution of the fatty acids 15:0 and 17:1(n-8), this lack of a correlation most likely suggests that the organisms producing the 15:0 and 17:1(n-8), either do not produce sterols, or produce them in very low quantities. This precludes algae as a likely source of these fatty acids, which are known to produce an abundance of sterols (Volkman et al. 1998). Prokaryotic organisms generally do not produce sterols (Volkman In Press) and are, therefore, a likely source of these unusual fatty acids. Neither 15:0 nor 17:1(n-8) were detected in the lens tissue extract precluding cyanobacteria as a source of these fatty acids. Given the evidence presented here, it would seem most likely that bacteria, possibly of the genus *Pseudoalteromonas* and *Alteromonas* are the most likely source of the fatty acids 15:0 and 17:1(n-8). Furthermore the branched 15:0 fatty acids occur in much lower proportions than the 15:0 and 17:1(n-8) fatty acids in *Pseudoalteromonas* and *Alteromonas* (Ivanova et al. 1998; Ivanova et al. 2001). This is consistent with the earlier suggestion based on compound specific stable isotope analysis, that the 15:0, 17:1(n-8) and 19 PUFA arise from different sources to the branched 15:0 bacterial fatty acids.

Stable isotopes as indicators of sources of organic carbon within the sediments

Figure 2.7 shows a dual isotope plot of $\delta^{15}N$ versus $\delta^{13}C$, for the mudflat sediments at both sites, as well as a range of possible sources of organic matter. It can be seen that two end-members can be clearly established using carbon stable isotope values. Terrestrial sediments from the Huon River and smaller creeks as well as plant detritus had a depleted carbon isotopic signature with a $\delta^{13}C$ of between -27 to -29‰. Another potential source of organic matter, which I have considered to be terrestrial, was the marsh reed *Juncus kraussi*, which formed large stands on the edges of both mudflats (personal observation). The data presented here for this species represents a composite for live, standing dead and detrital plant material. The $\delta^{13}C$ of this was -26‰, slightly more enriched in ^{13}C than the other terrestrial sources of organic matter. In contrast, organic carbon derived from estuarine sources including MPB and macroalgae consistently showed a much greater relative

enrichment in ^{13}C, with $\delta^{13}C$ values being "heavier" than -20‰. At Site CF, the MPB was particularly enriched, with $\delta^{13}C$ values of around -15‰ being observed. The heavy $\delta^{13}C$ values observed at Site CF are consistent with previous studies of the isotopic signature of MPB, in particular where cyanobacteria make a significant contribution to the algal community (Currin et al. 1995 and references therein), as was the case here – see later discussion "Taxonomic composition of microphytobenthos". The average isotopic signature of sediments from the marine end of the Huon Estuary, as well as a typical sediment sample taken from the upper Huon Estuary are also shown in order to place the isotopic end members considered for the mudflats, within the broader context of the estuary.

Signatures of the stable isotopes of nitrogen also allowed a distinction between marine and terrestrial sources of organic matter at Site PC. MPB and macroalgae at this site both exhibited an enrichment in ^{15}N ($\delta^{15}N$=7–8‰) compared to terrestrially derived organic matter which was relatively depleted in ^{15}N ($\delta^{15}N$ = -2–2‰). At Site CF, however, the $\delta^{15}N$ isotopic signature of MPB was less than 3‰, and indistinguishable from that of organic matter derived from terrestrial sources. Low $\delta^{15}N$ values in benthic and pelagic algae are most likely to arise from nitrogen fixation by cyanobacteria (Currin et al. 1995; MacGregor et al. 2001). This accords with the observation that the MPB at Site CF contained a high proportion of cyanobacteria which exhibited high rates of N_2 fixation at times (Chapter 5). The $\delta^{15}N$ of 7.1‰ for the MPB at Site PC was much more enriched than at Site CF. This is consistent with the observation that this algal fraction was dominated by diatoms (as evidenced by microscopic examination) and that N_2 fixation rates were much lower at this site (Chapter 5). The $\delta^{15}N$ of the diatoms, measured at Port Cygnet is in the upper range of $\delta^{15}N$ literature values reported for benthic diatoms (Riera 1998 and references therein). The high $\delta^{15}N$ values of MPB were most likely caused by the drawdown of NO_3^- within the Huon Estuary over the summer months (Butler et al. 2000), which results in a greater uptake of $^{15}NO_3^-$ by microalgae due to Rayleigh fractionation (Rau et al. 1998).

At this stage it should be considered whether the $\delta^{15}N$ and $\delta^{13}C$ values measured for the separated MPB at Site CF in are representative of the entire MPB community.

As discussed earlier it was found that cyanobacteria were preferentially separated using the lens tissue method. The isotopic signatures measured here, may, therefore, be weighted towards this taxanomic class. Whether or not the $\delta^{13}C$ and $\delta^{15}N$ values measured are representative of the wider MPB community, will depend upon the major mechanism of isotopic fractionation. For example, if the fractionation observed here is caused by a metabolic pathway unique to a particular algal class (e.g. N_2 fixation), then the isotopic signature is not likely to be representative of the wider community. However, if the observed isotopic signature arises from a more general phenomenon, such as the draw-down of C or N, then the isotopic signature is more likely to reflect that of the entire community. If it is assumed that the snails collected at this site fed solely on MPB, and that there was no discrimination in the snail's diet for a particular fraction of the MPB community, then their tissue will reflect the isotopic composition of the entire MPB community. In addition, the isotopic signature of the snail tissue will provide a more time-integrated signature of the MPB community than a single sample of MPB will. For carbon, animals will usually have a $\delta^{13}C$ within 2‰ of their diet (Fry and Sherr 1984), and for nitrogen an enrichment of ~3.5‰ relative to their diet is often assumed (e.g. Riera et al. 1999). The isotopic signatures of the snails ($\delta^{15}N$~3‰ and $\delta^{13}C$~ -14‰) then, are consistent with the measured isotopic signatures of the MPB community presented here suggesting these values are representative of the majority of the community.

The isotopic end-members described above are qualitatively similar to those observed elsewhere (Heip et al. 1995 and references therein), where carbon and nitrogen are isotopically enriched at the marine end-member of the estuary compared to the riverine end-member. In a study of the Tay Estuary, Thornton and McManus (1994) compared the use of C:N ratios, $\delta^{13}C$ and $\delta^{15}N$ as tracers of marine and terrigenous organic matter. In that study it was concluded that $\delta^{13}C$ provided the best means of estimating the relative contribution of organic matter arising from marine and terrestrial sources because the isotopic signature was more likely to be conserved during organic matter degradation. C:N ratios and $\delta^{15}N$ are both likely to change through the degradation of organic matter. During organic matter degradation, an increase in the C:N ratio is usually observed as a consequence of preferential degradation of low C:N ratio sub-fractions of organic matter. For $\delta^{15}N$ values, an increase is usually observed, caused a preferential loss of ^{14}N from the

organic matter undergoing degradation. In this study, the isotopic signature of carbon gave a much clearer separation of the marine and terrestrial end-members than did that of nitrogen. Given the problems associated with the changes in C:N ratio during organic matter degradation, the $\delta^{13}C$ was used to estimate the relative contributions of marine and terrigenous organic matter to the sediments. Assuming a simple two component mixing system, the relative contribution of different sources of organic matter to the total organic matter pool in the sediment can be estimated using the terrestrial and marine end-member compositions and the mixing equation of Schultz and Calder (1976):

$$\delta^{13}C_s = F_t\delta^{13}C_t + F_m\delta^{13}C_m$$

Where F is the fraction of organic matter in the sample and the subscripts s, t and m denote sample, terrigenous and marine. Thus, the fraction of terrigenous organic matter can be calculated

$$F_t = \frac{\delta^{13}C_s - \delta^{13}C_m}{\delta^{13}C_t - \delta^{13}C_m}$$

For the purposes of this study, it was assumed that the input of organic matter at the two study sites was either derived from "terrestrial" or "algal" (MPB and macroalgae) sources and this equation was used to estimate the relative proportions of organic matter from these two sources. At Site CF, the isotopic signature of MPB was defined as one end-member with a $\delta^{13}C$ value of -15‰. The terrestrial end-member at this site was considered likely to consist of a mixture of the reed *Juncus kraussi* and terrestrial organic matter, therefore, a mean $\delta^{13}C$ value of -27‰ was assumed. At Site PC, the algal end-member was assumed to consist of MPB and *Gracilaria* sp. and a mean $\delta^{13}C$ of -20‰ was assumed. A terrestrial $\delta^{13}C$ value of -27‰ was used at Site PC, which was measured in sediment taken from Agnes Rivulet, which discharges into Port Cygnet. These end-members neglect the possible contribution of phytoplankton blooms within the estuary, which could conceivably be deposited on the mudflats. Recent algal blooms within the Huon Estuary have generally been dominated by dinoflagellates in terms of their biomass (Butler et al. 2000). As the sterol (4,23,24-trimethyl-5α-cholest-22E-en-3β-ol) and pigment

(peridinin) biomarkers for dinoflagellates were only occasionally detected in small quantities or not at all, it seems unlikely that the remnants of these pelagic algal blooms were deposited at these sites. The input of detritus from spring diatom blooms observed in the Huon Estuary cannot be discounted, although a significant input compared to the sources considered here seems unlikely given their short duration (Butler et al. 2000).

The endmembers defined in this manner are also consistent with the compound specific analysis of biomarkers from these sediments. Fatty acids are generally depleted in ^{13}C compared to the bulk material of both higher plants and algae (Collister et al. 1994; Schouten et al. 1998). The $\delta^{13}C$ of the terrestrial fatty acids 22:0, 24:0 and 26:0 fatty acids were -28‰ on average at site CF and -29‰ at site PC (Table 2.4), consistent with the terrestrial endmember of -27‰ estimated at these sites. The algal grouped fatty acids (Figure 2.5) for which the $\delta^{13}C$ were measured were generally enriched in ^{13}C, with an average $\delta^{13}C$ value of -19‰ and -22‰ measured for these fatty acids at Site CF and PC respectively (Table 2.4). Once again, this is consistent with the estimated "algal" endmember signatures of -15 and -20‰ at Sites CF and PC respectively.

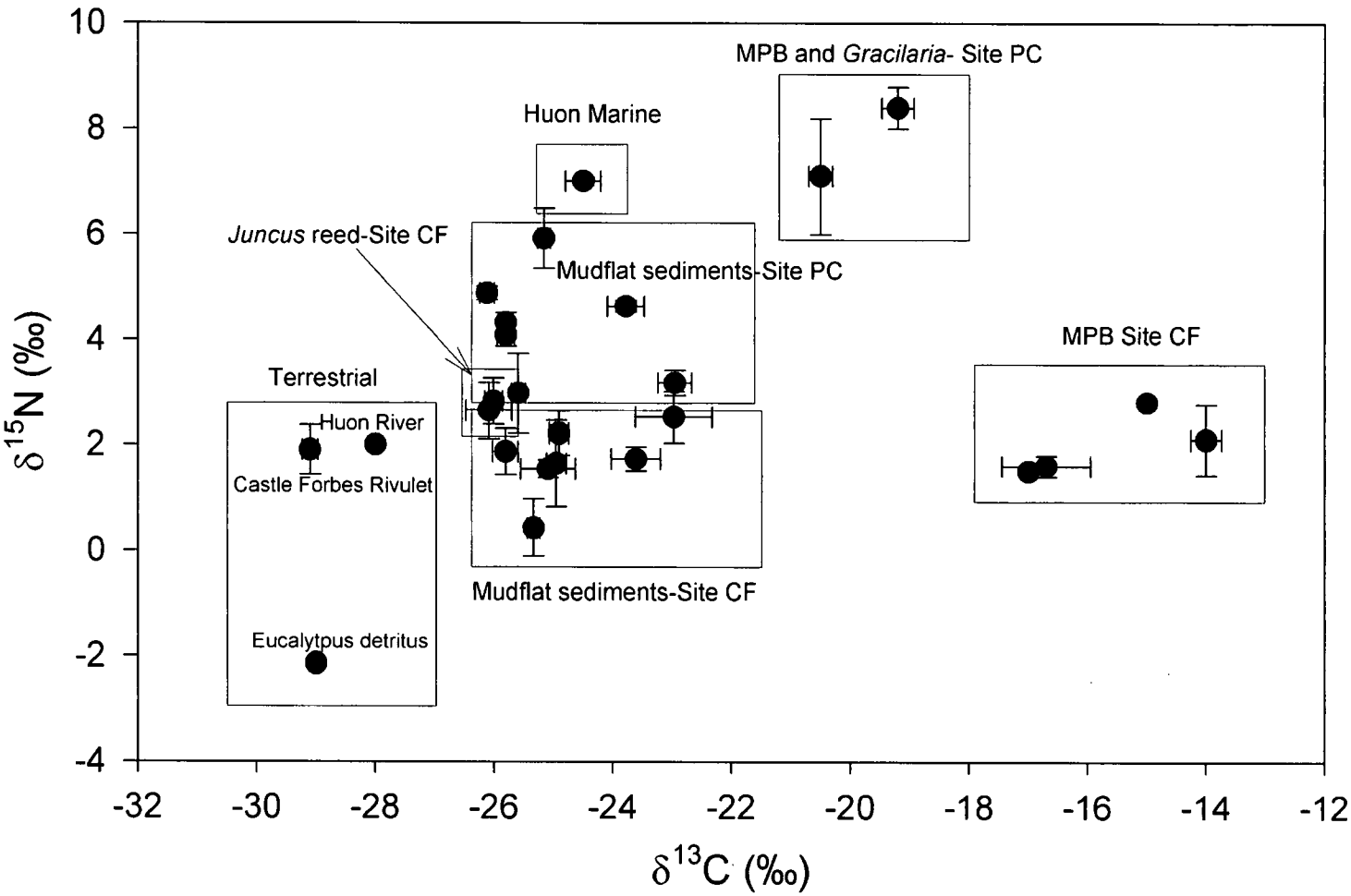


Figure 2.7. A dual isotope plot of $\delta^{15}N$ versus $\delta^{13}C$ for sediment samples taken from Castle Forbes Bay and Port Cygnet over the study period in relation to potential sources of organic matter to the sediments (as outlined in Table 2.1) at each site. Error bars represent the standard error of the mean (n=3–4).

Figure 2.8a shows the estimated contribution of organic carbon from algal and terrestrial sources on the upper and lower mudflat at Site PC and CF over the study period. It can be seen that organic matter derived from terrestrial sources dominated the organic matter pool in the sediments at all sites, with the exception of Site PC lower, where algal sources of organic matter dominated during autumn. The dominance of terrestrially derived organic matter at both sites is reflected in the high C:N ratios in the sediments, which were generally close to, or in excess of 12. The finding that the bulk of the organic matter is derived from terrestrial sources may seem inconsistent with the lipid data (Figure 2.6), which showed that terrestrially derived fatty acids make up only a small fraction of total fatty acids. However, when comparing these two data sets it must be remembered that the long-chain fatty acids will make up a much smaller proportion of the organic carbon pool for higher plants than will the fatty acids in MPB and bacteria. For example, Canuel and Martens (1993) assumed that the long-chain fatty acids comprised 4% of the extractable lipids and that the extractable lipids comprised 10% of the total organic carbon in higher plants. For algae, Canuel and Martens (1993) converted lipids to carbon by assuming that fatty acids accounted for 40% of their lipid content, and that lipids comprised 20% of the carbon in these organisms. As an example, the lipid data were converted into carbon derived from algae and terrestrial plants using these ratios for the sediment data from CF upper during autumn. This resulted approximately in an 80:20 split in the carbon from terrestrial and algal sources respectively, which is consistent with the stable isotope data.

The contribution of live MPB biomass to the "algal"-derived organic carbon pool was calculated using the measured carbon-to-chl *a* ratio of 19 as well as chl *a* data which is presented in Chapter 3. It can be seen (Figure 2.8b), that live MPB generally only comprised less than 20% of the algal organic matter pool at Site CF, and generally much less than 10% at Site PC. If the "algal" organic matter was derived from MPB, then the majority of this organic matter was present as detritus for which the chl *a* had been degraded, or heterotrophic biomass synthesised from carbon derived from MPB. At Site CF, evidence to support the contention that the majority of MPB derived carbon is present as detritus or incorporated into heterotrophic biomass, comes from the significant correlation between phytol and the algal fraction of organic matter within the sediment ($r=0.72$, $p<0.05$). The

correlation between the algal fraction and chl *a* was not significant. As a degradation product of chl *a*, phytol provides a more time-integrated measure of recent autotrophic carbon input than chl *a*. This was manifested at Site CF with phytol equivalents generally in excess of chl *a* by three to six times. As such phytol remains in the sediment as a recent record of senescent algal biomass or that which has been consumed and incorporated into biomass by heterotrophs. Phytol itself, is also rapidly degraded in sediments, with a recent study showing that 85–90% of phytol will be degraded within three months (Grossi et al. 1998). The turnover time for chlorophyll by contrast, was estimated to be in the order of hours to days, this was estimated by dividing the algal biomass (calculated using a carbon to chl *a* ratio of 19) by the rates of primary production (see Chapter 3). Therefore, the phytol present, could be regarded as an integrated measure of algal degradation within the sediments over a period of days to weeks.

At Site PC, there was no significant relationship between phytol or chl *a* and the "algal" fraction of organic matter. At this site, the macroalgae *Gracilaria* sp. was also likely to contribute to organic matter deposited within the sediments, as it was observed to cover up to 50% of the mudflat (personal observation). *Gracilaria* sp was found to have a C:N ratio of 27 (Table 2), which suggests that this macroalgal derived carbon will be refractory. As such, it seems highly likely that macroalgae will contribute refractory organic matter to the sediment, which will maintain a "algal" isotopic signature, but is likely to have lost any associated chl *a*, phytol and fatty acids due to preferential degradation of these relatively labile compounds. It is, therefore, suggested that a large fraction of the "algal" organic matter at Site PC was refractory carbon derived from macroalgae. As will be discussed subsequently, it seems unlikely that significant amounts of fresh macroalgal detritus were present within the sediments because only small amounts of the fatty acid 20:4(n-6), which is found in high quantities in *Gracilaria* sp (Vaskovsky et al. 1996), were detected. As a consequence of this, any relationship between phytol and labile "algal" organic matter derived from MPB would be obscured by an additional input of refractory "algal" organic matter from degraded macroalgae.

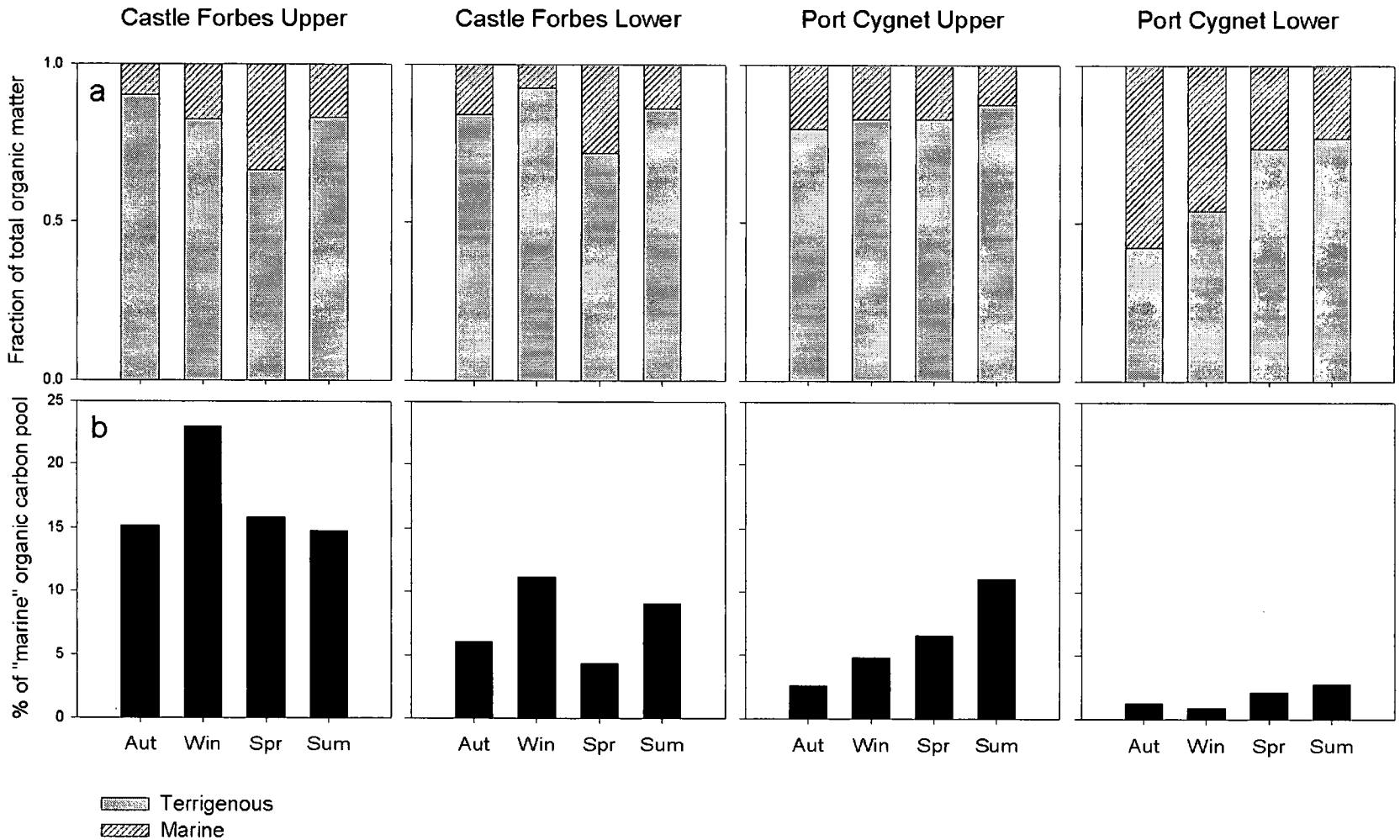


Figure 2.8. The fraction of organic matter derived from marine and terrestrial sources calculated using carbon stable isotope ratios (a); and the proportion of the "marine" organic pool accounted for by live BMA biomass calculated using chl *a* data and a carbon to chl *a* ratio of 19 (b) in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) at the two study sites during the year 2001.

Taxonomic composition of microphytobenthos

To date, most studies of MPB on estuarine mudflats have shown a predominance of diatoms. (Cariou-Le Gall and Blanchard 1995; Barranguet et al. 1997; Brotas and Plante-Cuny 1998; Lucas and Holligan 1999; Cahoon and Safi 2002). The dominance of diatoms within these microalgal communities resulted in relatively high concentrations of fucoxanthin with fucoxanthin:chl *a* ratios of between 0.35–1.6 being observed. In the Huon Estuary mudflats, the observed fucoxanthin:chl *a* ratios were much lower, generally being less than 0.1. The accessory pigments lutein and zeaxanthin were also present in comparable or greater quantities, indicating that chlorophytes and cyanobacteria were also present (Jeffrey and Vesk 1997)(Figure 2.3). The presence of chlorophytes and cyanobacteria have also been recorded in other MPB communities (Cahoon 1999), including intertidal sediments (Barranguet et al. 1997; Brotas and Plante-Cuny 1998). During spring at Site CF, the xanthophyte *Vaucheria* sp. was observed, but no pigments characteristic of xanthophyceae were detected.

Using pigment data to estimate the relative abundance of various algal classes is imprecise due to the variability of pigment ratios caused by a range of factors, including growth conditions as well as differing pigment ratios between different species within the same class (Jeffrey and Vesk 1997; Porra et al. 1997). The use of mathematical analysis techniques, such as that applied in the program CHEMTAX, have been shown to give realistic and reliable estimates of the relative abundances of algal classes in the open ocean (Mackey et al. 1996). While this approach has not been previously used in sediments, it has been used here to obtain a first-order estimate of the relative abundance of diatoms, cyanobacteria and chlorophytes. Because the accessory pigments characteristic of xanthophyta were not detected, this taxa was neglected from the estimate. As a consequence of this the proportion of diatoms, cyanobacteria and chlorophytes have most likely been overestimated at the expense of xanthophytes during spring. The proportions of diatoms, cyanobacteria and chlorophytes relative to one another, are however, likely to be as accurate as the estimates at other times of the year.

In order to conduct the CHEMTAX analysis, the data were separated into the two sites, for each sampling date and location. A prerequisite of CHEMTAX is that each

sample is independent. Four replicate pigment samples were included individually in the CHEMTAX analysis as there was generally a high degree of heterogeneity, as can be seen by the size of the standard errors for the pigment data in Figure 2.3. This resulted in ~30 samples being run for each site. For cyanobacteria a zea:chl *a* ratio of 0.06 was used at Site CF. This was based on the ratio of zea:chl *a* in the lens tissue separated fraction of MPB. It was assumed that this was dominated by cyanobacteria based on visual observations, as well as pigment and fatty acid data (Figure 2.4, Table 2.5). The low zea:chl *a* ratio in *Oscillatoria* spp. was confirmed by the analysis of the pigment composition of *Oscillatoria animalis*, obtained from the CSIRO culture collection (Osc 326) which was found to have a zea:chl *a* of 0.015. At Site PC, no *Oscillatoria* spp. were observed during the year 2001, so a zea:chl *a* ratio of 0.2 was used, which is typical of *Synechococcus* found in the southern ocean (Mackey et al. 1996) and a cultured species (*Synechococcus* sp.) in the CSIRO culture collection (Syn 94). For chlorophytes, a lut:chl *a* of 0.4 was estimated based on the data from winter at Site PC lower, when lutein dominated the other pigments (Figure 2.3). It was assumed that chlorophytes did not contribute to zeaxanthin. Diatoms were assumed to have a chl *c*:chl *a* ratio of 0.2, a fuc:chl *a* of 0.2 based on the data of Mackey et al. (1996). Diadinoxanthin and diatoxanthin were summed and it was assumed they had a total ratio to chl *a* of 0.3. The maximum change in the final pigment ratios, as estimated by CHEMTAX, was an increase of 50% in the ratio of fucoxanthin: chl *a* at Site PC, which indicates that the pigment ratios estimated here are close to their true values.

Figure 2.9a, shows the relative contribution of each algal class to chl *a* as calculated by CHEMTAX. In general, it shows a mixed community of diatoms cyanobacteria and chlorophytes. At Site CF, the community was generally dominated by cyanobacteria, which is in agreement with the high rates of nitrogen fixation observed at this site (Chapter 5). Cyanobacteria also apparently dominated the MPB community at PC lower during spring and summer. In addition to cyanobacteria another likely source of zeaxanthin at Site PC is from the red macroalgae *Gracilaria* sp. (Jeffrey and Vesk 1997). It has been inferred (stable isotopes discussion) that a large fraction of the organic matter within the sediment was derived from this macroalgae. It is most likely that this organic matter is highly degraded, because β,ε carotene produced by rhodophyta (Jeffrey and Vesk 1997) was not detected. The

fatty acid 20:4(n-6), which is produced in large amounts by *Gracilaria* sp. (Vaskovsky et al. 1996) was also only detected in very small quantities (Figure 2.6). As such, it seems most likely that any zeaxanthin associated with the *Gracilaria* sp. detritus would also have been degraded, and not contributed significantly to the zeaxanthin measured here. The relative proportion of chlorophytes was greatest at all sites during autumn and winter, suggesting conditions during these periods were more favourable for this algal class. Diatoms comprised a nearly constant fraction of the MPB community of between 20–40% at both sites, this was with the exception of Site PC Lower where diatoms were generally insignificant except during autumn.

The lipid biomarker data were generally in broad agreement with the pigment data. The presence of diatoms at all sites and all times of the year was indicated by the fatty acid 20:5(n-3), 24-methylcholesta-5,22E-dien-3β-ol, as well as 24-methylcholesta-5,24(28)-dien-3β-ol which has recently been suggested as a marker for diatoms (Volkman et al. 1998). 24-methylcholesta-5,24(28)-dien-3β-ol is also produced by some dinoflagellates and prasinophytes (Volkman et al. 1998). However, the xanthophyll pigments peridinin and prasinoxanthin - particular to each of these groups respectively (Jeffrey and Vesk 1997) were not detected. 4,23,24-trimethyl-5α-cholest-22E-en-3β-ol, which is specific to dinoflagellates, was also only detected occasionally and in relatively small quantities. Therefore, it would appear that in this system 24-methylcholesta-5,24(28)-dien-3β-ol is likely to arise mostly from diatoms. The presence of chlorophytes was indicated by the fatty acids 18:2(n-6) and 18:3(n-3) (Volkman et al. 1980; Volkman et al. 1989), particularly at Site PC, where the pigment data suggested these algae were generally dominant. Although there are no generally accepted fatty acid markers for cyanobacteria, the 18:4 PUFA was found to be present in relatively high proportions in the extract of separated MPB dominated by cyanobacteria. (Table 1). Since this 18:4 PUFA is not generally found in such high quantities in diatoms or chlorophytes, it was used as a marker for cyanobacteria at Site CF. While hopanoid alcohols were detected within the sediment (data not shown) they were apparently not derived from cyanobacteria because no hopanoid alcohols were detected in the separated MPB extract. This is consistent with the data of Summons et al. (1999), which showed that a number of species of *Oscillatoria* (the dominant genus in the separated fraction of MPB) are among the few cyanobacteria which do not produce hopanols.

Fatty acid data were also used to provide a comparative estimate of the relative importance of the different algal classes. A meaningful estimate using such data can only be made at Site CF, where I was able to obtain fatty acid data from a separated fraction (dominated by *Oscillatoria* spp.) of the community. In order to obtain a fatty acid: chl *a* ratio for the algal extract, the amount of chl *a* in the extract was calculated by multiplying the phytol concentration by 3. It was assumed that all the phytol present within the separated MPB was in the form of chlorophyll and the correction of 3 was applied to account for the differences in molecular weight. The ratio of the PUFA 18:4:chl *a* (3) was then used to calculate the contribution of *Oscillatoria* spp. to chl *a* in the sediments. It was assumed that the contribution of 18:4 by chlorophytes was negligible because previous studies have shown that chlorophytes only produce small amounts of 18:4 compared to total lipids (~3%) (Volkman et al. 1989; Ahlgren et al. 1992). The diatom contribution to chl *a* was estimated by assuming that 20:5(n-3) comprised 20% of total fatty acids (Volkman et al. 1980; Volkman et al. 1989) and using a total fatty acid-to-chl *a* ratio of 5.2. (Volkman et al. 1989). The contribution of chlorophytes to chl *a* in the sediment was estimated using the fatty acid 18:2(n-6). This fatty acid was not found in significant quantities in the separated MPB extract, and generally only occurs in small quantities in diatoms (~<2%) (Volkman et al. 1989). It was assumed that 18:2(n-6) comprised 10% of total fatty acids and that chlorophytes had a total fatty acid-to-chl *a* ratio of 4 (Volkman et al. 1989; Ahlgren et al. 1992). Using this approach, the amount of chl *a* attributable to each algal class can be expressed as a percentage of the measured concentration of chl *a* (Figure 2.9b). The proportions do not necessarily add up to 100% because the ratios of fatty acid - to - chl *a* are only estimates of the true values. Despite the potentially large errors in such estimates, the sum of the chl *a* attributable to each algal class was generally in agreement with the measured values of chl *a* suggesting the fatty acid:chl *a* ratios used were on average close to the true values. At Site CF, using fatty acid data, it was estimated that cyanobacteria, chlorophytes and diatoms comprised an average of 53, 15 and 32% of the community, respectively, on the upper and lower mudflats on an annual basis. Using pigment data cyanobacteria, chlorophytes and diatoms were estimated to comprise 41, 25 and 34%, respectively.

The estimates of the relative composition of the MPB community using both pigment and fatty acid data were in broad agreement in that they both revealed a mixed community of diatoms, cyanobacteria and chlorophytes. At Site CF upper, the fatty acid data suggest that chlorophytes accounted for a much greater proportion of the community than the pigment data. This highlights a number of problems of using fatty acid and pigment data to estimate the relative contributions of the algal classes present. The ratios of fatty acids to chl *a* in particular, are likely to vary from the literature values used here. Diatoms may have a highly variable 20:5(n-3) content (Volkman et al. 1989) and the light regime to which algae are exposed may affect the fatty acid content of microalgae (Thompson et al. 1990). This is particularly evident during winter when estimates of total chl *a*, using fatty acids exceed the measured value by up to 100%. This suggests that the total fatty acid-to-chl *a* ratio at this time of the year was lower than at other times, which is consistent with the expectation that more chl *a* would be produced relative to other bio-molecules during times of low light availability (Geider 1987). Differential rates of degradation between pigments and fatty acids may also give rise to differences observed in the estimates of relative abundances.

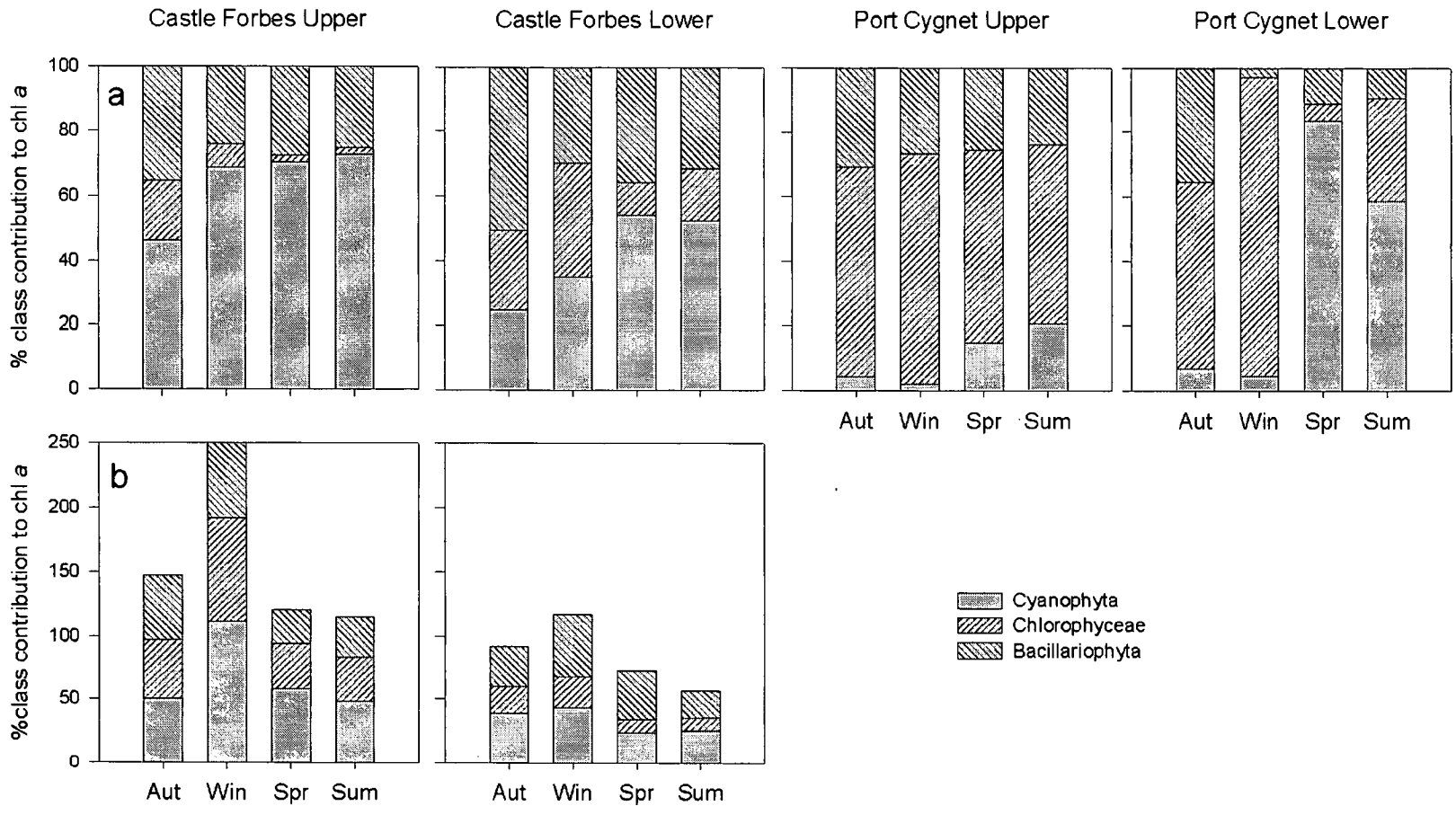


Figure 2.9. The percent contribution of the algal classes Cyanophyta, Bacillariophyta and Chlorophyceae to total chl *a* calculated using pigment data and CHEMTAX (a) and fatty acid data (b) in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) at the two study sites during the year 2001 (see text for details).

General classification of the mudflat ecosystem

The fact that a significant fraction of the MPB consisted of cyanobacteria (which were dominated by *Oscillatoria* spp.) raises the question as to whether the MPB community at Site CF should be described as a microbial mat. To date, most reports of benthic *Oscillatoriaceae* have been from ecosystems described as being microbial mats (Stal 1995 and references therein). From an aesthetic point of view, the mudflat studied does not match the general description given by Stal (1995), in that (i) no mats of microbial organisms were observed, and (ii) no laminations were apparent within the sediment. High rates of sulphate reduction are also cited as a characteristic of microbial mats (Stal 1995). In the Huon Estuary mudflats, significant sulphate reduction was shown to occur within the sediment only on the upper mudflat (Chapter 3). When sulfate reduction did occur, it was confined to the deeper layers of sediment. Evidence for the absence of sulfate reduction in the surface sediments comes from the absence of fatty acids such as 10Me16:0 and cy19:0, which are markers for sulfate reducing bacteria (Findlay and Dobbs 1993), in the surface 0.5 cm of sediment. Anoxygenic photosynthesis by purple sulfur bacteria was also apparently not occurring as indicated by the absence of bacteriophyll *a* (Millie et al. 1993), which further suggests the description of the Huon Estuary mudflats as a microbial mat is inappropriate. The presence of significant quantities of diatoms and chlorophytes within these sediments also suggest that its description as a MPB community is more appropriate and is consistent with other descriptions of mixed MPB communities consisting of diatoms and cyanobacteria (Kristensen 1993; Sundbäck et al. 2000)

Summary and conclusions

Organic matter from a variety of sources was identified on the mudflat (Table 5). The organic matter pool within the sediments was dominated by terrestrial sources of organic matter. This was most likely refractory and not undergoing remineralisation to a significant extent. Organic matter derived from microalgae made up a minor fraction of the sediment organic matter pool. This fraction of organic matter was likely to be much more labile than that derived from terrestrial sources as indicated by much lower C:N ratios of this organic matter, as well as its high PUFA content. It is the input this MPB-derived material that drives bacterial production within the sediment as suggested by the stable isotope ratios of bacterial fatty acids.

The MPB at Site CF consisted of a mixed community of diatoms, cyanobacteria and chlorophytes. Separation of MPB from the sediment at this site using the lens tissue technique was found to significantly fractionate the algal community with cyanobacteria being enriched in the separated fraction. Nitrogen fixation was likely to contribute significantly to the growth requirements of cyanobacteria at this site as indicated by the low $\delta^{15}N$ of the cyanobacteria. At Site PC, chlorophytes and diatoms generally dominated the MPB, with the exception of Site PC lower during spring and summer, when cyanobacteria dominated the MPB.

Table 2.6. Summary of the sources of organic matter identified at Castle Forbes Bay and Port Cygnet.

Organic matter source	Evidence	Reference or justification
MPB	Stable carbon isotopes, chl *a*	$\delta^{13}C$ of MPB measured assumed to be major "endmember"
Cyanophyta	Zeaxanthin, 18:4(n-3)	(Jeffrey and Vesk 1997), 18:4(n-3) measured in separated cyanobacteria
Bacillariophyta	Fucoxanthin, chl *c*, 20:5(n-3), 16:1(n-7), 24-methylcholesta-5,22E-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol	(Volkman et al. 1989; Jeffrey and Vesk 1997; Volkman et al. 1998)
Chlorophyceae	Lutein, 18:3(n-3), chl *b*	(Jeffrey and Vesk 1997)
Bacteria	Fatty acids i15:0, ai15:0, 17:0, ai17:0	(Findlay and Dobbs 1993)
Alteromonas, Pseudoalteromonas?	15:0, 17:1(n-8)	(Ivanova et al. 2000)
Terrestrial sources	Stable carbon isotopes, β-amyrin, fatty acids 22:0, 24:0, 26:0, 28:0, 30:0	$\delta^{13}C$ of detritus and terrestrial sediments measured assumed to be major "endmember" (Volkman et al. 1987) (Wannigama et al. 1981)
Macroalgae	Stable carbon isotopes, 20:4(n-6), observation	$\delta^{13}C$ of macroalgae measured assumed to be major "endmember" (Vaskovsky et al. 1996)

Chapter 3

Benthic metabolism on intertidal mudflats in a temperate Australian estuary

Abstract

The light and dark fluxes of O_2 and TCO_2 as well as the concentrations of chlorophyll *a* and pheopigments were measured across the inundation gradients of two mudflats, one in the upper Huon Estuary, and one in a marine side-arm of the estuary, over four seasons. Annual rates of gross community production measured at these sites were comparable to those measured in other systems. At the more sheltered site in upper estuary, it was estimated that gross community production on mudflats accounted for 12% of primary production in the water column. At this site, significantly greater rates of gross community production were measured on the upper mudflat. High concentrations of coloured dissolved organic matter (CDOM) in the water most likely limited light penetration on the lower mudflat, resulting in lower rates of gross community production and MPB biomass. By contrast, no significant difference in rates of gross community production were observed across the inundation gradient at the site in the marine side arm of the estuary, where much lower levels of CDOM meant that light penetration was not likely to be limited over the inundation gradient. Despite the apparent lack of light limitation, rates of gross community production were much lower than on the upper mudflat in the upper estuary. It is proposed that a greater exposure to wave energy at the marine site was the cause of the lower rates of gross community production. On an annual basis, both the upper and lower mudflats in the upper estuary were net autotrophic while the upper and lower mudflats in the marine side arm were net heterotrophic. At the site in the upper estuary, rates of benthic respiration were apparently controlled by temperature and MPB biomass, whereas at the marine site temperature was the only variable that could explain the variation in respiration rates. Rates of TCO_2 consumption in the light were generally greater than O_2 production. It is suggested that O_2 effluxes are greatly reduced in the light as a consequence of the reoxidation of sulfides within the sediments. Similarly, at high sediment respiration rates there was an excess of TCO_2 produced compared to O_2 consumed, suggesting a net reduction of sulfur. Therefore, rates of TCO_2 uptake and production will provide a better measure of gross community production and sediment respiration rates than O_2 production and consumption, respectively.

Introduction

Intertidal sediments act as important zones for organic matter deposition within estuaries (Jickells and Rae 1997). As such, high rates of organic matter remineralisation may be observed within these zones. For example, it has been estimated that intertidal sediments in the Westerchelde Estuary account for 25–30% of the estuarine retention of carbon (Middelburg et al. 1996a). An availability of light and nutrients, as well as sheltered conditions means that intertidal sediments may also be an important habitat for microphytobenthos (MPB) (Heip et al. 1995). MPB have been shown to contribute significantly to total primary production in a number of estuaries (Underwood and Kromkamp 1999). It is not surprising then that MPB have been shown to be a significant food resource for benthic food webs (Herman et al. 2000; Middelburg et al. 2000). Intertidal sediments are, therefore, likely to play an important role in both in the production and remineralisation of organic matter within estuarine ecosystems.

The balance between these heterotrophic and autotrophic processes is of fundamental importance in determining how an ecosystem functions (Heip et al. 1995). Where community production is greater than respiration (net autotrophy), one would expect a net import of inorganic constituents (NH_4^+, CO_2, NO_3^-, HPO_4^{2-}) and an export of organic matter through the food web. Where respiration is greater than community production (net heterotrophy), one would expect an import of organic detritus and an export inorganic constituents. The trophic status of benthic systems has many potential implications for factors including water quality (Rizzo et al. 1996), nutrient cycling (Eyre and Ferguson 2002; Ferguson 2002) and ecosystem stability (de Wit et al. 2001). In order to understand the ecological role played by tidal flats in estuarine ecosystems, it is essential to understand the balance between heterotrophy and autotrophy within these ecosystems.

In order to evaluate the importance of community production and respiration on mudflats and the balance between these two processes it is necessary to consider the rates of the processes across spatial and temporal scales likely to exert important influences on these processes. Environmental factors controlling community production by MPB include light, sediment type, exposure and temperature

(MacIntyre et al. 1996; Underwood and Kromkamp 1999). Seasonality is well recognised as exerting an important influence on the biomass and rates of primary production by MPB. Peaks in primary production have generally been previously observed during spring and sometimes in autumn (MacIntyre et al. 1996). Of key importance on mudflats, is the inundation gradient which gives rise to large changes in some of these important environmental variables, in particular irradiance. This gradient has been shown to significantly affect MPB biomass and production in a number of turbid macrotidal systems, with greater biomass and productivity generally being observed in the upper intertidal zone (Underwood and Kromkamp 1999). Exposure to hydrodynamic energy as indicated by sediment grain size has also been shown to be a significant factor affecting microalgal biomass and primary production on intertidal flats with lower biomass and rates of primary production generally being observed at more exposed sites (MacIntyre et al. 1996).

Factors controlling respiration rates include temperature, the quantity and quality of organic matter; the availability of electron acceptors will determine the respiratory pathways by which organic matter is degraded (Heip et al. 1995). Because organic matter derived from MPB is likely to drive secondary production by both bacteria (Cammen 1991; Middelburg et al. 2000) and macrofauna (Herman et al. 2000) in intertidal sediments, sediment respiration rates are likely to reflect the input organic matter derived from MPB. Mineralisation rates have also been shown to change considerably over an estuarine salinity gradient, most likely as a consequence of changes in the quality and quantity of organic matter delivered across the estuary (Kelley et al. 1990; Middelburg et al. 1996a). In order to gain a representative understanding of how tidal flats function in terms of the balance between respiration and production, it is necessary to sample across spatial and temporal gradients which are likely to exert important controls on the system

The vast majority of studies of benthic productivity and respiration in estuaries have been conducted in European and North American systems (Heip et al. 1995; MacIntyre et al. 1996; Underwood and Kromkamp 1999). In order to gain a broader understanding of the factors controlling production and mineralisation, it is necessary to obtain data from a range of geographic locations. To date, no studies of benthic primary production or respiration have been conducted in Australian intertidal

sediments with the notable exceptions of Alongi and Co-workers (Alongi 1994; Alongi et al. 1999; Alongi et al. 2000), however, these studies were conducted in tropical systems. No published studies of primary production or respiration exist in Australian temperate intertidal sediments. Australian estuarine systems differ in a number of key respects to North American and European systems (Eyre 1998), most relevant in this instance perhaps are the low rates of nutrient runoff due to low population densities and relatively intact catchments (Harris 2001). It has been proposed that a ready supply of nutrients remineralised within the sediments means that MPB production is unlikely to be nutrient limited, particularly in muddy habitats (MacIntyre et al. 1996; Underwood and Kromkamp 1999). As such, MPB may be an important source of organic carbon in the relatively low nutrient Australian systems. Furthermore, an understanding of less disturbed temperate Australian estuaries may provide insights into how eutrophication has changed the ecological function of other mudflat systems.

This chapter evaluates the relative importance of production and remineralisation (and hence, the net trophic balance) on the intertidal flats of a cool temperate microtidal estuary. These processes were studied across the inundation gradient of two mudflats, one saline and one euryhaline (Salinity range ~8–30), over four seasons. Community respiration and primary production were measured using O_2, TCO_2 and alkalinity fluxes. These techniques have only been occasionally used in conjunction with one another over light/dark cycles (Kristensen 1993; Eyre and Ferguson 2002); yet they allow estimates of net anoxic respiration (Anderson et al. 1986), as well as giving insights into changes in sediment processes when shifting from dark to light (Eyre and Ferguson 2002).

Methods

Sampling

A description of the study sites is given in Chapter 1. Samples were collected from Castle Forbes Bay (Site CF) in March, June, September and December 2001. At Port Cygnet (Site PC) samples were collected during April, June September and December 2001. An additional sampling was undertaken earlier in November 2000 at CF upper, for which the data have also been presented. To facilitate comparison with other measurements, March and April are referred to as autumn. June,

September, November and December are referred to as winter, early spring, late spring and summer, respectively. Four intact sediment cores (25 × 14.5 cm i.d.) were taken at each site as described in Chapter 1. The cores were then placed in a padded box and returned to the laboratory within 1 h. Upon return to the laboratory, the cores were submerged in a water bath of site water (160 l) at in-situ temperature. Transparent lids with a paddle stirrer (4.5 × 1.5 cm) rotating at 60 rpm were then propped over the cores such that a free exchange of water between the cores and the water bath could take place. The paddle stirrer in each core driven by an electric motor mounted on the edge of the lid. After all flux experiments had finished, the top 0.5cm of sediment was removed for chlorophyll *a* (chl *a*) and pigment analysis. Details of water temperature and salinity are presented in Chapter 4, Figure 4.1.

O_2 and TCO_2 exchange

Rates of O_2, TCO_2 and nutrient exchange in the light (nutrient data presented in Chapter 4) were measured the day after collection coincidental with the period of in-situ low tide. Cores were illuminated at 500 μE m^{-2} s^{-1} at the sediment surface using a 50 W halogen lamp placed above each core. Measurements showed that this was reduced to ~250 μE over an area equivalent to ~10% of the core immediately below the electric motor. Before starting the flux measurements, the cores were illuminated for ~1 h, the cores were then flushed with fresh site water, and capped. Rates of O_2 and TCO_2 and nutrient exchange were similarly made in the dark on the following day using the same cores. Four samples of the water – for nutrient and alkalinity determinations– were taken from the core by withdrawing 65 ml of sample into a plastic syringe through a Luer Lock valve fitted to the lid. The water withdrawn from the core was simultaneously replaced with water from a gravity-fed reservoir. Water samples taken for nutrients and alkalinity were filtered through a precombusted Whatman GF/F filter into 10-ml screw-cap polypropylene containers; alkalinity samples were stored in the dark at 4°C and analysed within two weeks. Dissolved oxygen (DO) and pH were measured in the core during the incubations using electrodes. An incubation time was used which allowed the DO in the dark incubation to drop by ~20% from its saturated concentration, this ranged from 3–6 h. In the light, DO readings were taken before bubble formation. Observed DO and TCO_2 fluxes were always linear and generally had an r^2>0.98 (data not shown). The flux across the sediment-water interface was calculated as

$$flux = \alpha - \alpha_w \times \frac{V}{A}$$

where

α = linear regression slope of analyte concentration (corrected for the addition of replacement water) versus time in sediment core ($\mu mol\ l^{-1}\ h^{-1}$)

α_w = linear regression slope of analyte concentration (corrected for the addition of replacement water) versus time in "blank" core.

V = water column volume (l)

A = sediment surface area (m^{-2}).

The linear regression typically used four data points. The flux was only taken as being significant if the standard error of the slope was less than the magnitude of the flux.

Gross rates of production in the light were calculated for O_2 and TCO_2 as follows.

Gross benthic O_2 production (sediment efflux)= light net O_2 flux (positive)-dark O_2 flux (negative)

Gross benthic carbon production (sediment influx) = light net TCO_2 flux (negative) – dark TCO_2 flux (positive).

These rates are referred to as gross community production in this chapter. After the completion of core incubations the top 0.5 cm of sediment was collected from half of the core and frozen in liquid nitrogen for pigment analysis.

Analytical methods

Oxygen was measured in the cores using a calibrated dissolved oxygen probe (WTW) (precision = ±5%). TCO_2 was calculated from pH (precision = ±0.002) and alkalinity (precision = ±1%) according to Almgren et al. (1983), dissociation constants of carbonic acid were calculated according to Roy et al. (1993). pH was measured in the core using an Orion (91-55) pH probe connected to a PHM85 (Radiometer) pH meter. Alkalinity was measured using a Gran titration which was performed using an Orion 960 Autochemistry system. Chl *a* and pheopigments were extracted and analysed as described in Chapter 2, with the exception of the sampling

taken in November 2000, when pigments were determined spectrophotometrically as described in Chapter 5. Sediment grain size was determined by wet sieving using Endecott (London, UK) test sieves through 500, 250 150 and 63 μm meshes. Photosynthetically available radiation (PAR) at the sediment surface was measured using a Biospherical Intruments QSL-100 light meter.

Annual production rates

Annual production rates were estimated using five models as applied by Thornton et al. (2002). For consistency, these have been referred to by the same names Maximum, Minimum, Regresssion, Photosynthesis and Gross TCO_2. Here, I have used TCO_2 fluxes to estimate production instead of O_2. The Maximum, Minimum and Regression models were taken from Santos et al. (1997). For the maximum and minumum models, mean annual chl *a* (μg g^{-1}) in the sediment was converted to production using a factor of 5 for the minimum rate of production and a factor of 25 for the maximum rate of production. The Regression model was a regression equation derived by Santos et al. (1997) from published data sets of mean annual chl *a* concentrations and production. The equation derived was $\ln PP = 0.419 + 0.974 \ln$ chl *a*, where PP = annual primary production (g C m^{-2} yr^{-1}) and chl *a* = concentration of chl *a* in mg m^{-2}. The Photosynthesis model was based on data obtained by Wolfstein and Hartig (1998) which gave a maximum production rate of 2.75 mg C mg chl a^{-1} h^{-1}. Daily estimates of production were then obtained from the average seasonal photosynthetic day length, which was calculated for each day of the year at latitude 43°S was calculated from the equations given in Kirk (1994). The average seasonal daylength was then calculated by dividing the days up into the seasons defined as follows: summer; December-February, autumn; March-May, winter; June-August, spring; September-November.

It was observed that when there was an onshore breeze at each of the sites, the water became highly turbid, thus potentially light limiting photosynthesis. For calculation purposes, it was assumed that when the wind blew onshore at each of the sites (easterly for Site CF and south westerly for Site PC), no photosynthesis occurred, even during periods of exposure. I consider this reasonable as the upper mudflat at both sites was inundated for greater than 50% of the time and also disturbance caused by an onshore wind most likely meant that photosynthesis would not resume immediately upon re-exposure because MPB would require time to migrate back to

the sediment surface. The period of time the wind blew onshore at each site during daylight hours for each season (as defined above) was calculated from half hourly meteorological data on wind speed and direction recorded at Hobart (approx 40 Km north of the study sites). The effective photoperiod was then calculated as the average day length – 2 h – daily onshore wind hours (the two hours were subtracted as it was assumed significant rates of photosynthesis were not likely to be occurring at dawn and dusk). It was assumed that MPB production was saturated at the irradiance of 500 $\mu E\ m^{-2}\ s^{-1}$ used in the laboratory and that MPB production was also always saturated in the field during the effective photoperiod. Measured TCO_2 fluxes were scaled up to give daily estimates using seasonal photosynthetic day length as calculated above. Daily estimates were summed to give seasonal production rates, which were in turn summed to give annual production rates. Estimates of production at Site PC and the upper estuary (Site CF) were extraploated using 1:25000 topographic maps (Tasmap) which denoted the intertidal areas of the estuary.

No measurements of primary production exist for the water column of the Huon Estuary so this was estimated according to the relationship derived by Cole and Cloern (1987).

$$P = 150 + 0.73BI_0Z_p$$

Where

P = net photic zone primary production (mg C $m^{-2}\ day^{-1}$)

B = biomass (mg chl *a* m^{-3})

Z_p = photic zone depth (m)

I_0 = daily irradiance (E $m^{-2}\ day^{-1}$)

Biomass data were obtained from surveys conducted at three monthly intervals from 1996–1998 as part of the Huon Estuary Study (Butler et al. 2000). Photic zone depth was estimated from Secchi depth data from Parker (2002) converted to euphotic zone depth using a multiplication factor of three (Holmes 1970). Average annual daily irradiance was calculated from satellite estimates of insolation (obtained from the Australian Bureau of Meteorology) as follows. Insolation was converted from total

energy to photosynthetically available radiation using a factor of 0.45 (Baker and Frouin 1987) and from joules per day to moles per day using 2.5×10^{18} quanta W^{-1} (Morel and Smith 1974). Estimates of productivity were made for the upper estuary and Port Cygnet (the areas of which are defined in Figure 1.2, Chapter 1). Minimum and maximum estimates of primary production were made using data from the surveys with the minimum and maximum average chl *a* concentrations for each particular zone of the estuary. The average rate of production was estimated from the chl *a* concentration in each particular zone of the estuary averaged across all surveys.

Statistical analysis.

Statistical analysis was carried out using Statistica Vers 6.0 (StatSoft Inc). 1-way and 2-way analysis of variance (ANOVA) were carried out on log transformed data with time and position as factors. Correlation and multiple regression analysis were used to explore relationships between variables. The level of significance for rejection of the null hypothesis was set at $p<0.05$.

Results

Sediment grain size

Table 3.1. summarises the percent of each grain size measured on the upper and lower mudflats of the two study sites. It can be seen that the sediments at Site CF had a much greater fraction of fine silt (<63) (68–83%) than those at PC (28–48%). Coarse sand and fine sand (250–500μm and 125–250μm respectively) comprised a much greater fraction of the grain sizes at Site PC (20–30%) than at Site CF (2–9%). The consistently larger sediment grain size at Site PC suggests this is a higher hydrodynamic energy environment than Site PC.

Table 3.1. Measured percent composition for sediment grain sizes during autumn (Aut) and spring (Spr) on the upper and lower mudflats at Castle Forbes Bay (CF) and Port Cygnet (PC).

	CF Upper		*CF Lower*		*PC Upper*		*PC Lower*	
	Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr
%>500μm	0.40	0.42	0.18	0.73	11.4	17.1	8.48	7.22
%250–500μm	0.89	0.74	1.32	3.13	10.5	15.2	12.2	12.5
%125–250μm	1.24	2.20	4.64	5.82	9.77	14.7	12.2	13.0
%63–125μm	14.3	15.3	25.2	19.2	23.6	24.6	18.7	23.3
%<63μm	83.2	81.4	68.6	71.2	44.8	28.3	48.4	44.0

Chlorophyll a and degradation products

Figure 3.1 summarises the chl *a* concentration at each site over the course of the year. At Site CF, chl *a* concentrations were significantly greater on the upper mudflat than the lower mudflat (2-way ANOVA, $p<0.01$). A significant interaction between position and time, however, indicated that time affected the upper and lower mudflats differently. A Fisher LSD post-hoc analysis showed that chl *a* on the lower mudflat did not vary significantly throughout the year, however, on the upper mudflat chl *a* was highest in spring and lowest in autumn, summer and winter chl *a* concentrations were not significantly different. At Site PC, chl *a* concentrations were significantly greater on the upper mudflat (2-way ANOVA, $p<0.01$), time did not significantly affect chl *a* concentrations throughout the year on the upper or lower mudflat. On an annual basis, chl *a* concentrations were significantly different between mudflats (2-way ANOVA, $p<0.01$), Fisher LSD post-hoc analysis indicated that chl *a* concentrations were greater on both the upper and lower mudflats at Site CF than either of the positions at Site PC. Chl *a* was signficantly correlated with respiration (TCO_2) at CF ($r=0.74$ $p<0.05$). Pheopigment to chl *a* ratios were significantly less at Site CF upper and highest at Site PC lower over the course of the year (1-way ANOVA, Fisher LSD post-hoc analysis $p<0.01$), there was no significant difference in pheopigment:chl *a* between Site PC upper and CF lower.

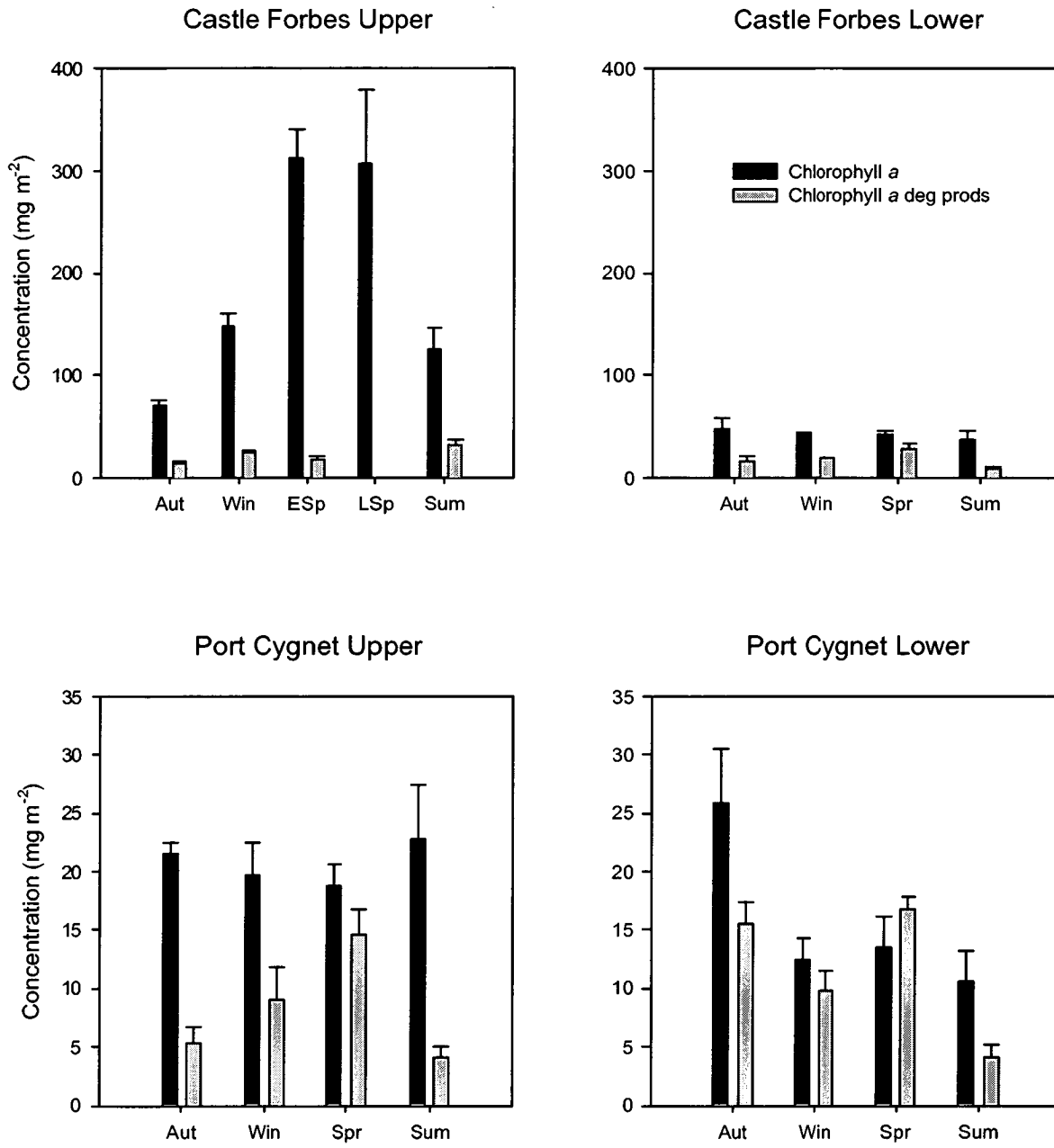


Figure 3.1. Summary of chlorophyll *a* and chlorophyll *a* degradation products during autumn (Aut), winter (Win), early spring (Esp), late spring (LSp) and summer (Sum) on the upper and lower mudflats at Port Cygnet and Castle Forbes Bay (Note: different vertical scale for the two sites). Error bars represent the standard error of replicate samples (n=3–4)

Gross community production and respiration

Castle Forbes Bay

Figure 3.2 summarises net TCO_2, O_2 and alkalinity fluxes under light and dark conditions over the course of the year at Sites PC and CF. It can be seen that the sediments at Site CF were net productive (as measured by O_2 and TCO_2 fluxes) in the light throughout the year on both the upper and lower mudflat. Gross rates of productivity ranged from 1100 μmol m^{-2} h^{-1} O_2 (-650 μmol m^{-2} h^{-1} TCO_2) on the lower mudflat in winter up to 13300 μmol m^{-2} h^{-1} O_2 (-15000 μmol m^{-2} h^{-1} TCO_2) on the upper mudflat during early spring. Gross community production quotients (CPQ = O_2/CO_2 flux ratio) for gross community production averaged 0.88 and ranged between 0.57 and 1.7. Gross production (as measured by O_2 and TCO_2 fluxes) was significantly greater (2-way ANOVA, $p<0.001$) on the upper mudflat at Site CF over an annual cycle. A significant interaction (2-way ANOVA, position × time, $p<.001$) showed that time affected the upper and lower mudflats differently. This was manifested as a much greater increase in rates of gross community production on the upper mudflat compared to the lower mudflat in spring.

Sediment respiration rates ranged between -560 μmol m^{-2} $h^{-1}O_2$ (390 μmol m^{-2} h^{-1} TCO_2) on the lower mudflat during winter and -2600 μmol m^{-2} $h^{-1}O_2$ (6900 μmol m^{-2} h^{-1} TCO_2) on the upper mudflat during late spring. Gross community respiration quotients (CRQ = TCO_2/O_2 flux ratio) averaged 1.4 and ranged from 0.71 to 2.7. The highest respiration quotient was measured at CF upper during November when respiration rates were highest. Respiration (as measured by TCO_2 and O_2 fluxes), was significantly greater on the upper mudflat than on the lower mudflat (2-way ANOVA, $p<0.001$) but a significant interaction (position × time, $p<0.001$) indicated that the upper and lower positions on the mudflat were affected differently by time. Respiration rates (TCO_2 effluxes) were correlated with chl *a* ($r=0.75$, $p<0.05$) and gross production (TCO_2) ($r=-0.74$, $p<0.05$). Standard multiple regression analysis showed that chl *a*, temperature (T) and net production (NP, TCO_2 uptake) controlled 84% (adjusted r^2) of the variability for respiration, the resulting regression equation was:

$$TCO_2 \text{ flux} = -2458 + 13.9\text{Chl a} + 264\text{T} + 0.15\text{NP} \qquad (F(2,30) = 57, p<0.001)$$

The semi-partial correlations were 0.65 for chl *a* and 0.65 for temperature indicating that they contributed similarly to the variation in TCO_2 fluxes. For net production the semi-partial correlation was 0.2, indicating that it had a smaller unique contribution to the variability in sediment respiration rates. Chl *a*, temperature and net production all had a significant unique contribution to the model as indicated by the significance of the semi-partial correlations (t-test, $p<0.01$).

Port Cygnet

Gross community production at Site PC ranged from 570 μmol m^{-2} h^{-1} O_2 (-1100 μmol m^{-2} h^{-1} TCO_2) on the lower mudflat in summer up to 5700 μmol m^{-2} h^{-1} O_2 (-7700 μmol m^{-2} h^{-1} TCO_2) on the lower mudflat in autumn. Productivity (as measured by O_2 and TCO_2 fluxes) was not significantly different on the upper and lower mudflats (2-way ANOVA). A significant interaction with time × position ($p<0.01$) indicated time did affect productivity on the upper and lower mudflats differently. This primarily manifested itself as high rates of production on the lower mudflat in autumn which were not observed on the lower mudflat.

Respiration rates were not significantly different on the upper and lower mudflats (as measured by both O_2 and TCO_2), however, they were significantly affected by time (2-way ANOVA, $p<0.01$). The CPQ for gross production averaged 0.87 and ranged from 0.39 to1.31. The CRQ averaged 1.0 and ranged between 0.72 and 1.48. Gross community production was not correlated to temperature. Respiration was significantly correlated to temperature ($r = 0.86$, $p<0.01$, O_2 flux). Multiple regression analysis indicated that only temperature, and not chl *a*, contributed significantly to the variability in dark TCO_2 fluxes.

Port Cygnet and Castle Forbes Bay

Site CF upper had significantly greater production rates (light TCO_2 uptake) than all of the other sites, (1-way ANOVA, Fisher LSD post-hoc analysis $p<0.01$). Respiration rates (dark TCO_2 fluxes) were significantly highest at CF upper and lowest at CF Lower. Respiration at PC upper and lower was not significantly different to each other (1-way ANOVA, Fisher LSD Post-hoc analysis, $p<0.05$). CPQ was not significantly different for CF and PC (1-way ANOVA), but RQ was significantly greater at CF (1.4) than PC (1).

Alkalinity fluxes

Alkalinity fluxes were generally positive in the dark and negative in the light, with the exception of the lower mudflat at Site PC, which had a significant negative alkalinity flux during the dark in summer. Alkalinity fluxes were generally similar to TCO_2 fluxes with the highest uptake occurring during early spring on the upper mudflat at Site CF in the light (-5800 $\mu Eqm^{-2}\ h^{-1}$) and the highest efflux occurring during late spring in the dark (4200 $\mu Eqm^{-2}\ h^{-1}$) at the same site. Light alkalinity fluxes were highly correlated with TCO_2 flux ($r=0.99$). At Site PC, neither position on the mudflat nor time significantly affected dark alkalinity fluxes (2-way ANOVA, $p>0.05$). At Site CF, dark alkalinity fluxes were significantly greater on the upper mudflat (2-way ANOVA, $p<0.01$). Time also significantly affected alkalinity fluxes, with no significant interaction between position $\times$ time, indicating the impact of seasonality on alkalinity was consistent regardless of position on the mudflat.

Estimated annual gross community production

Rates of annual gross community production were estimated according to the five models outlined in the methods section. The Chl *a* and the Maximum model gave much greater estimates of annual gross community production at Site CF than did the TCO_2 model (Table 2). The Minimum model gave the best agreement with the TCO_2 model at Site CF. By contrast the Chl *a* and the Maximum model were in close agreement with the TCO_2 model at Site PC. The Regression and the Minimum models both gave much lower estimates of gross community production than the TCO_2 model at Site PC. On an annual basis, it was estimated that the mudflats in the upper estuary produced 180 t of carbon per year, and those in Port Cygnet produced 57 t of carbon per year using the TCO_2 model. This represented approximately 12% of the estimated gross community production in the water column in the upper estuary and 2.5% in Port Cygnet (Table 3.3)

Table 3.2. Estimated rates of annual gross community production on the mudflats (g C m^{-2} yr^{-1}) using the Regression (Reg), Minimum (Min), Maximum (Max), TCO_2 and Chl *a* models outlined in the methods section. Figures in parentheses are annual remineralisation rates of carbon (g C m^{-2} yr^{-1}). Total production in each section of the estuary was estimated accounting for the area of the mudflats in each part of the estuary.

Model	CF Upper	CF Lower	PC Upper	PC Lower	Total upper Estuary tC/yr	Total PC tC/yr
Reg	219	59	29	22	101	16
Min	490	143	31	34	229	20
Max	2452	716	153	169	1145	98
TCO_2	340 (250)	150 (110)	83 (150)	105 (130)	180	57
Chl a	1300	320	160	120	580	80
Mean	960	280	91	89	450	55

Table 3.3. Estimates of annual gross community production in the Upper Huon Estuary and Port Cygnet based on chlorophyll *a*, insolation and Secchi depth data. See methods section for details.

Section of Estuary	Annual production (av) g C $m^{-2}yr^{-1}$	Annual production (max) g C $m^{-2}yr^{-1}$	Annual production (min) g C $m^{-2}yr^{-1}$	Total annual production TC yr^{-1} (based on av)	Total production by MPB as % of total production by phytoplankton[1]
Upper Estuary	86	280	55	1500	12%
Port Cygnet	210	2200	63	2300	2.5%

[1]Estimated using gross annual production data estimated from the TCO_2 flux method in Table 3.2.

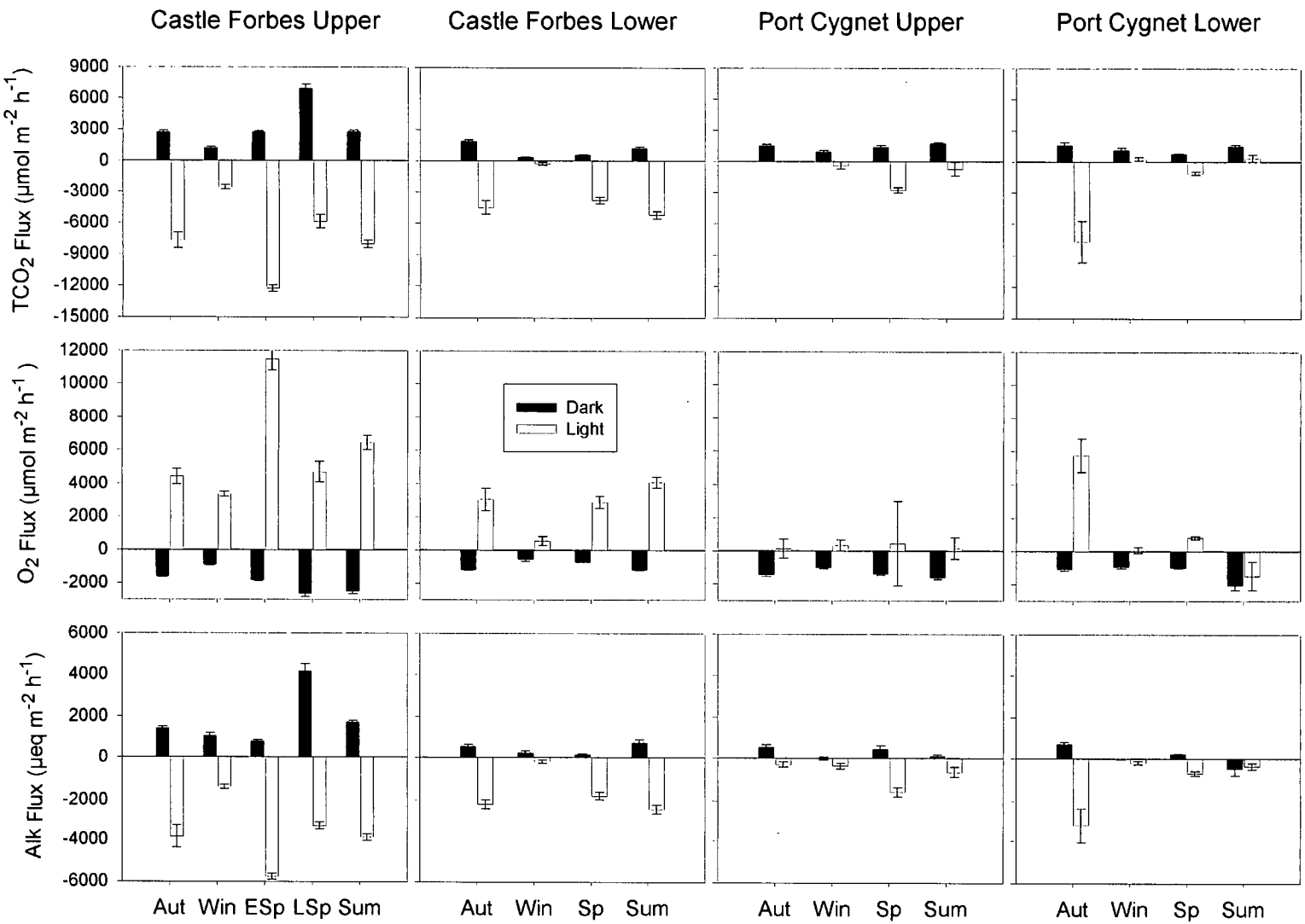


Figure 3.2. Summary of TCO_2, O_2 and alkalinity fluxes measured during autumn (Aut), winter (Win), early spring (Esp), late spring (LSp) and summer (Sum) on the upper and lower mudflats at Port Cygnet and Castle Forbes Bay. Error bars represent the standard error of replicate samples (n=3–4)

Discussion

The light environment

Intertidal sediments are exposed to an extremely dynamic light environment. In turbid macrotidal and mesotidal sediments, investigators generally assume that gross community production only occurs during tidal exposure. In this study, it was assumed that benthic community production continued during tidal inundation, the validity of this is considered here. The waters of the Huon Estuary have a low turbidity with concentrations of suspended particulate matter being <6mg kg^{-1} (Butler et al. 2000). The incoming freshwater discharge from the Huon River contains concentrations of dissolved organic carbon (DOC) of up to 18 mg l^{-1} (Butler et al. 2000). This results in light attenuation coefficients (Kd) in excess of 2 m^{-1} being observed in the riverine waters (Kirk 1994). At Site PC, the waters generally have a salinity above 30 (Butler et al. 2000), and therefore, freshwater-derived DOC has a relatively small influence on light penetration at this site.

Using tidal data (National Tidal Facility Unpublished Tidal Data – Hobart Tide Gauge) it was calculated that the lower mudflats would not be inundated by greater than 1 m of water for more than 5% of the time. The annual average surface irradiance was estimated to be 500 μE m^{-2} s^{-1} during daylight hours based on satellite insolation data and average day length (see methods section). Average Kd values based on at least fortnightly sampling over a three year period (1996–1999) were obtained from Parker (2002). At Port Cygnet, an average Kd of 0.46 was reported, which would result in a light intensity of 300 μE m^{-2} s^{-1} reaching the sediment through 1 m of water when the surface irradiance was 500 μE m^{-2} s^{-1}. At Site CF, the salinity was highly variable and ranged between 5 and 32. At a study site 5km down the estuary from Site CF (Brabazon Park), Parker (2002) reported an average Kd of 0.67, which was significantly greater than at Port Cygnet (Tukey Test, $p<0.05$). Based on these data, it was estimated that mean a light intensity of 250 μE $m^{-2}s^{-1}$ would reach the sediment through 1 m of water when the surface irradiance was 500 μE m^{-2} s^{-1}. 250–350 μE m^{-2} s^{-1} has generally been found to be saturating for photosynthesis of benthic microalgae from several intertidal systems (Davis and MacIntyre 1983; Rasmussen et al. 1983; Pinckney and Zingmark 1991; Brotas and Catarino 1995; Rizzo et al. 1996), although this may not always be the case (e.g. Underwood and Kromkamp 1999). Nevertheless, it still does seem likely that there

will be periods when little light reaches the sediment surface at Site CF lower, such as during times of high freshwater inflow. Significantly lower rates of productivity at Site CF lower also suggest that light limitation may occur at this site (see later discussion – temporal and spatial patterns and controlling factors). As such, the irradiance of 500 μE m^{-2} s^{-1} used in these experiments is likely to be significantly higher than those encountered in the field at site CF lower, particularly during winter. Consequently, the rates of production presented for CF lower (Figure 3.2) most likely represent an upper estimate of the true in-situ rates occurring at this site.

Gross community production

Temporal and spatial patterns, and controlling factors

At Site CF, there was a clear spatial difference in gross community production on the upper and lower mudflat. Greater MPB biomass and benthic productivity were measured on the upper zone of the mudflat compared to the lower zone. This appears to be a generally observed characteristic in macrotidal and mesotidal estuaries, where high turbidity means that photosynthesis is limited to periods of tidal exposure (Underwood and Kromkamp 1999). As discussed earlier, light penetration at Site CF lower is likely to be limited across the inundation gradient as a consequence of high CDOM concentrations in the water column. It might be argued that another possible explanation for the lower rates of productivity on the lower mudflat is greater nitrogen limitation at this position, because porewater NH_4^+ concentrations were much greater on the upper mudflat than the lower mudflat (Chapter 4). This seems unlikely because the greater concentrations of NH_4^+ in the porewater on the upper mudflat most likely arose as a consequence of the greater rates of organic matter remineralisation on the upper mudflat. It is evident in this chapter, and in Chapter 2 that the organic matter being remineralised within the sediment is derived from MPB. As such, the greater NH_4^+ concentrations on the upper mudflat arose as a consequence of the greater MPB biomass and activity on the upper mudflat. Light limitation is, therefore, the most likely explanation for the observed gradient in biomass and productivity observed at Site CF.

The strong seasonal interaction of gross community production with biomass between the upper and lower sites at Site CF arose from a peak in production on the upper mudflat in spring; this was not reflected on the lower mudflat, which reached

its production peak in summer. Similar observations were made by Santos et al. (1997) in the Gironde Estuary in France, and also by Admiraal and Peletier (1980) and Colijn and Dijkema (1981) in the Netherlands. It, therefore, appears to be a general phenomenon that benthic algae on the upper mudflat are able to bloom earlier in the season than those on the lower mudflat due to lifting of light limitation earlier on the upper mudflat.

In contrast to Site CF, the highest productivity recorded at site PC was observed on the lower mudflat (Figure 3.2), yet there was no consistent pattern between the upper and the lower mudflat. Davis and MacIntyre (1983) interpreted the lack of any observable gradient in production in the intertidal zone as indicating that light was not a limiting to gross community production. The waters of Site PC generally have a salinity > 30 (Butler et al. 2000), and thus, CDOM concentrations are negligible, and light attenuation less compared to Site CF, resulting in significantly lower light penetration in the upper estuary compared to Port Cygnet (Parker 2002). Despite the apparent absence of light limitation, however, rates of gross community production and chl *a* at Site PC were significantly less than at Site CF. In fact, Site CF lower had significantly greater rates of production than PC upper (1-way ANOVA, Fisher LSD Post-hoc analysis $p<0.05$). As such, a factor other than light appears to be the dominant control on biomass and gross community production.

Other factors that might influence gross community production between the two sites include salinity, nutrient availability, grazing pressure and physical disturbance. Salinity differences between Sites PC and CF are unlikely to have a negative affect on rates of gross community production as previous studies have either shown that salinity does not significantly affect MPB production on intertidal flats (Admiraal 1977; van Es 1982), or that it had a positive affect up to a salinity of 30 (Rasmussen et al. 1983). Nutrient availability is also unlikely to be limiting to gross community production, because NH_4^+ concentrations in the porewater were generally greater than those at Site CF; (Chapter 4). Low rates of grazing are generally accepted as creating a positive feedback in rates of gross community production, but above a certain threshold a negative feedback may occur (Andersen and Kristensen 1988). While macrofauna were not quantified, large numbers of crabs were observed at PC, compared to CF throughout the year. Pheophorbides were never detected in

significant quantities at either Site PC or CF (data not shown), which may indicate a lack of grazing pressure (Bianchi et al. 1988), although a recent study has suggested pheophorbides may be a poor indicator of grazing (Ford and Honeywill 2002). It is thus most likely that a factor other than those discussed above is exerting a controlling influence on gross community production by MPB.

Sediment resuspension may be an important factor controlling biomass and gross community production of MPB (MacIntyre et al. 1996) and it has previously been observed that gross community production was lower on a more exposed mudflat in the Bay of Fundy (Hargrave et al. 1983). The mudflat at Site PC has a southerly aspect and was thus much more exposed to the prevailing south westerly winds, than Site CF which had an easterly aspect. By using sediment grain size as a proxy for hydrodynamic energy, it has been found that biomass is generally lower in high energy environments where grain size is larger (de Jong and de Jonge 1995; Lucas and Holligan 1999). The sediments at Site PC were coarser than those at Site CF (Table 3.1), further suggesting that the mudflat at PC is a higher energy environment. It, therefore, seems most likely that the lower rates of gross community production and biomass at Site PC were caused by a greater exposure to resuspension events. This finding is, however, in contrast to those of Barranguet et al. (1997) and Sundbäck et al. (2000) who found that sediment type did not necessarily affect MPB biomass and productivity.

There was a significant relationship between hourly rates of gross community production and chl *a* content in the sediment (Figure 3.3) for the pooled data from both mudflats. A weak, but significant, relationship is generally observed between chl *a* and gross community production for MPB, with changes in chl *a* generally accounting for 30–40% of the variation in gross community production (MacIntyre et al. 1996). It has been suggested that the variation in this relationship is due to the inclusion of photosynthetically inactive biomass from deeper sediment layers in the chl *a* measurements (Underwood and Kromkamp 1999). As the data here was pooled from samples taken throughout the year, it is also likely that changes in assimilation number (production rate per unit of chlorophyll *a*) occurred in the photosynthetically active biomass. Changes in grazing pressure (Kristensen 1993)

and possibly also temperature and light intensity are likely to account for such changes in assimilation number.

Comparison with estuaries in other regions.

Despite the differences between the Huon Estuary and the more widely studied macrotidal European estuaries, the gross rates of production estimated from the TCO_2 flux data for the Huon Estuary (Table 3.2) were within the range of those reported for those estuaries (Underwood and Kromkamp 1999). The exception was for rates of gross community production measured at Site CF upper, which were slightly greater than rates reported from other intertidal sediments. The high rates of gross community production reported here may be because of a real difference, or possibly due to artefacts associated with different techniques used to measure gross community production between the different studies. The ^{14}C and bell-jar O_2 techniques generally employed may underestimate production (Epping and Jorgensen 1996; Underwood and Kromkamp 1999). Measurements of TCO_2 flux using the bell-jar technique are also subject to the problems associated with the bell-jar O_2 flux method, however, TCO_2 fluxes will not underestimate productivity to the same extent as the O_2 fluxes will (see later discussion – light, O_2 and TCO_2 stoichiometry).

Annual rates of production were estimated using a number of models outlined in the methods section, the results are presented in Table 3.2. Estimates of production made from the Chlorophyll and Maximium model were always greater than those estimated from TCO_2 flux data. Thornton et al (2002) also found these models to give much greater estimates of productivity than O_2 flux measurements. The Minimum and Regression models tended to give a lower estimate of gross community productivity that the TCO_2 flux data. However, Thornton et al. (2002) found that the Regression and the Minimum model were in close agreement with the data obtained from O_2 flux measurements. Gross community production on the lower mudflat at Site PC in particular, was five times greater than that predicted by the Regression model. At Site PC, the chl *a* concentrations were below the range of the data set analysed by Santos et al. (1997), and therefore, the applicability of the model to this site is questionable. While the estimates of production using the Regression model were also low compared to the TCO_2 model at Site CF, the rates of

production using the TCO_2 model were within variability of the data set analysed by Santos et al. (1997). Therefore, the relationship between chl *a* and annual production in the Huon Estuary is not dissimilar to that observed in other estuaries.

The regression equation of gross community production per unit chlorophyll *a* derived for hourly rates of production in the Huon Estuary (GPP = 32.5 + 0.44chl *a*) was also compared with published regression equations derived for Netarts Bay, Oregon, GPP = 9.57+0.35chl *a* (Davis and MacIntyre 1983), and the Ems Dollard Estuary in Europe GPP = 21.74+0.22chl *a* (Colijn and de Jonge 1984) (Figure 3.3). It can be seen that the slope of the regression of the Huon Estuary data is unlikely to be significantly different to those derived for Netarts Bay and the Ems Dollard estuary because they are within two standard errors of the gradient of the regression equation for the Huon Estuary. This suggests that regressions describing the relationship between chl *a* and gross hourly production from European and North American systems may be successfully applied to gain a first-order estimate of production in other disparate systems.

Comparing the estimated gross community productivity of the whole mudflat areas to that measured in other estuarine systems is somewhat more difficult due to the varying sizes of the estuary in question. Perhaps of more relevance is to compare the relative importance of community production by phytoplankton and MPB within a system. Here, primary production by phytoplankton in Port Cygnet and the upper estuary were estimated using the relationship derived by Cole and Cloern (1987) (Table 3.3). As the data for the regression equation derived by Cole and Cloern (1987) were taken under nutrient-replete conditions' one might expect this regression to give an overestimate of production in the Huon Estuary. For the upper Huon Estuary, the euphotic zone depth was calculated from data at Brabazon Park which is at the lower end of this section of the estuary, consequently, the euphotic zone depths used here are likely to be overestimates.

In the upper estuary, it was estimated that gross community production by MPB represented about 12% of the calculated average production in the upper Huon Estuary. In Port Cygnet, it was estimated that MPB represented only 2.5% of total production by phytoplankton (Table 3.3). These proportions of productivity by MPB

are low compared to other estuaries studied (Underwood and Kromkamp 1999), and stem primarily from the fact that intertidal areas in the upper estuary and Port Cygnet both represent a relatively small proportion of the estuary at approximately 5% of subtidal areas. Intertidal areas in the upper estuary, nevertheless, represented a significant proportion of production in this section of the estuary. This was due to a combination of high rates of production by MPB at Site CF and low calculated rates of production by phytoplankton in the upper estuary.

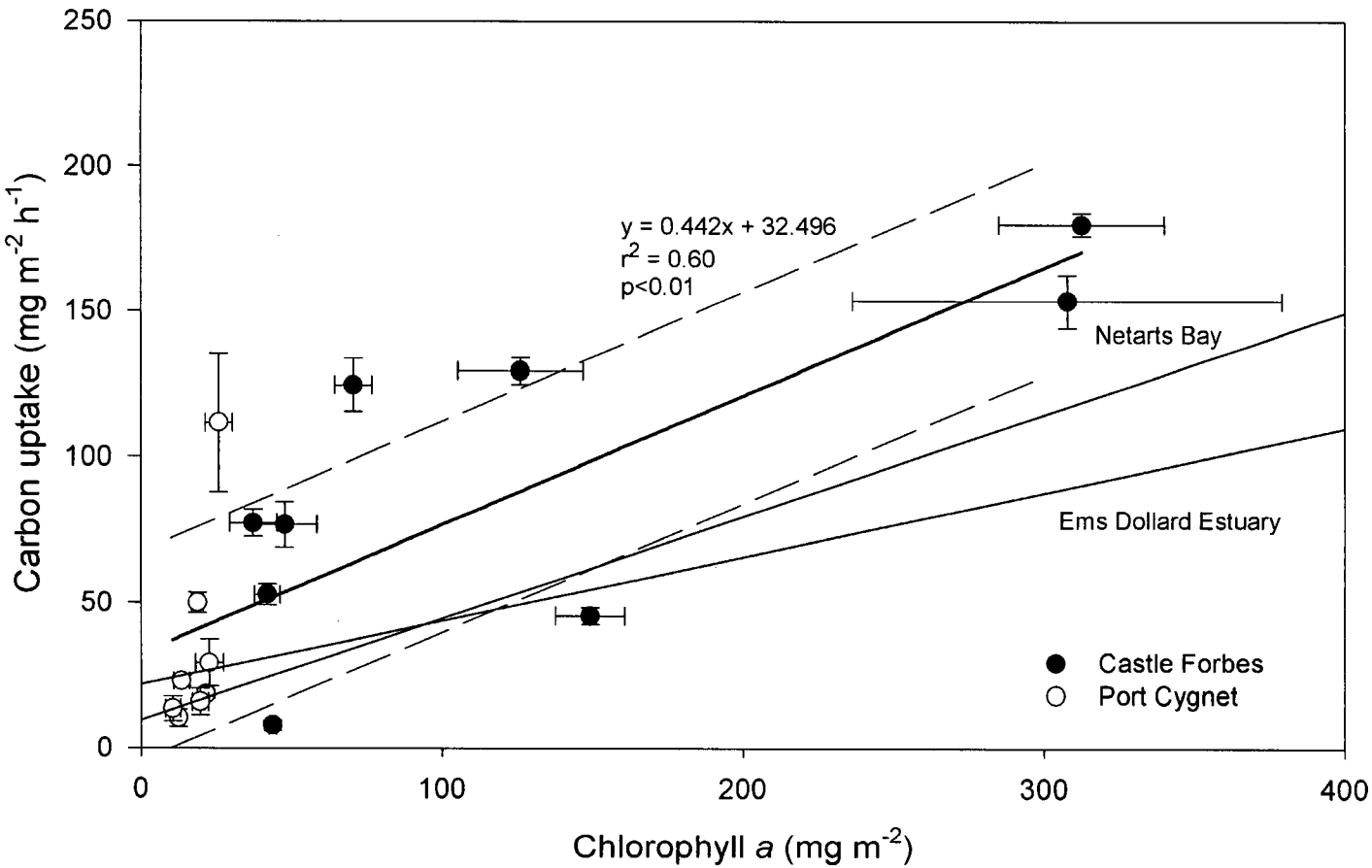


Figure 3.3. A plot of gross carbon uptake versus chlorophyll *a* at Port Cygnet and Castle Forbes Bay. The best fit line (thick) for the data is shown. Dashed lines represent the standard error of the y estimate. The best fit regression lines (thin) obtained from similar studies in Netarts Bay, Oregon (Davis and MacIntyre, 1983) and the Ems Dollard Estuary in Europe (Van Es, 1982) have been overlaid for comparison. Error bars are standard errors for replicate measurements (n=3–4).

Respiration

Temporal and spatial patterns, and controlling factors

The supply of organic matter and temperature are both likely to be important regulators of respiration rates within sediments. Organic matter inputs from MPB on intertidal flats have been shown to stimulate bacterial production (Cammen 1991), and a rapid and significant transfer of carbon derived from MPB into the sediment bacterial pool has also been demonstrated (Middelburg et al. 2000). In this study too, it is most likely that bacterial respiration was driven by organic matter derived from MPB as indicated the compound-specific stable isotope analysis of bacterial fatty acid presented in Chapter 2. It can be hard to distinguish the effects of temperature and organic matter supply from MPB, as the acivity of MPB is also controlled by temperature, although to a lesser extent than bacterial respiration (Davis and MacIntyre 1983; Kristensen 1993).

At Site PC, there was a relatively high and significant correlation between temperature and respiration (O_2 flux, $r = 0.86$, $p<0.01$) Multiple regression analysis showed that chl *a* and gross community production made no significant contribution to explaining the variability in sediment respiration rates suggesting that temperature was the major controlling factor on respiration in this system. This is in agreement with other observations from temperate systems (Kristensen 1993; Trimmer et al. 1998; Sundbäck et al. 2000). The fact that chl *a* did not contribute significantly to the variability observed at PC does not exclude MPB production from being an important supply of organic carbon for sediment respiration. Even with the relatively low rates of production observed at PC, MPB biomass was apparently the major source of fresh labile organic carbon to the sediments as suggested by the stable isotope ratios of bacterial fatty acids (See Chapter 2). The relatively constant biomass at Site PC throughout the year suggests a more constant rate of organic matter supply to the sediments and, hence, rates of respiration are governed by temperature to a greater extent.

At Site CF, temperature was more weakly correlated with respiration rates and the results of multiple regression analysis showed that sediment chl *a* content (an indicator of biomass) actually explained slightly more of the variability than temperature. While gross production was highly correlated with respiration it could

not explain a significant proportion of the variability in respiration when temperature and biomass were also considered in the multiple regression. As such, MPB biomass appears to be more important in controlling sediment respiration rates than rates of gross community production. It has previously been observed that bacterial biomass on intertidal flats is more closely related to MPB biomass than to rates of production. It was suggested that this was due to reliance by bacteria on extracellular organic material released by MPB, rather than consumption of algal cells (Cammen and Walker 1986). The stimulation of bacterial productivity by extracellular organic carbon (EOC) seems plausible given that it is highly labile (Goto et al. 2001; Köster and Meyer-Reil 2001) and that up to 73% of total carbon fixation in MPB may be excreted as EOC (Goto et al. 1999). Middelburg et al. (2000) also proposed that a direct link between bacteria and algae occurred via the production of EOC in order to explain the rapid transfer of freshly fixed ^{13}C from MPB into bacteria. In support of the contention that EOC was an important driver of respiration within the sediment in my study, strong correlations were observed between total bacterial fatty acids and indicators of live MPB biomass including total algal fatty acids and chl *a* (r=0.75 and r=0.67 respectively). Indicators of senescent algal biomass such as phytin a and phytol showed slightly weaker but also significant correlations with bacterial fatty acids (r=0.65 and r=0.58, respectively). This strongly suggests that both EOC and senescent algal cells were an important driver of bacterial respiration within the sediment.

Based on the above discussion, it is most likely that the significantly greater rates of respiration at Site CF upper are due to the greater amount of organic matter derived from MPB biomass at this site. Other possible causes of the observed differences in respiration rates may include: additional sources of organic matter other than MPB (e.g. wracks of seaweed) on the upper mudflat and warming of the upper mudflat during atmospheric exposures. Additional sources of organic matter other than MPB can be ruled out on the basis of observations and biomarker analysis (Chapter 2). Warming of the upper mudflat also seems unlikely given that no similar stimulation in respiration was observed on the mudflat at Site PC.

Comparison with other estuaries

A direct comparison of respiration rates is difficult as most previous studies have used sediment O_2 uptake as a measure of sediment respiration rates. When

respiration rates are high, increased rates of sulfate reduction will result in a decoupling between sediment remineralisation rates and O_2 uptake (e.g. Canfield and Marais 1993). This is exemplified during late spring at Site CF upper, when sediment O_2 uptake rates were only about a third of sediment respiration rates as measured by TCO_2 release. Eyre and Ferguson (2002) similarly observed that TCO_2 fluxes were well in excess of O_2 fluxes when sediment respiration rates were high. In contrast, Alongi et al. (1999) found that O_2 fluxes were generally in excess of TCO_2 fluxes by up to 4 times; this was ascribed to the reoxidation of reduced solutes. The measurement of O_2 fluxes are, therefore, likely to provide a poor measure of carbon remineralisation rates within sediments.

TCO_2 fluxes may also underestimate rates of carbon remineralisation, where rates of methanogenesis are high. However, Capone and Kiene (1988) state that methanogenesis is only likely to be a significant carbon oxidation pathway in freshwater systems. In agreement with this, studies have shown that methanogenesis was a significant carbon oxidation pathway in sediments where salinities were below ~10 (Middelburg et al. 1996a; Hopkinson et al. 1999).

Table 3.4 shows the range of sediment respiration rates measured as O_2 uptake and TCO_2 release across a range of marine aquatic systems. When compared with other temperate intertidal systems, it can be seen that sediment O_2 uptake rates in the Huon Estuary are generally in the lower range of sediment respiration rates measured. This is most likely a result of the greater particulate loads from anthropogenically derived terrestrial run off entering the estuaries in the other studies. Few studies have directly measured TCO_2 fluxes in intertidal sediments, but the rates of TCO_2 production in the Huon Estuary are comparable to those measured in Flax Pond Marsh on Long Island. Most of the organic matter deposited at this American site was derived from MPB and Spartina detritus (Mackin and Swider 1989), rather than anthropogenic sources; so one might expect to see similar respiration rates in these two systems.

Despite the much warmer temperatures in the tropics, sediment respiration rates as measured by TCO_2 efflux are generally much lower in mangrove sediments than those measured in the Huon Estuary and other temperate intertidal sediments. The

generally lower respiration rates measured in mangrove sediments when compared to those in temperate systems may be ascribed to a limited supply of labile organic carbon. This situation was caused by low rates of MPB production as well as the high C:N ratio of mangrove-derived detritus (Alongi 1994; Alongi et al. 1999).

The data set presented here generally showed that respiration rates in temperate intertidal sediments were in the upper range of sediment TCO_2 release rates compared to other temperate subtidal sediments. On this basis, it might be speculated that respiration rates within temperate intertidal sediments are generally high compared to subtidal coastal and estuarine sediments as a result of high rates of organic matter deposition derived from terrestrial, estuarine sources as well as MPB. Given the limited data set and the different methods used to measure respiration rates within intertidal sediments, it would seem premature to make comparisons of remineralisation rates in intertidal compared to sub-tidal systems.

Table 3.4. Summary of benthic O_2 and TCO_2 fluxes reported in intertidal and subtidal sediments from a range of systems (mmol m^{-2} day^{-1})

Location	System	O_2	TCO_2	Reference
N Queensland, Australia	Tropical Intertidal/ mangrove	2.8–61	1.8–21.9	(Alongi et al. 1999)
Falmouth Harbour, Jamaica	Tropical Mangrove	38–62	24–241	(Nedwell et al. 1994)
NW Malaysia	Tropical Mangrove	11.5–21.4	8.9–20.9	(Alongi et al. 1998)
Western Australia	Tropical Mangrove		28.5–52.9	(Alongi et al. 2000)
Average		**17–48**	**16–84**	
Huon Estuary, Tasmania, Australia	Temperate intertidal	12–62	9–165	This Study
Colne Estuary, UK	Temperate intertidal	24–192		(Dong et al. 2000)
Tagus Estuary, Portugal	Temperate intertidal	6–120		(Cabrita and Brotas 2000)
Westerschelde Estuary, SW Netherlands	Temperate intertidal		0–1200	(Middelburg et al. 1996a)
Great Ouse Estuary, UK	Temperate intertidal	12–168		(Trimmer et al. 1998)
Long Island, New York	Temperate intertidal	23–137	33–160	(Mackin and Swider 1989)
Bay of Fundy, Canada	Cold-temperate intertidal	2–40		(Hargrave et al. 1983)
Average		**13–119**	**14–508**	
White Oak River Estuary, North Carolina	Temperate estuarine		12–48	(Kelley et al. 1990)
Parker River Estuary, Massachusetts	Temperate estuarine	13–130	21–170	(Hopkinson et al. 1999)
Chesapeake Bay, Eastern USA	Temperate Estuarine	31–93		(Boynton and Kemp 1985)
Galveston Bay, Texas	Subtropical Estuarine	0.9–10	0.06–0.6	(Zimmerman and Benner 1994)
Average		**15–78**	**11–73**	
Svalbard, Norway	Arctic, coastal		11–24	(Kostka et al. 1999)
Disko Bay, West Greenland	Arctic coastal	8–14	6.8–13	(Rysgaard et al. 2000)
Continental shelf, Massachusetts	Temperate coastal	10.6–14.3	12.1–29.8	(Hopkinson et al. 2001)
Boston Harbour, Massachusetts	Temperate coastal	10–200	10–200	(Giblin et al. 1997)
Port Phillip Bay, Victoria, Australia	Temperate coastal	19–102	18–120	(Berelson et al. 1998)
Northern Adriatic Sea	Temperate coastal	15–18	20–22	(Hammond et al. 1999)
Average		**15–83**	**15–93**	
New South Wales, Australia	Warm temperate lagoon	12–24	12–48	(Eyre and Ferguson 2002)
Island of Fyn, Denmark	Cool Temperate Lagoon	7–80	7–80	(Kristensen 1993)

Trophic status

The trophic status (heterotrophy versus autotrophy) of a system can provide a useful tool for assessing ecosystem stability (de Wit et al. 2001), the likely effect of a benthic system on water quality (Rizzo et al. 1996) and nutrient cycling (Eyre and Ferguson 2002). A number of indices have been used to classify the status of benthic production *vs* respiration. The benthic trophic state index (BTSI) (Rizzo et al. 1996) and the trophic oxygen state indicator (TOSI) (Viaroli et al. 1996), both give an indication of the balance between production and respiration. Here, I have applied these indices to the TCO_2 data instead of the O_2 data, as TCO_2 is likely to more accurately reflect actual rates of production and respiration (see later discussion). Applying these indices (Figure. 3.4), it can be seen that Site CF sediments were generally highly autotrophic (BTSI 3 region), while the Site PC sediments ranged from being net heterotrophic to highly autotrophic (BTSI 1–3). TOSI and BTSI do not take into account the trophic status over a diurnal cycle, therefore, a sediment that appears to be autotrophic on the basis of these indices may in fact be net heterotrophic over a diurnal cycle when the day length is short.

More traditional P:R ratios as applied by Eyre and Ferguson (2002) give an indication of the tropic status of the sediments over a diurnal cycle and may thus be more ecologically relevant, especially in more temperate latitudes where day length can vary greatly between winter to summer. The extrapolation of data measured on an hourly scale (as is the case here) up to diurnal and annual scales is, however, subject to large uncertainties (Rizzo et al. 1996). Figure 3.4a shows a plot of daily respiration vs daily production. Daily respiration was simply calculated as hourly dark TCO_2 flux multiplied by 24 h. Daily production was calculated as hourly gross production multiplied by the photosynthetic day length (see methods). Using P:R ratios, the sediments are classified as either net heterotrophic (where $P:R<1$) or net autotrophic (where $P:R>1$). For this data set, all sediments with a classification of highly autotrophic (BTSI 3) were also classified as net autotrophic using P:R ratios. Sediments classified as net heterotrophic and net autotrophic (BTSI 1,2), were net heterotrophic using P:R ratios. As such, it appears that the two approaches result in similar trophic classifications in this instance.

Rizzo et al. (1996) investigated a range of factors affecting BTSI and came to the conclusion that the most autotrophic sediments were the coarser sandy sediments. It was suggested that this was primarily due to increased respiration rates in the more organic-rich, finer sediments. Yet, Sundbäck et al. (2000) found no clear differences in the trophic status of a silty and sandy site. In the current study, it was found that the most autotrophic sediments were generally those at Site CF, which were the more fine and organic-rich (Chapter 2 Figure 2.1) sediments. As discussed earlier, the most likely cause of the greater rates of productivity observed at Site CF is its more sheltered position. I believe that hydrodynamic energy is a key factor controlling the balance between production and respiration in this system.

On an annual basis, the upper mudflat at CF remineralised 250 g C m^{-2} compared to an annual production of 340 g C m^{-2}. While on the lower mudflat, 110 g m^{-2} of carbon was remineralised compared to 150 g Cm^{-2} produced (Table 3.2). As such, both the upper and lower mudflats at Site CF were net producers of carbon. Despite the fact that the mudflat at Site CF was autotrophic, only a small fraction of carbon within the sediment was derived from MPB (Chapter 2). Therefore, burial of the unmetabolised carbon would seem an unlikely fate, rather, an export to the estuary - possibly via grazing and resuspension of MPB - is a more likely outcome. At Site PC, it was estimated that 150 g Cm^{-2} was remineralised on an annual basis compared to 83 g Cm^{-2} produced. On the lower mudflat, 130 g Cm^{-2} was remineralised compared to 105 g Cm^{-2} produced (Table 3.2). Consequently, both the upper and lower mudflat were net heterotrophic on an annual basis.

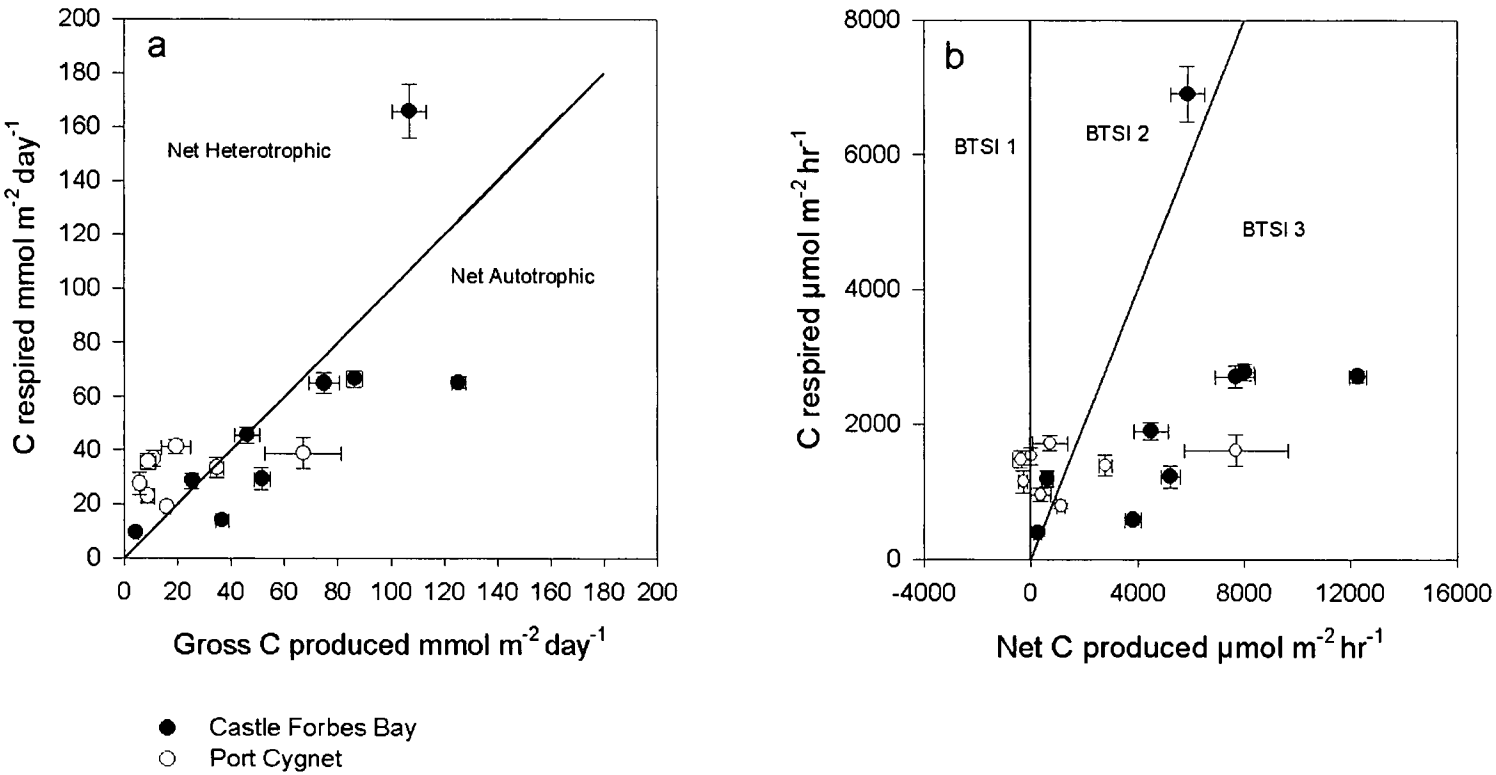


Figure 3.4. **(a)** The balance of respiration and primary production at the two study sites estimated on a daily basis using the effective photoperiod as calculated in the methods section. **(b)** A plot of dark TCO_2 flux versus light TCO_2 flux as applied by Viaroli et al, (1996) to oxygen fluxes, over the study period at Castle Forbes Bay and Port Cygnet. The Benthic Trophic State Index (BTSI) as applied by Rizzo et al (1996) is marked on plot b. BTSI 1–sediments net heterotrophic, BTSI 2–sediments net autotrophic, BTSI 3–sediments highly autotrophic. Error bars are standard errors for replicate measurements (n=3–4)

Light O_2 and TCO_2 stoichiometry

The use of both O_2 and TCO_2 fluxes in this study has allowed two estimates of production using the net flux or "bell jar" technique. The average production quotient (PQ) for Sites PC and CF were very similar to the value of 0.87 reported by Kristensen (1993) using the same methodology, but were well below the ratio of 1.1–1.4 expected for phytoplankton assimilating NH_4^+ and NO_3^-, respectively (Laws 1991) (Figure 3.5a). Recent studies have generally assumed a PQ of 1.2 for MPB (Cabrita and Brotas 2000; Sundbäck and Miles 2000). For sediment systems, it seems reasonable to assume most of the nitrogen is assimilated in the form of NH_4^+, recycled within the sediment, especially during times of low nutrient availability in the water column (Kristensen 1993; Sundbäck et al. 2000). In this system, it was found that NO_3^- generally only made up a small fraction of nitrogen assimilated by MPB (see Chapter 5) hence a PQ of 1.1–1.2 seems reasonable under such conditions.

The average PQ value, however, was less than 1 at both sites and most likely further highlights the limitation of measuring gross sediment production using direct fluxes. Measuring gross production in this way assumes that respiration rates in the dark will be the same as those in the light. This has been shown not to be the case in intertidal sediments (Epping and Jorgensen 1996), and is most likely a consequence of increased respiration by both algae and bacteria as well as increased chemical oxygen demand (Epping and Jorgensen 1996; Epping et al. 1999; Fenchel and Glud 2000). Increased respiration by algae and bacteria in the light will not significantly alter the ratio of O_2 released to TCO_2 consumed, or appreciably alter alkalinity fluxes. Therefore, it is not possible to make an estimate of the magnitude of the increased rates of respiration using this data set. The reoxidation of Fe^{2+}, FeS and HS^- will consume alkalinity and O_2 without producing TCO_2. Removal of TCO_2 and increases in pH caused by photosynthesis may promote precipitation of $CaCO_3$ (Berner 1971), consuming TCO_2 and alkalinity, without affecting O_2 fluxes. Thus, carbonate precipitation and the reoxidation of reduced solutes are both possible explanations for the observed negative alkalinity fluxes and low PQ.

In order to estimate the likely magnitude of $CaCO_3$ precipitation and sulfide oxidation, gross fluxes of O_2 and TCO_2 were corrected using alkalinity data as follows. The alkalinity fluxes in the dark and light were first corrected by adding the

sediment NO_3^- flux and subtracting the NH_4^+ flux (Berelson et al 1996). Primary production by "Redfield-type" algae assimilating NH_4^+ will reduce alkalinity by 14 eq for every 106 moles of TCO_2 consumed. This alkalinity flux attributable to photosynthesis (calculated as gross TCO_2 flux × 14/106) was also subtracted from the alkalinity flux measured in the light. In summary:

$$Alk_{corD} = JAlk_{measD} + JNO_3^-{}_D - JNH_4^+{}_D$$

$$Alk_{corL} = Jalk_{measL} + JNO_3^-{}_L - JNH_4^+{}_L - (TCO_{2G}*14/106)$$

Where

Alk_{cor} = corrected alkalinity flux
$Jalk_{meas}$ = the measured alkalinty flux
JNO_3^- and JNH_4^+ = the fluxes of NO_3^- and NH_4^+
TCO_{2G} = gross community production.
L and D refer to light and dark.

It was assumed that processes giving rise to positive alkalinity fluxes in the light continued during the dark (see later discussion). Gross alkalinity fluxes in the light were estimated by subtracting the dark alkalinity fluxes from the light fluxes.

$$Alk_{corG} = Alk_{corL} - Alk_{corD}$$

Where Alk_{corG} is the gross alkalinity flux in the light.

Alk_{corG} is either attributable to carbonate precipitation or to the oxidation of reduced species such as HS^-, FeS_2 and Fe^{2+}. The amount of O_2 consumed in relation to alkalinity consumed will depend upon the stoichiometry of the oxidation reaction. Here I have considered two scenarios, the oxidation of FeS_2 as suggested by Fenchel and Glud (2000) and the precipitation $CaCO_3$. For the sulfide oxidation scenario, the O_2 fluxes were corrected assuming sulfide oxidation via the following equation (Stumm and Morgan 1996).

$FeS_2(s) + 15/4O_2 + 3\ ½\ H_2O \rightarrow Fe(OH)_3\ (s) + 4H^+ + 2SO_4^{2-}$

The O_2 flux was then corrected as $O_2 - (0.92 \times Alk_{corG})$. For the carbonate precipitation scenario, TCO_2 fluxes were corrected for $CaCO_3$ precipitation as $TCO_2corr = TCO_{2G} - (0.5 \times Alk_{corG})$ (Berelson et al. 1996).

Looking at Figure 3.5b and c it can be seen that the data corrected for either $CaCO_3$ precipitation or sulfide oxidation fall closer to the expected O_2:TCO_2 of 1.1–1.2:1 than the uncorrected data. The data corrected for $CaCO_3$ precipitation on average had a PQ of 0.88 as indicated by the gradient of the best fit line. The data corrected assuming sulfide oxidation on average, fell slightly below the expected PQ range with the gradient of the best fit line having a gradient of 1.06. Correcting the data for sulfide oxidation also resulted in a tighter coupling between between O_2 production and TCO_2 uptake as indicated by the greater correlation coefficient (r=0.98) than for the uncorrected and $CaCO_3$ corrected data (r=0.95). On the evidence, it seems most likely that the dominant process decoupling O_2 and TCO_2 fluxes is the reoxidation of reduced sulfides within the sediments during illumination. This does not preclude the possibility of calcium carbonate precipitation occurring to a limited extent. It is, however, unlikely that the observed alkalinity fluxes were being driven solely by $CaCO_3$ precipitation. The finding that sulfide oxidation in the light was the most likely process giving rise to the excess of TCO_2 consumed over O_2 produced, further highlights the fact that direct O_2 flux measurements in sediment systems will underestimate gross production. Measuring TCO_2 fluxes may give a better estimate, if re-oxidation of reduced solutes is the major oxygen consuming process which increases during illumination.

The assumption that the processes giving rise to the alkalinity flux in the dark continue during the light requires some further consideration. If the processes giving rise to the positive alkalinity fluxes was sulfate reduction, then this assumption seems reasonable as the bulk of sulfate reduction will predominantly occur below the top two cm of sediment (e.g. Alongi et al. 2000). O_2 production by MPB is unlikely to penetrate below this depth, and hence, sulfate reduction rates within the sediments will proceed essentially unaltered whether or not the sediments are illuminated. This assumption is, however, questionable for $CaCO_3$ precipitation and dissolution.

Anderson et al. (1986) found that $CaCO_3$ dissolution occurred at the sediment-water interface. If this were the case here, it seems unlikely to continue to occur during illumination because of the high pH likely to be encountered at the sediment surface (de Jong et al. 1988). Once again the TCO_2 flux was corrected for $CaCO_3$ precipitation, except this time using the Alk_{corL} flux instead of the Alk_{corG} flux to correct for $CaCO_3$ precipitation. An even lower PQ of 0.79 was obtained by performing the calculation in this manner. Thus, the conclusions remain essentially unchanged, since the PQ must be one or greater it appears $CaCO_3$ precipitation is not occurring to a great extent in the light.

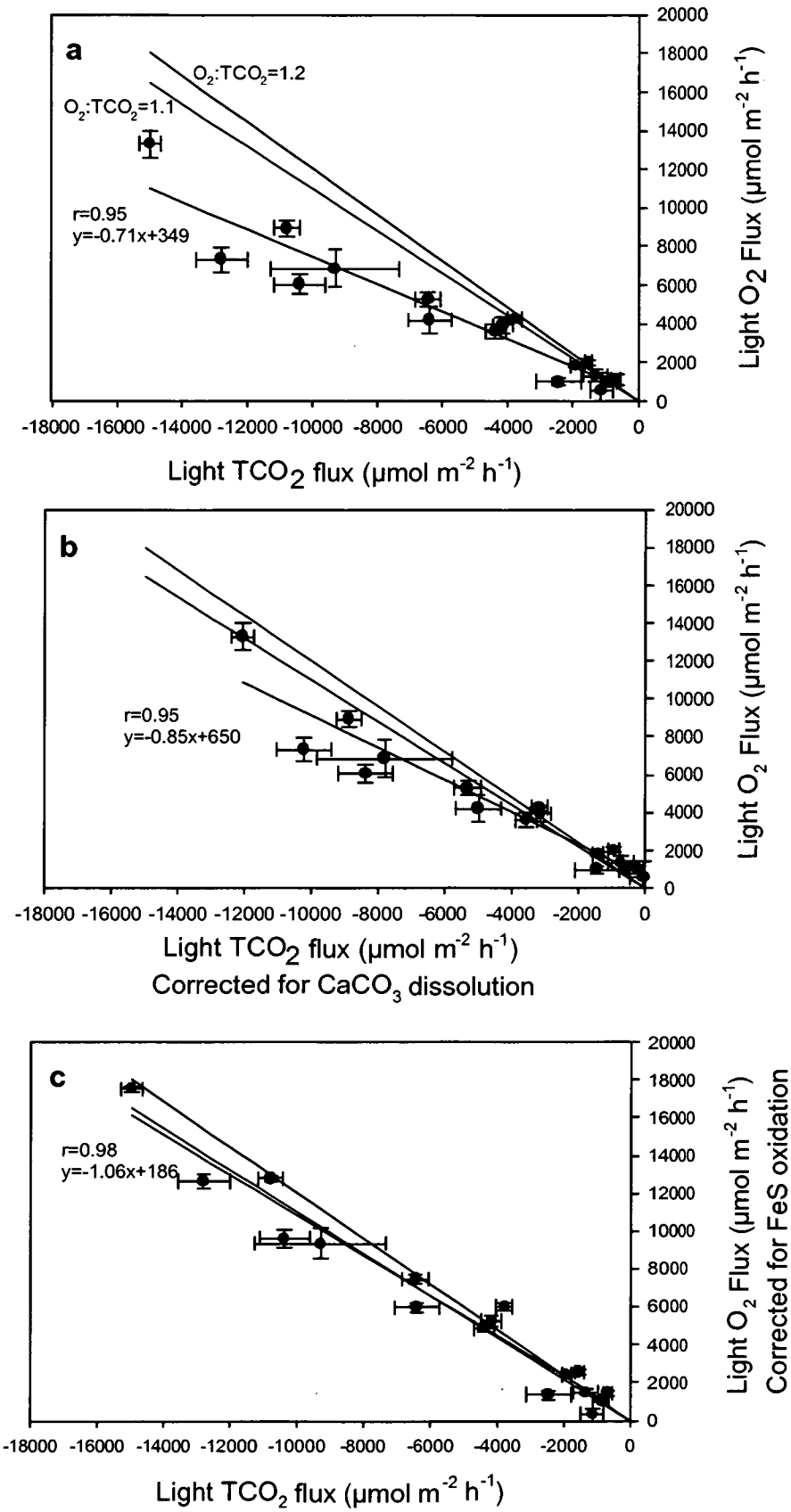


Figure 3.5. **(a)** A plot of gross light O_2 flux versus gross light TCO_2 flux, **(b)** gross light O_2 flux versus gross light TCO_2 flux corrected for $CaCO_3$ dissolution and **(c)** gross light O_2 flux corrected for FeS oxidation versus gross light TCO_2 flux. Data shown are from the two study sites over the course of the year. Error bars are standard errors for replicate measurements (n=3–4)

Dark O_2 and TCO_2 stoichiometry.

The ratio of TCO_2 produced to O_2 consumed will depend upon the organic matter undergoing remineralisation. Anderson et al. (1986) calculated the theoretical TCO_2:O_2 ratios for a range of different types of organic matter undergoing decomposition would fall within the range of 0.63–1.08. In Figure 3.6a it can be seen that at high sediment respiration rates, the TCO_2:O_2 ratio was often above this expected range. Anaerobic respiration and $CaCO_3$ dissolution are both plausible explanations for the excess of TCO_2 produced compared to O_2 consumed. Positive alkalinity fluxes are also consistent with these explanations (Berelson et al. 1998).

Once again alkalinity data were used to correct the observed TCO_2 and O_2 fluxes in order to gain an insight into the likely processes affecting the TCO_2:O_2 flux ratios. Two possible scenarios have been considered here. First, the entire alkalinity flux was assumed to be due to $CaCO_3$ dissolution, dark TCO_2 flux data were corrected accordingly as follows $TCO_{2Cacor} = TCO_2 - (0.5 \times Alk_{corD})$. Second, the entire alkalinity flux was assumed to be due to sulfate reduction and subsequent precipitation of iron sulphides, the data were corrected as follows $TCO_{2Scor}=TCO_2-(0.82\times Alk_{corD})$ (Berelson et al. 1998). On a number of occasions negative alkalinity fluxes were observed in the dark, in these cases it was assumed that a net oxidation of HS^- or precipitation of $CaCO_3$ was taking place. For the HS^- oxidation scenario the sediment O_2 consumption was corrected as $O_{2Scor} = O_2 - (0.92 \times Alkcor)$. Figures 3.6b and c show plots of the corrected dark O_2 and TCO_2 fluxes. When the TCO_2:O_2 ratio is highest, it can be seen that an alkalinity correction assuming sulfate reduction brings the TCO_2:O_2 ratio much closer to the expected range than does correction for $CaCO_3$ precipitation. Thus, it seems likely that sulfate reduction was driving the alkalinity fluxes in these sediments. In sediments where the observed TCO_2:O_2 ratio is close to that expected for the oxidation of organic matter however, the assumption that the alkalinity flux is driven by sulfate reduction results in an apparent TCO_2:O_2 ratio below theoretical expectations, suggesting that $CaCO_3$ dissolution may be driving the alkalinity fluxes in these instances. This generally occurred in sediments with the lowest TCO_2 production rates. The preceding discussion suggests that at high sediment respiration rates sulfate reduction will make a large contribution to total respiration. Under such conditions there may be a build up of reduced sulfides within the sediment. These reduced sulfides may then be reoxidised during the

illumination period, resulting in a decoupling of the O_2 and TCO_2 fluxes (Fenchel and Glud 2000).

Summary

Rates of gross community production were significantly greater on the upper mudflat at Site CF. This difference was most likely caused by the presence of high concentrations of CDOM in the water, which severely limited light penetration across the inundation gradient. At Site PC, no significant difference was observed between the upper and the lower mudflat. It is proposed that gross community production at Site PC was limited by an exposure to greater wave energy compared to Site CF as indicated by aspect and sediment grain size. On an annual basis, the mudflat at Site CF was net autotrophic and it was estimated that gross community production on the mudflats in the upper estuary were about 12% of that occurring in the water column. By contrast the mudflat at Site PC was net heterotrophic and it was calculated that mudflats in the Port Cygnet only accounted for 2.5% of primary production in the water column.

Benthic respiration rates at Site CF were controlled primarily by temperature and algal biomass. Rates of gross community production could only explain a small amount of the variability in the observed sediment respiration rates. As such, it is suggested that sediment respiration at this site was driven by the production of extracellular organic carbon to a greater extent than the consumption of senescent algal cells.

The use of both O_2 and TCO_2 fluxes to measure benthic community production and respiration showed that oxygen fluxes may underestimate rates of gross community production and respiration, particularly, where rates of both these processes are high. During illumination, the reduced oxygen efflux from the sediment compared to TCO_2 uptake was most likely caused by a reoxidation of reduced sulfides within the sediment. In the dark, the production of TCO_2 in excess of O_2 consumed at high respiration rates was most likely caused by a net reduction of sulfate.

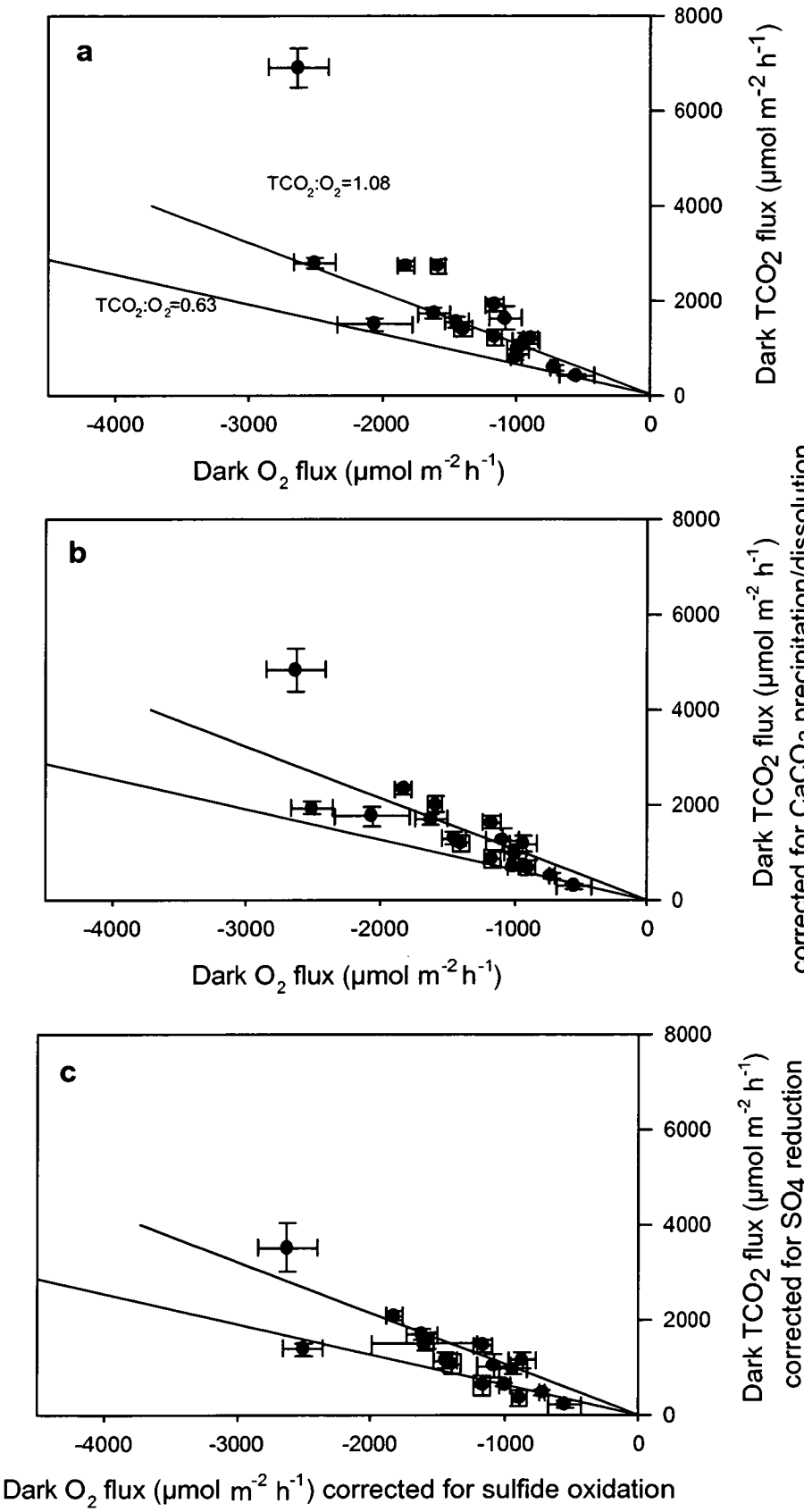


Figure 3.6. **(a)** A plot of dark TCO_2 flux versus dark O_2 flux, **(b)** dark TCO_2 flux corrected for $CaCO_3$ precipitation and dissolution versus dark O_2 flux, and **(c)** dark TCO_2 flux corrected for sulfate reduction versus dark O_2 flux corrected for sulfide oxidation. Data shown are from the two study sites over the course of the year. Error bars are standard errors for replicate measurements (n=3–4)

Chapter 4

Nitrogen remineralisation on intertidal mudflats in a temperate Australian estuary

Abstract

Benthic fluxes of dissolved nitrogen, rates of denitrification as well as NH_4^+ production within the sediment were measured on the upper and lower mudflats at two study sites in the Huon Estuary. Rates of these nitrogen cycling processes were considered with regard to benthic metabolism data presented in Chapter 3. Estimated rates of NH_4^+ production within the sediment were generally in excess of measured benthic fluxes, suggesting that NH_4^+ was reassimilated at the sediment surface by microphytobenthos (MPB). The ratio of TCO_2:NH_4^+ produced within the sediment was generally in excess of 15 and in some cases in excess of 60. It is suggested this was because the organic matter undergoing remineralisation had a high C:N ratio and that reassimilation of nitrogen by sediment bacteria was occurring. Benthic metabolism was found to be a key parameter controlling benthic fluxes of dissolved inorganic nitrogen (DIN), with an uptake of DIN being observed under autotrophic conditions and a release under heterotrophic conditions. Significant fluxes of dissolved organic nitrogen (DON) were measured where the activity of MPB was highest. At times, DON fluxes were well in excess of DIN fluxes highlighting the importance of measuring DON fluxes where the activity of MPB is high. Rates of denitrification were very low, and represented only a small loss of nitrogen compared to NH_4^+ production rates in the sediment. Fluxes of NO_3^- into the sediment were generally well in excess of uncoupled denitrification (D_w), indicating that most of the NO_3^- taken up by the sediment was assimilated by MPB. The activity of MPB had a significant negative impact on denitrification, most likely caused by competition for nitrogen between nitrifying / denitrifying bacteria and MPB.

Introduction

Mudflats are important zones of organic matter accumulation in coastal systems owing to their sheltered nature (Jickells and Rae 1997). The remineralisation of organic matter deposited within these zones results in the release of inorganic nitrogen and phosphorus to coastal waters (Rocha et al. 1995; Falcao and Vale 1998; Rocha 1998). In situations where a large fraction of this remineralisation proceeds via denitrification there may be a net loss of nitrogen from the system (e.g. Dong et al. 2000). As such, mudflats may act as both sources and sinks of dissolved inorganic nitrogen. Recently, much attention has been focused on the role of intertidal sediments in attenuating high inorganic nitrogen loads from the land to the sea in temperate European estuaries (Middelburg et al. 1995b; Ogilvie et al. 1997; Trimmer et al. 1998; Cabrita and Brotas 2000; Dong et al. 2000; Trimmer et al. 2000b; Magalhaes et al. 2002). High rates of denitrification are often observed in these estuaries driven by both NO_3^- from the water column (D_w) and from coupled denitrification of NH_4^+ produced within the sediment (D_n). In European estuaries, mudflats have been found to attenuate terrestrially derived dissolved inorganic nitrogen (DIN) loads by between 3 and 25% (Cabrita and Brotas 2000; Dong et al. 2000; Trimmer et al. 2000a).

Microphytobenthos (MPB) are ubiquitous on mudflats and may exert an important influence over nitrogen cycling processes in these environments (Underwood and Kromkamp 1999). Assimilation of nitrogen by MPB means that effluxes of NH_4^+ and NO_3^- may be drastically reduced or even reversed in the light (Sundbäck et al. 1991; Cabrita and Brotas 2000; Magalhaes et al. 2002). In addition, MPB have been found to compete effectively with nitrifying/denitrifying bacteria for NO_3^- and NH_4^+. Assimilation:denitrification ratios of between 2 and 10 have been reported on eutrophic mudflats, whilst ratios of between 7 and >100 have been reported in more oligotrophic sediments (Cabrita and Brotas 2000; Dong et al. 2000; Sundbäck and Miles 2000). This is significant as it suggests a far greater proportion of nitrogen remineralised within the sediments will be assimilated rather than denitrified, resulting in a retention of bioavailable nitrogen within the system. Recent studies have shown that dissolved organic nitrogen (DON) fluxes may exceed that of dissolved inorganic nitrogen (DIN) in the presence of MPB, suggesting that a significant fraction of nitrogen assimilated by MPB may be returned to the water

column as DON (Sundbäck et al. 2000; Eyre and Ferguson 2002). The production of oxygen by MPB may also significantly alter the rates of coupled and uncoupled denitrification. The increased oxygen penetration into the sediment during illumination will increase the diffusional path for NO_3^- from the water column to the denitrification zone resulting in decreased rates of uncoupled denitrification (D_w). Conversely increased O_2 penetration into the sediments may increase nitrification rates resulting in an increase in coupled denitrification (D_n) (Risgaard-Pedersen et al. 1994; Rysgaard et al. 1995). Any studies of nitrogen cycling on mudflats must, therefore, give due consideration to the central role MPB play in the cycling of nitrogen in these environments.

To date, all studies of benthic nutrient cycling on temperate tidal flats have been conducted in European systems that have generally experienced severe eutrophication (Middelburg et al. 1995a; Middelburg et al. 1995b; Ogilvie et al. 1997; Trimmer et al. 1998; Cabrita and Brotas 2000; Dong et al. 2000; Trimmer et al. 2000b). There is a lack of information on benthic nutrient processing in more oligotrophic estuaries (Nedwell et al. 1999). It has been proposed that prior to enrichment that European intertidal flats were net importers of nutrients from the sea, with tight recycling and storage of nutrients enabling these systems to support significant secondary production (Malcolm and Sivyer 1997). In order to further investigate this hypothesis, it is necessary to obtain data from temperate oligotrophic systems. Furthermore, the currently emerging conceptual understanding of nitrogen cycling from more eutrophic European systems may not be appropriate to more oligotrophic systems such as those found in Australia (Harris 2001; Ferguson 2002). Here, the results of a study into benthic nutrient cycling on two intertidal mudflats in a cool temperate mesotrophic Australian estuary are presented. In particular, I studied the role of MPB in controlling nitrogen cycling processes such as denitrification and dissolved organic and inorganic nitrogen fluxes.

Methods

Sampling

A description of the study sites is given in Chapter 1. Samples were collected at Castle Forbes Bay (Site CF) in March, June, September and December and during April, June, September and December at Port Cygnet (Site PC) during 2001. An additional sampling was undertaken in November 2000 at Site CF upper, for which the data have been presented. To facilitate comparison with other measurements, March and April are referred to as autumn. June, September, November and December are referred to as winter, early spring, late spring and summer, respectively. Four intact sediment cores (25 x 14.5 cm i.d.) were taken at each site as described in Chapter 1. The cores were then placed in a padded box and returned to the laboratory within 1 h. Samples for nutrient analysis were collected in 10-ml polypropylene screw cap vials after being filtered through a pre-combusted Whatman GF/F filter; samples were frozen for analysis within 2 h of collection. Upon return to the laboratory, the cores were submerged in a water bath of site water (160 l) at in-situ temperature and incubated as described in Chapter 3. While this methodology may not accurately reflect in-situ exchange rates it does allow for some of the controlling factors and interactions between benthic microbial metabolism and nutrient fluxes to be evaluated (Asmus et al. 1998). This was the aim of this study. After flux experiments had finished, 1–2 intact subcores were taken from each core for isotope-pairing measurements. The sediment remaining in the cores was sampled for chlorophyll *a* (chl *a*) and microbial biomass nitrogen. After the top 0.5cm of sediment had been removed for chl *a* and pigment analysis (data presented in Chapters 2 and 3), the 0.5–3.5 cm sediment horizon was sampled using a cut-off syringe barrel for "microbial biomass nitrogen". Sediment samples for microbial biomass nitrogen were stored in the refrigerator for up to 3 days before determination.

Porewaters

Cores for porewater analysis were taken in triplicate from the upper and lower mudflat at each site during April, August and late November 2001, and are referred to as autumn, winter and summer, respectively. An additional core was also taken at each site in April to measure porosity. Sediment porosity was determined according to Dalsgaard (2000) at 1 cm intervals to a depth of 6 cm. For porewater extractions,

cores were rapidly sectioned under atmospheric conditions at 0.5-cm intervals from 0–2 cm, 1-cm intervals from 2–4 cm, and 2-cm intervals from 4–8 cm. Slices were placed in a centrifuge tube under a stream of Ar and then sealed. The sediment was centrifuged at 2000 rpm for 10–15 mins. The centrifuge tubes were then placed in an Ar-purged glove box and filtered through Whatman GF/F filters. The filtrates were then frozen for later analysis of NH_4^+. Total nitrogen was also measured in the porewaters in cores taken during April and November. After NH_4^+ analysis, the porewaters from each depth interval were pooled for the three replicate cores for the analysis of total nitrogen. Preliminary experiments showed that NO_2^- and NO_3^- were not detected in the porewaters, suggesting that there were no artefacts from the oxidation of NH_4^+, and that net nitrification was not occurring within the sediment to a significant extent. Porosity was determined according to Dalsgaard (2000). In January, a core was taken from Sites CF and PC upper for a profile of microbial N.

Extraction of "microbial biomass nitrogen"

Microbial biomass nitrogen (hereafter referred to as microbial nitrogen) was determined according to Joergensen (1995), and the method is described briefly as follows. Sediment samples were first homogenised before two 10-g samples were taken. One sample was extracted straight away for total nitrogen using 40 ml of 0.5 M K_2SO_4. After shaking for 30 mins, the sample was placed in a refrigerator overnight to settle. The sample was then filtered under pressure through a pre-combusted Whatman GF/F filter. The nitrogen extracted in this way is hereafter referred to as free total nitrogen.

The second sediment sample was fumigated as follows. Samples were placed in a desiccator lined with moist paper towelling. 20 ml of ethanol-free chloroform was placed in the desiccator, as well as an open beaker of sodium hydroxide. The desiccator was then sealed and evacuated. It was then left for 24 h at ~20°C. The sediment samples were then extracted as described above. Total nitrogen in each sample was determined as NO_3^- after persulfate digestion. Microbial nitrogen was calculated as the difference between the concentration of total nitrogen in the fumigated and unfumigated (free total nitrogen) extracts.

Dissolved nutrient exchange and denitrification

Rates of nutrient exchange in the light and dark as well as dissolved oxygen and TCO_2 (data presented in chapter 3) were measured on the two days following sampling and coincided with the period of in-situ low tide. Cores were illuminated at 500 μE m^{-2} s^{-1} using a 50 W halogen lamp placed above each core. Before the commencement of flux measurements the cores were illuminated for approx ~1 h, flushed with fresh site water, and capped. Rates of O_2, TCO_2 and nutrient exchange were similarly made in the dark after flushing the cores with fresh site water. Four samples were taken over time for nutrient and alkalinity determinations. A "blank" core containing only water was also incubated and sampled in an identical manner to the sediment cores. Water samples taken for nutrients and alkalinity were filtered through a precombusted Whatman GF/F filter into 10 ml screw cap polypropylene containers. They were stored frozen for later analysis within three months for DIN and within six months for total nitrogen with the exception of porewater samples to be determined for total nitrogen, which were stored for up to 1.5 years before analysis. During the dark incubation, the dissolved oxygen (DO) was never allowed to drop below 80% of its saturated concentration. The flux across the sediment-water interface under light and dark conditions was calculated as

$$flux = \alpha - \alpha_w \times \frac{V}{A}$$

where

α = linear regression slope of analyte in sediment core (μmol/l/h)

α_w = linear regression slope of analyte in "blank" core.

V = water column volume (l)

A = sediment surface area (m^2).

The linear regression slope typically used four data points. The flux was only taken as being significant if the standard error was less than the magnitude of the flux.

Sample analysis

NH_4^+ was analysed using OPA derivatisation and fluorescence detection (Watson et al. In preparation) the precision of the method was generally between 5–8% with a detection limit of 0.07–0.2 μM. NO_2^- and NO_3^- were determined on a Technicon Autoanalyzer using sulphanilamide derivatisation of NO_2^-; NO_3^- was determined as

NO_2^- after cadmium reduction to NO_2^- (Modified from Grasshoff (1976)). The limit of detection of the analysis was 0.05 μM for NO_2^- and 0.1 μM for NO_3^-, the precision for both analyses was typically less than 3%. Total dissolved nitrogen was determined as NO_3^- after a persulfate digestion modified from Valderrama (1981); the precision was generally <5% and the limit of detection was 5 μM. Dissolved organic nitrogen (DON) was calculated as the difference between total nitrogen and DIN ($NO_2^- + NO_3^- + NH_4^+$) the precision for this analysis was 10%. Total dissolved primary amines (TDPA) were analysed according to the method of Petty et al. (1982). Samples were also run without OPA reagent in order to correct for the background fluorescence of humic substances present in the samples. The precision of the method was 1.5% and the limit of detection was <0.1 μM. Urea was analysed on a Technicon Autoanalyser according to the method of Price and Harrison (1987), the limit of detection was 0.25 μM and the precision was <5%.

Denitrification

Rates of denitrification were measured using the isotope pairing technique (Nielsen 1992) as described by Dalsgaard (2000). After the nutrient flux experiments were completed, sub-cores (4.8 cm id × 30 cm) were taken from the original cores such that there was ~8 cm of sediment and 17 cm of water column. A teflon-coated stirrer bar was then suspended ~5 cm above the sediment, this was driven by an external rotating magnet rotating at 60–70 rpm. Light and dark incubations were performed with four cores (1 subcore from each of the four replicate cores) from each site on the following day. Dark cores were double wrapped in aluminium foil, light was provided as described previously. Experiments commenced with the addition of stock $^{15}NO_3^-$ to a final concentration of 60 μM. This concentration was chosen after a concentration series experiment as described by Rysgaard et al. (1995) had shown that concentrations of $^{15}NO_3^-$ above 20 μM gave constant values of D_{14} (see results section). Samples were taken for the analysis of NO_3^- before and after the addition of $^{15}NO_3^-$ in order to calculate the final ^{15}N enrichment. This analysis also confirmed that the nitrate concentration in the water column of the cores was still similar to in-situ levels. Cores were then capped and left for 2 h to allow the added $^{15}NO_3^-$ to diffuse into the denitrification zone and come to equilibrium. Cores were sacrificed over a time span which allowed the DO to drop by no more than 20% from saturation. They were sacrificed as follows. One ml of 50% $ZnCl_2$ was added to the core before the sediment was gently slurried with the water column using a metal

rod, coarser particles were allowed to settle for about a minute before a sample was taken using a gas tight syringe. The sample was then placed in a 12.5-ml Exetainer (Labco, High Wycombe, UK) to which 250 µl 50% w/v $ZnCl_2$ had been added. A headspace of He was introduced into the Exetainer within 2 weeks and the samples were then analysed within several months. Sample analysis for $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$ was carried out using a Finnigan MAT delta S isotope ratio mass spectrometer in line with a 5890 Hewlett Packard gas chromatograph. A Cu reduction column heated to 640°C was used to remove oxygen from the sample, CO_2 and H_2O were removed using a liquid nitrogen cryotrap. Denitrification rates were calculated according to the isotope pairing equations in Dalsgaard (2000) as follows.

The production rates of $^{29}N_2$ and $^{30}N_2$ were calculated as

$$p(^{xx}N_2) = \frac{\alpha_{xx}}{A} \times 10000$$

where

$p(^{xx}N_2)$ = production rate of $^{29}N_2$ or $^{30}N_2$

α_{xx} = slope of the regression line of amount of $^{xx}N_2$ versus time

A = sediment surface area in the core

The rates of denitrification were then calculated as

$$D_{15} = p(^{29}N_2) + 2p(^{30}N_2)$$

$$D_{14} = D_{15}\frac{p(^{29}N_2)}{2p(^{30}N_2)}$$

Where

D_{14} = the rate of denitrification of $^{14}NO_3^-$

D_{15} = the rate of denitrification of $^{15}NO_3^-$

The part of D_{14} that is based on NO_3^- derived from the water column (D_w) is calculated from D_{15} and the ^{14}N:^{15}N ratio of the water column NO_3^-:

$$D_w = D_{15} \frac{[^{14}NO_3^-]_w}{[^{15}NO_3^-]_w}$$

Where:

$[^{14}NO_3^-]_w$ = concentration of $^{14}NO_3^-$ in the water column

$[^{15}NO_3^-]_w$ = concentration of $^{15}NO_3^-$ in the water column

Denitrification of NO_3^- produced by nitrification within the sediment is calculated as

$$D_n = D_{14} - D_w$$

In order to verify the isotopic analysis method, samples taken during this study (Sed 2–4) as well as sediment taken from a nearby subtidal location (Sed 1) were also sent to the NERI (National Environment Research Institute) laboratory in Silkeborg, Denmark for analysis on a Europa dual inlet mass spectrometer.

Modelled sediment NH_4^+ production rates

Production of NH_4^+ within the sediments was modelled using the "PROFILE" modelling routine of Berg et al. (1998) for which the executable code is freely available. The procedure involves fitting a series of least squares fits to the measured data followed by statistical F testing to find the simplest production/ consumption profiles that fits the measured concentration profiles. This model assumes the observed porewater profiles represent steady state. The model can consider three types of vertical transport, diffusion, bioturbation and irrigation. Here, I considered diffusion as well as bioturbation. An estimate of the bioturbation coefficient (D_B) was obtained by comparing the measured fluxes of oxygen to those calculated from O_2 micro-profiles. Micro-profiles were taken During August 2001 at all sample sites using OX 25 (25-μm tip diameter) O_2 micro-electrodes (Unisense, Aarhus, Denmark). The calculated O_2 consumption rates were then compared to those measured in intact cores taken in June (Site CF) and September (Site PC). It was assumed that sediment respiration rates were the same in the cores taken for the flux and micro profile measurements. D_B was calculated according to Berg et al. (2001):

$$D_B = D_S\left(\frac{F_{meas}}{F_{calc}} - 1\right)$$

Where D_s is estimated according to Iversen and Jorgensen (1993) F_{meas} is the measured flux in intact cores and F_{calc} is the calculated flux using the modelled profile assuming $D_B = 0$. Diffusion coefficients for NH_4^+ were obtained from, and corrected for temperature according to Li and Gregory (1974). Diffusion coefficients for O_2 were obtained from Broeker and Peng (1974).

Statistical analysis.

Statistical analysis was carried out using Statistica Vers 6.0 (StatSoft Inc). 1-way and 2-way analysis of variance (ANOVA) were carried out on log-transformed data. Where log transformation failed to correct heteroscadisticity as indicated by a Cochran's C test, a non-parametric test was used. Correlation and multiple regression analysis were used to explore relations between variables.

Results

Water column and site characteristics

A summary of relevant water column parameters measured during each survey is presented in Figure 4.1. The salinity at Site PC was greater and less variable than that at Site CF, reflecting the lower freshwater discharge into Port Cygnet. During September, the salinity at Site PC did drop markedly reflecting a heavy rainfall event, the week before sampling. The salinity at Site CF was highly variable reflecting its position in the mixing zone of the estuary, where "outcrops" of marine waters are observed (Butler et al. 2000). Water temperature varied with season, consistently remaining at temperatures around 18°C between late spring and autumn and between 5–10°C during winter and early spring at both sites. NO_2^- + NO_3^- (NOx) concentrations were low reflecting the low nutrient runoff from the catchment, peaking during winter when it was derived from the marine waters of the estuary (Butler et al. 2000). NO_3^- was always the dominant NOx species, but at Site PC NO_2^- concentrations were up to 50% of those of NO_3^- and is a previously observed phenomenon at Port Cygnet (Butler et al. 2000). NH_4^+ concentrations were generally low and variable reflecting the low inputs to the estuary and its high bioavailability to phytoplankton. At Site CF, NH_4^+ concentrations covaried inversely with salinity suggesting that it was derived from the fresh or brackish waters of the

estuary, while at Site PC NH_4^+ concentrations covaried with salinity suggesting that it was derived from the marine end-member at this site. Dissolved organic nitrogen (DON) was the dominant form of dissolved nitrogen. DON concentrations ranged between 8–19 μM at Site CF and between 7 and 12 μM at Site PC. The ave rage sediment porosity (0–6 cm) was lowest on the upper mudflats, being 0.64 at Site PC upper and 0.73 at Site CF upper. On the lower mudflats the porosity was 0.8 and 0.9 at Sites PC and CF respectively (data not shown).

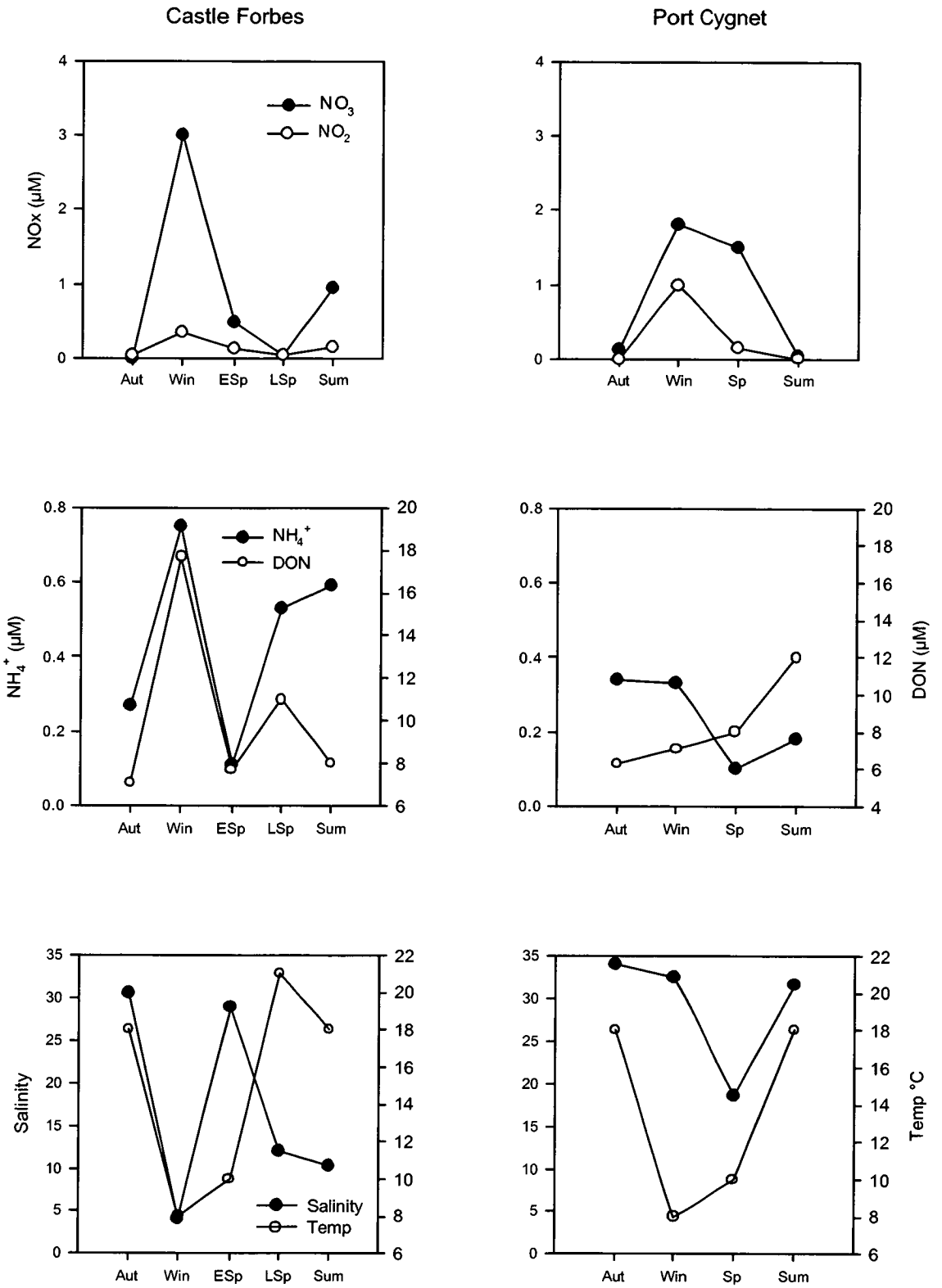


Figure 4.1. Summary of NO_3^- and NO_2^- concentrations (top panels), NH_4^+ and dissolved organic nitrogen (DON) concentrations (middle panels), temperature and salinity (bottom panels) of the water column on the days of sampling for the sediment flux measurements at Castle Forbes Bay and Port Cygnet, during autumn (Aut) winter (Win) spring (Sp) and summer (Sum) during 2001. ESp and LSp refer to early spring and late spring.

Dissolved inorganic nitrogen fluxes.

Figure 4.2 summarises the measured dissolved nitrogen fluxes at both study sites. Light and dark conditions had a marked influence on the benthic fluxes of NO_2^-, NO_3^- and NH_4^+ (DIN). Under light conditions, DIN was either taken up at a greater rate, or released at a lower rate, compared to dark conditions. Over the course of the year, none of the DIN fluxes were significantly different between the upper and the lower sites of either mudflat (Kruskal Wallis, $p>0.05$), with the exception of dark NO_3^- fluxes at Site PC. At this site, NO_3^- uptake by the sediment was more rapid on the upper mudflat (Kruskal Wallis $p<0.01$). All the data from the upper and lower mudflats were then pooled for each site and the two sites compared on an annual basis. Release of NH_4^+ from the sediment was significantly greater on average at Site PC than at Site CF (Kruskal Wallis, $p<0.01$). The sediments at Site CF were always a sink for NH_4^+ in the light, and also more generally during the dark. Flux rates ranged from an uptake (-28 μmol m^{-2} h^{-1}) on the upper mudflat in the light, up to an efflux (39 μmol m^{-2} h^{-1}) in the dark during summer. At Site PC, NH_4^+ was generally released from the sediments under both light and dark conditions, with the highest efflux of NH_4^+ occurring during summer (95 μmol m^{-2} h^{-1}). NO_3^- was taken up at a significantly greater rate in both the light and dark at Site CF than at Site PC (Kruskal Wallis $p<0.05$). NO_3^- fluxes into the sediment were significantly correlated to NO_3^- concentrations in the water column under both light and dark conditions, when the data from both sites were pooled ($r = 0.60$, $p<0.05$). Fluxes of NO_3^- and NO_2^- were not significantly correlated, with NO_2^- making up between 0 and 90% of NOx fluxes.

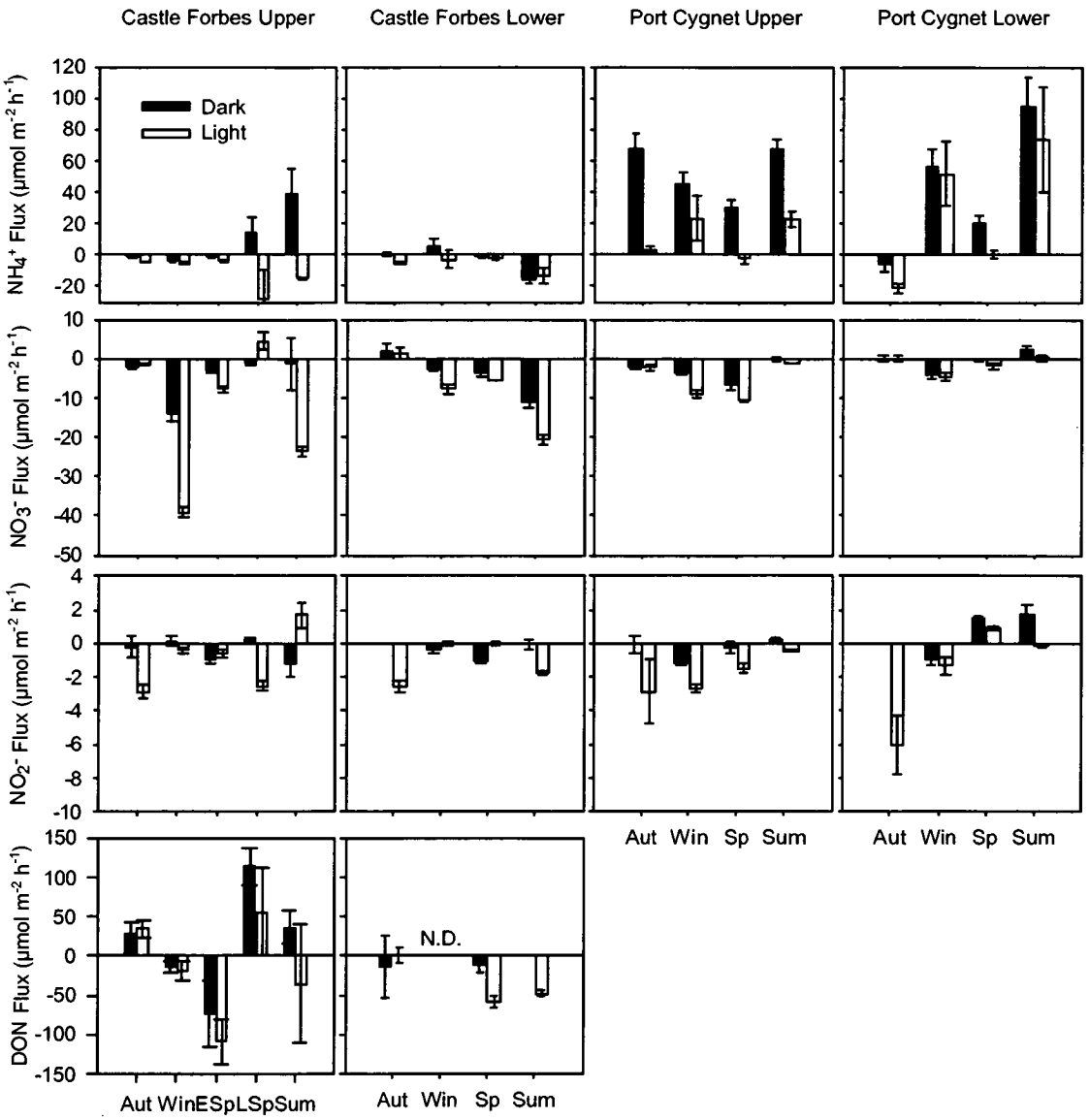


Figure 4.2. Summary of the measured fluxes of NH_4^+, NO_3^-, NO_2^- and dissolved organic nitrogen (DON) on the upper and lower mudflats of Castle Forbes Bay and Port Cygnet during autumn (Aut) winter (Win) spring (Sp) and summer (Sum) during 2001. ESp and LSp refer to early spring and late spring. Error bars represent the standard error of the mean (n= 4). N.D. is not determined.

Dissolved organic nitrogen fluxes

No significant fluxes of DON were detected at Site PC. Significant DON fluxes were always detected at Site CF on the upper mudflat and occasionally on the lower mudflat (Figure 4.2). Fluxes of DON were not measured on the lower mudflat in winter and were assumed to be insignificant. Treatment (light/dark) was not found to have a statistically significant effect on DON fluxes (Kruskal Wallis $p>0.05$), however, there was generally a greater release (or lower uptake) of DON in the dark compared to the light. Highest DON effluxes were observed on the upper mudflat in late spring, (113 μmol m^{-2} h^{-1}), coinciding with highest respiration rates (Chapter 3, figure 3.2). Highest uptake rates were observed in early spring (-109 μmol m^{-2} h^{-1}) coinciding with highest productivity (Chapter 3 Figure 3.2). In this study, I measured both urea and total dissolved primary amines (TDPA). Even when the highest fluxes of DON were seen during November, urea and TDPA fluxes were insignificant (<5% of total fluxes)

Denitrification

Experimental validation of the isotope pairing technique

Rates of D_{14} calculated using the measured isotope ratios from the NERI laboratory were in close agreement with those measured at the CSIRO Marine Laboratories (Figure 4.3a). The concentration series experiment also showed that constant rates of D_{14} were when $^{15}NO_3^-$ was added in excess of 20 μM (Figure 4.3b).

Castle Forbes Bay

Figure 4.4 summarises the D_{14}, D_w and D_n at the two study sites. Denitrification rates at Site CF were not significantly affected by time or position on the mudflat (2-way ANOVA). Denitrification rates were undetectable during light incubations on the upper mudflat for all seasons, rates in the dark ranged from 0.3 μmol m^{-2} h^{-1} in autumn up to 3 μmol m^{-2} h^{-1} in summer. On the lower mudflat denitrification was detected in both the light and dark; rates varied between undetectable in spring and 2.3 μmol m^{-2} h^{-1} in autumn. A large difference between light and dark denitrification rates was seen in autumn on the lower mudflat, when benthic productivity was highest.

Port Cygnet

Total denitrification at Site PC was significantly greater on the lower than the upper mudflat. (2-way ANOVA, $p<0.01$). A significant interaction between position and

time showed this varied significantly with time, however, the F value for the interaction (F = 6.2) was much smaller than for the effect of position alone (F = 32), indicating that seasonality had a relatively small influence. The $\%D_n$ of total denitrification was not significantly affected by treatment (light/dark) on an annual basis (2-way ANOVA), but, time did affect $\%D_n$ significantly ($p<0.01$). The $\%D_n$ was significantly lower in winter, when NO_3^- concentrations were highest and denitrification was primarily driven by D_w. There was a strong positive correlation between D_n and NO_2^- efflux ($r = 0.85$, $p<0.01$), but not NO_3^- efflux and D_w or D_{14}. D_{14} was not significantly correlated to temperature.

Castle Forbes Bay and Port Cygnet pooled

On an annual basis the rates of denitrification were significantly highest at Site PC lower (1–way ANOVA, Fisher LSD Post Hoc analysis, $p<0.01$) and not significantly different among the other 3 sample sites. D_{14} was dominated by D_n, presumably because of the low NO_3^- concentrations in the water column. D_w was highest at all sites in winter, coinciding with the highest NO_3^- concentrations in the water column. D_{14} was not related to temperature in either the light or the dark. Light and dark D_{14} had positive relationships with light and dark NH_4^+ fluxes, the relationship was strongest under light conditions ($r = 0.77$, $p<0.01$) indicating the availability of NH_4^+ played a role in controlling rates of denitrification. Photosynthesis by MPB apparently had a negative effect on D_{14} in the light as indicated by the significant correlations with TCO_2 ($r = 0.50$, $p<0.05$) and O_2 fluxes ($r = -0.53$, $p<0.05$).

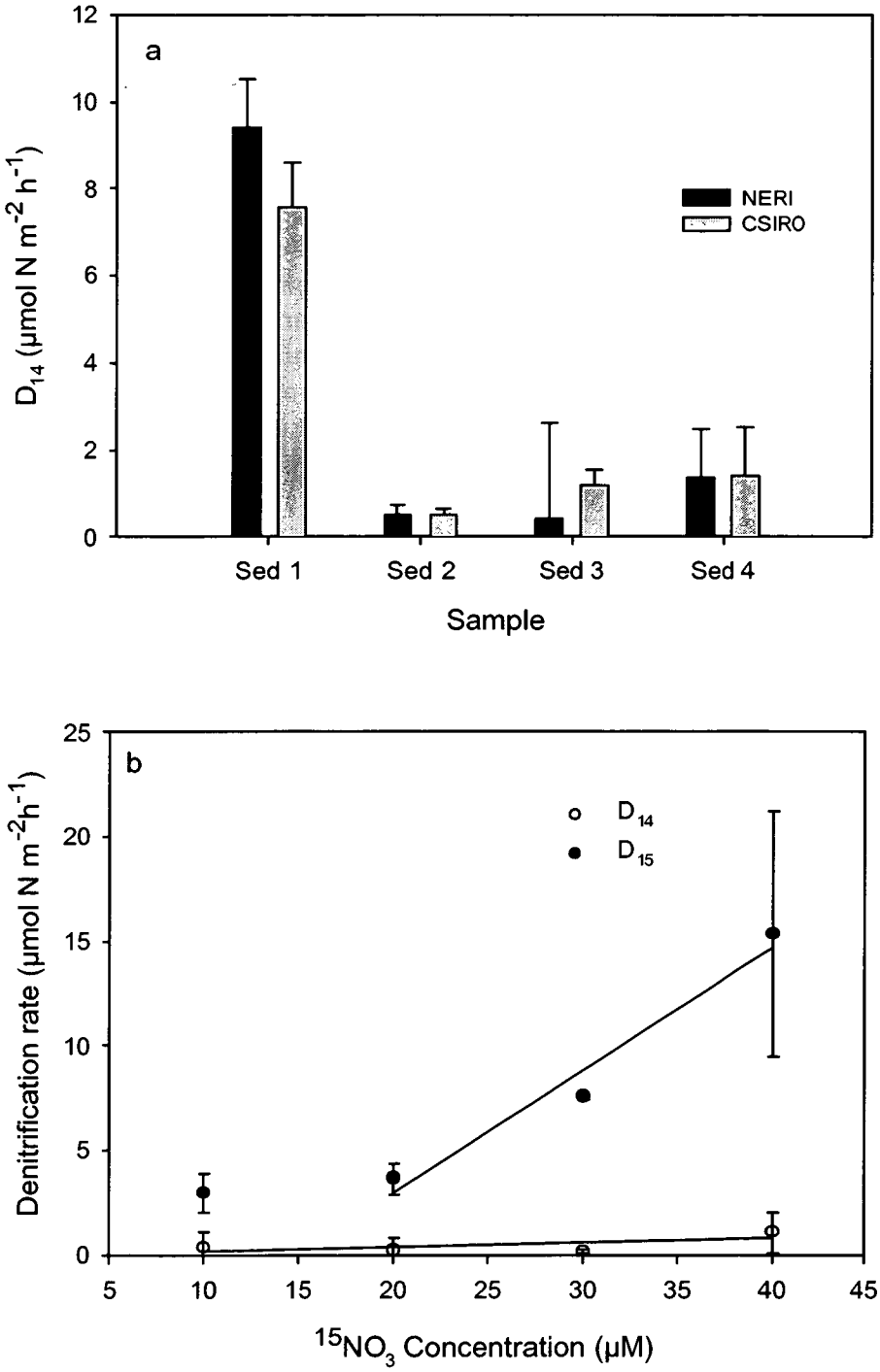


Figure 4.3. **(a)** The rates of denitrification (D_{14}) calculated for duplicate samples taken from the same core and analysed at the CSIRO Marine Laboratories in Hobart (CSIRO) and NERI in Silkeborg, Denmark (NERI). Sed 1 is a subtidal sediment taken from a site in southern Tasmania and Seds 2–4 are sediments sampled as part of this study. **(b)** The measured rates of D_{15} and D_{14} for sediment samples taken from Castle Forbes Bay incubated with different $^{15}NO_3^-$ concentrations. Error bars represent the standard error of the mean (n=4).

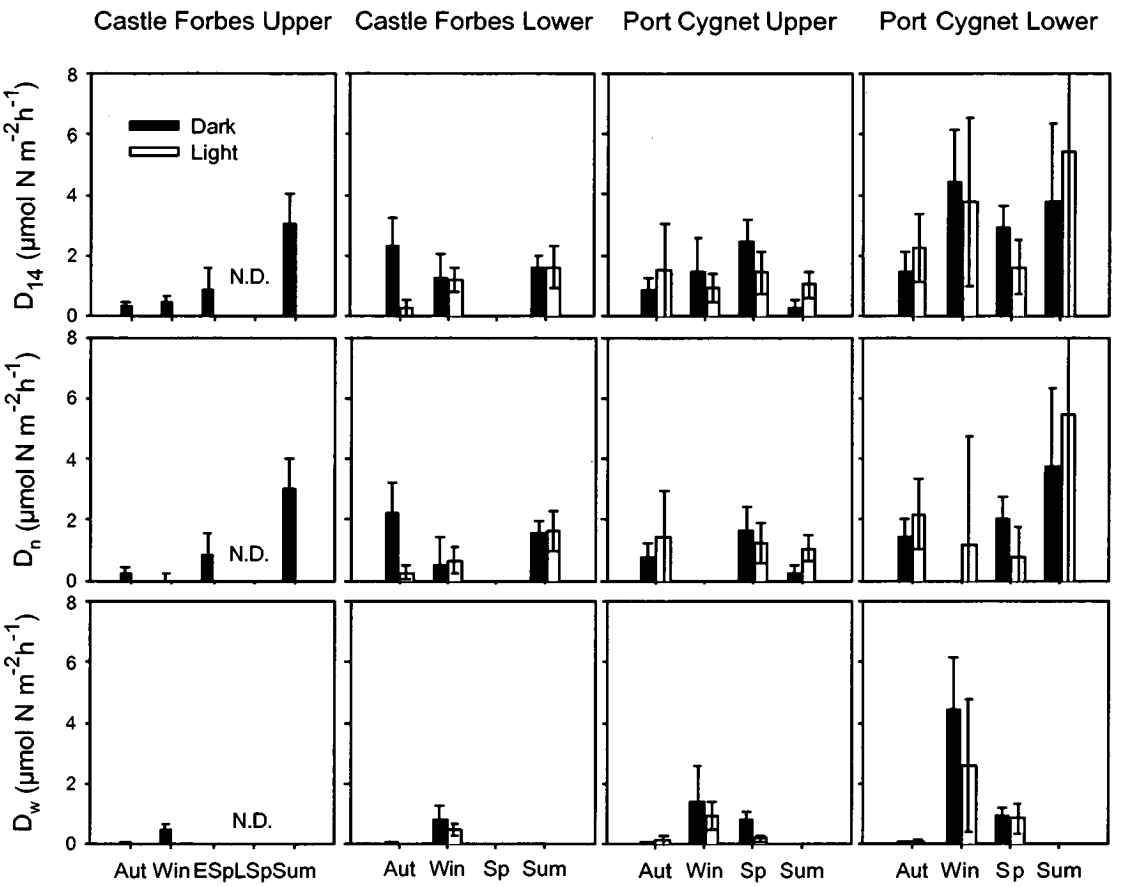


Figure 4.4. Summary of total denitrification (D_{14}), coupled denitrification (D_n) and uncoupled denitrification (D_w) on the upper and lower mudflats of Castle Forbes Bay and Port Cygnet during autumn (Aut) winter (Win) spring (Sp) and summer (Sum) during 2001. ESp and LSp refer to early spring and late spring. N.D. not determined. Error bars represent the standard error of the mean (n=4).

Modelled sediment NH_4^+ production rates

Figure 4.5 shows the porewater profiles of NH_4^+ at Site CF (a) and PC (b). All porewater profiles showed smooth trends indicating the sediments at both sites were relatively undisturbed by resuspension and macrofaunal reworking. NH_4^+ concentrations at all sites generally increased down the profiles reflecting the production of NH_4^+ within the sediments. At both study sites, the porewater concentrations of NH_4^+ were highest on the upper mudflat. NH_4^+ concentrations were lowest during winter at all sites, and generally similar during autumn and late spring. From the "PROFILE" modelling routine, it was apparent that NH_4^+ was generally produced in the lower sediment layers, sometimes being consumed in the upper sediment layers. This was most apparent at Site CF during autumn when NH_4^+ concentrations within the porewaters were approximately 1 μM in the upper 3 cm of sediment on the upper mudflat and in the upper 6 cm on the lower mudflat. Calculated rates of NH_4^+ production were generally highest on the upper mudflat at PC and lowest on the lower mudflat at Site CF.

Profiles of DON (calculated as the difference between total nitrogen and NH_4^+) were generally highly variable, showing no consistent trend with depth (data not shown). Where a depth concentration gradient was apparent it was generally less than the gradient for NH_4^+. Blackburn and Blackburn (1993) suggested a diffusion coefficient of 5.8×10^{-6} cm^2 sec^{-1} for DON, which is approximately a third of that used for NH_4^+. This means that for DON to be produced at comparable rates to NH_4^+, it would have to have a concentration gradient 3 times greater than that for NH_4^+, which was not the case here. Therefore, rates of DON production within these sediments were considered to be negligible compared to NH_4^+ production rates, and are not considered further.

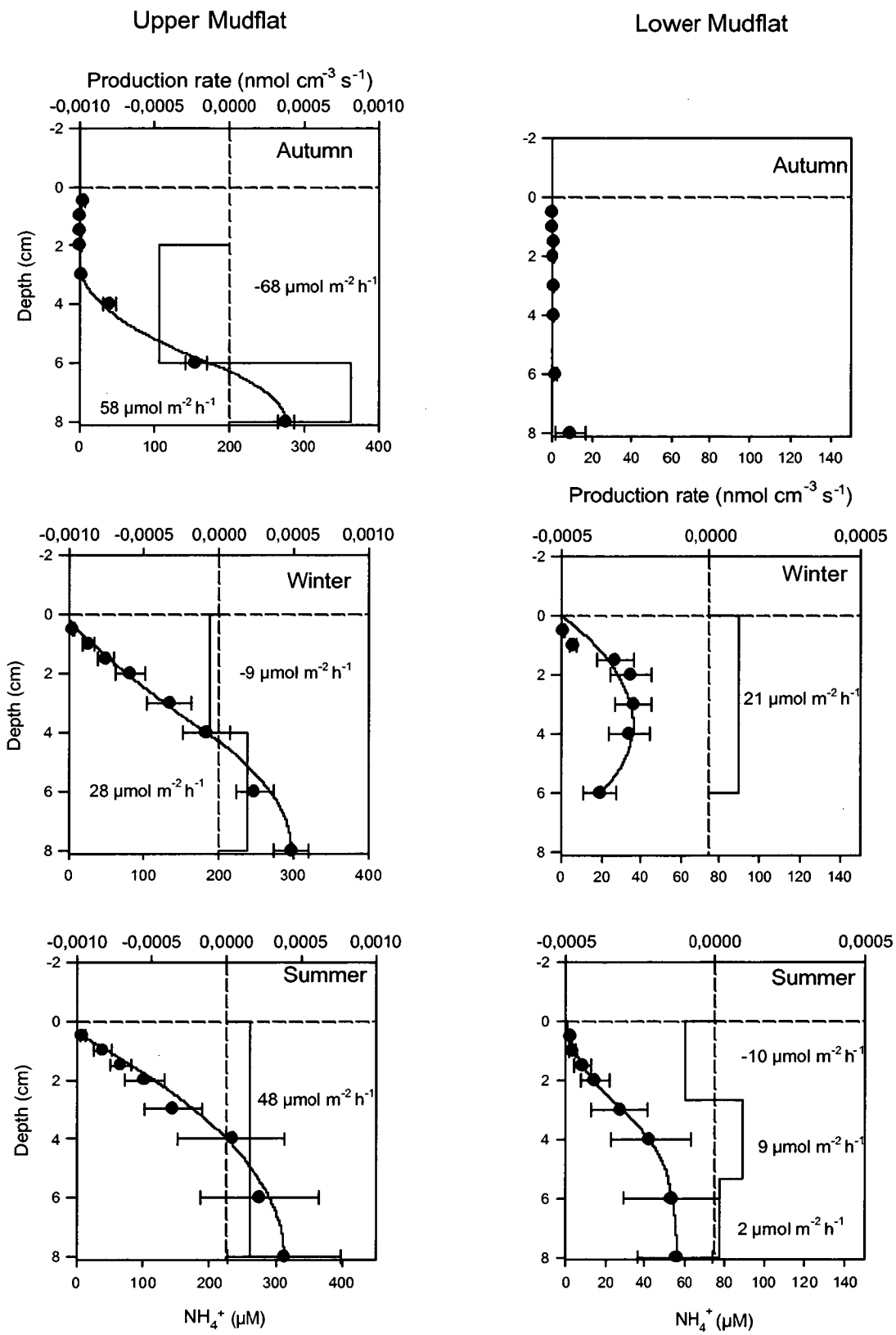


Figure 4.5a. Porewater profiles of NH_4^+ (circles) and the modelled best fit line using the "PROFILE" porewater model of Berg et al. (1998), at Castle Forbes Bay during autumn, winter and summer. Error bars represent the SE of the mean (n=3). The zones of production and consumption of NH_4^+ are denoted graphically with the net rate of consumption or production in each zone given on the graph.

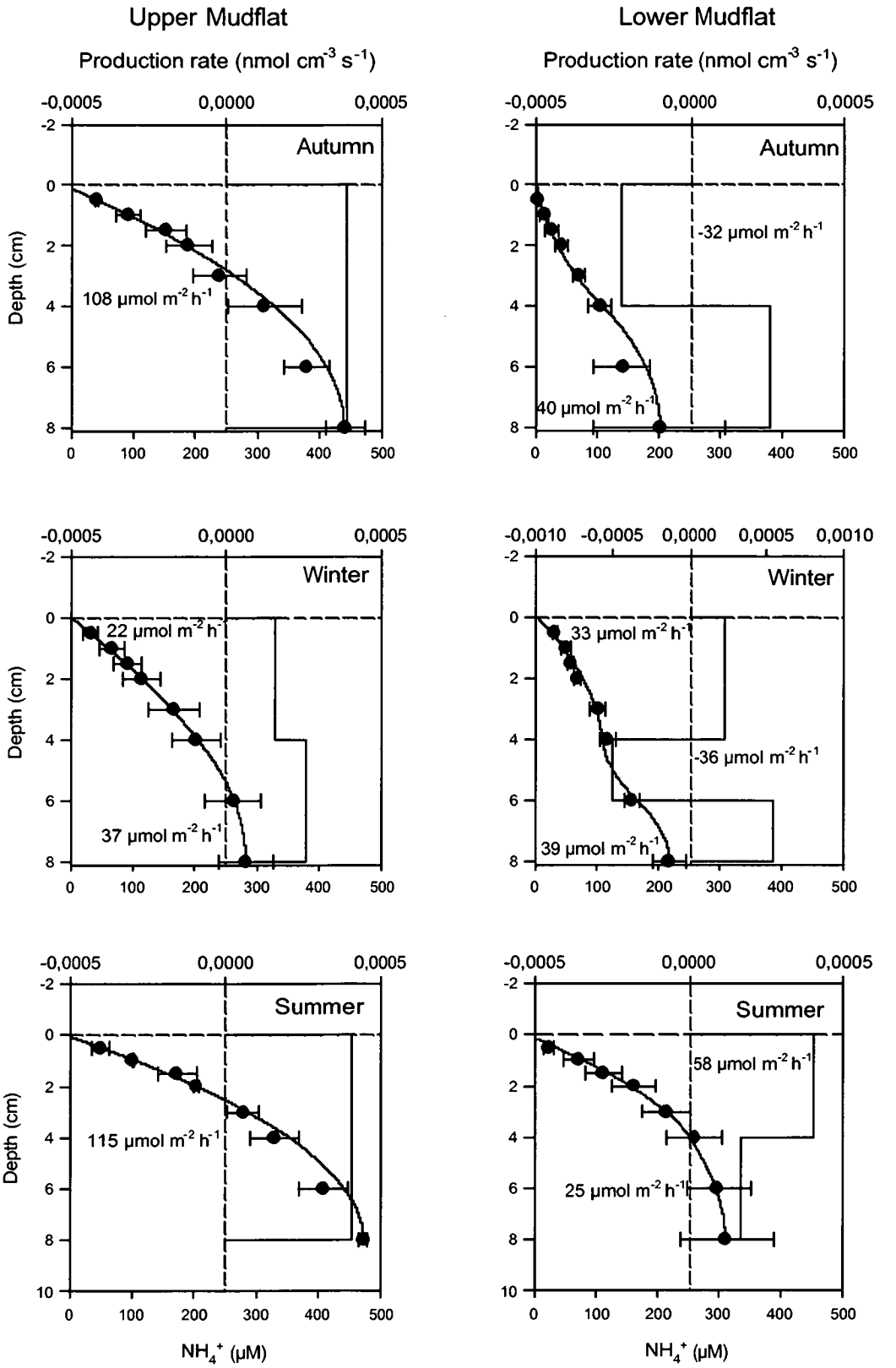


Figure 4.5b. Porewater profiles of NH_4^+ (circles) and the modelled best fit line using the "PROFILE" porewater model of Berg et al. (1998), at Port Cygnet during autumn, winter and summer. Error bars represent the SE of the mean (n=3). The zones of production and consumption of NH_4^+ are denoted graphically with the net rate of consumption or production in each zone given on the graph.

Microbial biomass nitrogen

Figure 4.6 summarises the microbial nitrogen concentrations in the 0.5–3.5 cm depth interval as well as profiles of microbial nitrogen taken in January 2001. Microbial nitrogen generally made up a greater fraction of total nitrogen than "free total nitrogen". This was most noticeable at Site CF upper where the highest microbial nitrogen concentrations were generally observed, particularly in relation to free total nitrogen. Microbial nitrogen:free total nitrogen ratios were generally highest in summer and autumn and lowest in winter and early spring (September) at all sites. Profiles of microbial nitrogen showed an increase in microbial nitrogen towards the sediment surface, while free total nitrogen decreased towards the sediment surface.

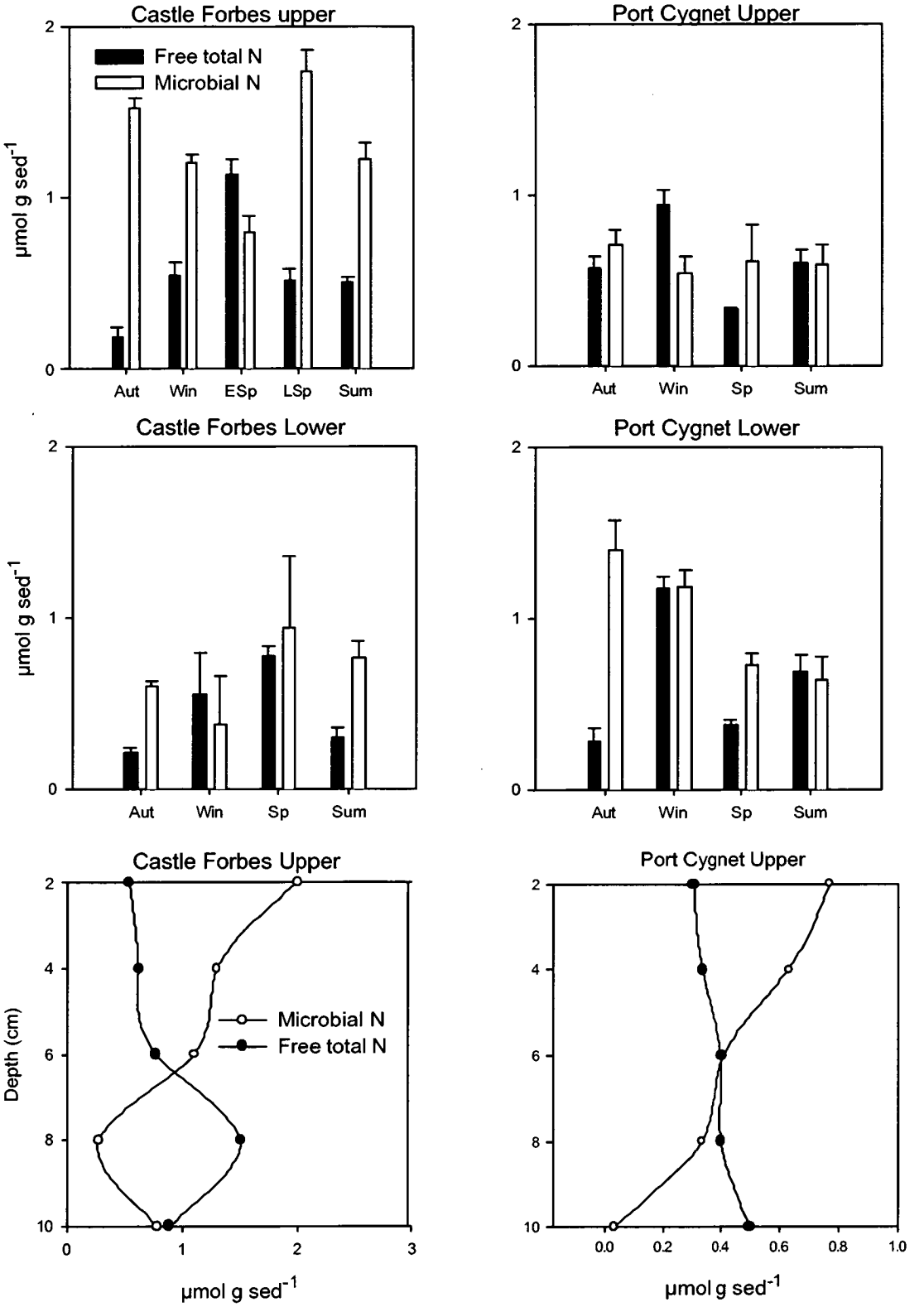


Figure 4.6. Upper four panels: Measured concentrations of "microbial biomass nitrogen" and "free total nitrogen" in the 0.5–3.5 cm sediment horizon on the upper and lower mudflats of the study sites during autumn (Aut) winter (Win) spring (Sp) and summer (Sum) during 2001. ESp and LSp refer to early spring and late spring. Lower two panels: Sediment profiles of "microbial biomass N" and "free total N" on the upper mudflats at Port Cygnet and Castle Forbes Bay taken in January 2001. Error bars represent the standard error of the mean (n=4) for the profiles n=1.

Discussion

The use of PROFILE to calculate sediment NH_4^+ production rates

Porewater profiles in combination with modelling techniques can provide a useful insight into benthic processes. The modelling of solute fluxes in sediments is complicated by transport processes other than diffusion, including bioturbation and bioirrigation. The PROFILE modelling routine employed here allows for the consideration of these two transport processes. In this study, the bioturbation coefficient was estimated using oxygen microelectrode profiles and the measured fluxes of O_2 in intact core incubations. The measured fluxes of O_2 were always in excess of those calculated from the micro profiles of O_2 assuming only diffusive transport. It was assumed that the additional O_2 transport was due to bioturbation. The bioturbation coefficient (D_B) was calculated as outlined in the methods section. A D_B of 10.2×10^{-6} was found for Site CF upper and lower, 38×10^{-6} for Site PC upper and 18×10^{-6} for PC lower. A D_B of 11.9×10^{-6} was reported at a shallow station in Disko Bay in Greenland (Rysgaard et al. 2000). Berg et al. (2001) cite D_B values occurring in the order of 10^{-6} to 10^{-4}. The D_B values measured here are, therefore, within the range of values expected in bioturbated sediments.

An indication of the accuracy of the production rates modelled by profile is perhaps best obtained by comparing the measured fluxes to the calculated production rates during periods of low productivity by MPB when assimilation of NH_4^+ at the surface is likely to be negligible (see following discussion). The lowest rates of primary production were observed at Site CF lower during summer, at this time there was also only a small difference between light and dark fluxes, indicating that the assimilation of NH_4^+ by MPB was negligible. At this time, the calculated production rate of NH_4^+ within the sediment was 83 $\pm$17μmol m^{-2} hr^{-1} compared to a measured flux of 94 $\pm$18μmol m^{-2} hr^{-1}. The overlapping standard errors of both these estimates suggest they are not significantly different, indicating that the calculated production rates of NH_4^+ within the sediment using the PROFILE model are close to the true values.

NH_4^+ production within the sediment

Figure 4.7a shows the observed dark NH_4^+ fluxes in relation to those predicted by "PROFILE". It can be seen that in all instances with one exception the predicted fluxes of NH_4^+ were greater than those actually measured. In many instances, particularly at Site CF, there was an uptake of NH_4^+ measured, which suggests that NH_4^+ was being consumed at the sediment surface. In some instances this zone of NH_4^+ reassimilation at the sediment surface occurred on a scale which could be resolved by the PROFILE model. Nitrification and subsequent denitrification are unlikely to be the NH_4^+ consuming process, because NO_3^- was not detected in these porewaters and rates of denitrification were extremely low. Assimilation by MPB is the most likely explanation given the high MPB biomass and productivity at these sites combined with the low water-column concentrations of DIN. This seems plausible given that MPB are able to assimilate NH_4^+ and NO_3^- for up to 60 h after darkening (Rysgaard et al. 1993). It is unclear whether MPB were responsible for the zones of NH_4^+ consumption at the sediment surface, which in some instances extended to a depth of 6 cm. Other studies have shown that MPB are capable of consuming nutrients in the surface sediment layers, in some instances down to depths of 3 cm (Sundbäck et al. 1991; Thornton et al. 1999). The MPB observed at this site were dominated by *Oscillatoria* spp., which are highly motile (see Chapter 2) and were observed to move several cm up and down the side of core tubes. It is, therefore, plausible that the MPB are capable of assimilating NH_4^+ from depths of several cm within the sediment.

Given that MPB were apparently consuming NH_4^+ at the sediment surface, the net rate of NH_4^+ production within the sediment was calculated neglecting any zones of NH_4^+ consumption at the sediment surface. Figure 4.7b shows a plot of the NH_4^+ production rate within the sediment versus the measured TCO_2 flux. It can be seen that the ratio of NH_4^+ produced to TCO_2 produced generally fell within the range of 1:10 to 1:60. This may suggest that the organic material undergoing decomposition had a very high C:N ratio. Material with such a high C:N ratio (particularly from terrestrial sources) would generally be considered highly refractory and unlikely to undergo decomposition at appreciable rates.

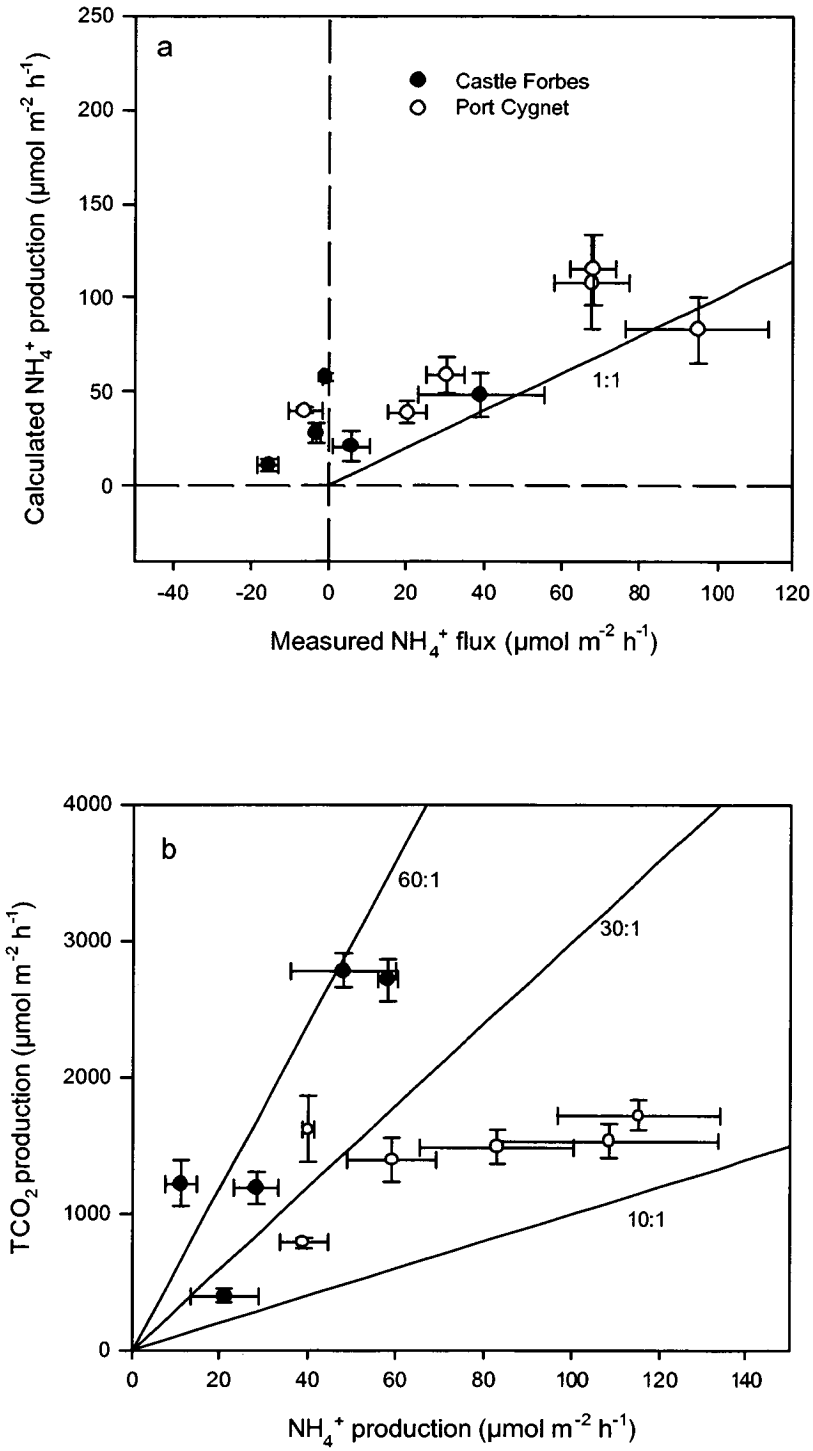


Figure 4.7. **(a)** Calculated production rate of NH_4^+ within the sediment versus the measured dark NH_4^+ flux at Castle Forbes Bay and Port Cygnet. The marked line shows the 1:1 relationship between the modelled and measured fluxes. **(b)** The dark TCO_2 flux versus the calculated production rate of NH_4^+ within the sediment at Castle Forbes Bay and Port Cygnet. Marked lines show the TCO_2:NH_4^+ ratios of 10, 30 and 60. Error bars represent the standard error of the mean (n=3–4).

In Chapters 2 and 3, it was suggested that the production of extracellular organic carbon (EOC) by MPB was likely to be a major driver of respiration within the sediment. The importance of EOC as a labile carbon source to benthic consumer animals and bacteria is now recognised (Decho 1990; Goto et al. 2001). This material is generally considered to consist predominantly of simple sugars and polysaccharides (Decho 1990; Underwood et al. 1995), the nitrogen content of EOC has been poorly investigated, however, it has been suggested that the importance of EOC as a nitrogen source to consumers is minimal (Decho 1990). A recent study has shown that amino acids comprised up to 5% of EOC (Granum et al. 2002), if it is assumed that all the nitrogen in EOC is present as amino acids with an average C:N ratio of 2, then a C:N ratio of ~40 for EOC is conceivable. EOC, therefore, may represent a labile, high C:N ratio source of organic carbon to bacteria and meiofauna within the sediment.

In support of this hypothesis, a significant positive relationship between net primary production and the ratio of net TCO_2:NH_4^+ fluxes was observed (Figure 4.8). During the periods of the highest productivity MPB was most likely to nutrient-limited (See Chapter 5). At times of nutrient limitation, the production rates of EOC are also likely to be highest, compared to cellular growth which will slow (Stal 1995; Staats et al. 2000; Engel et al. 2002). Thus, during the periods of highest productivity, higher C:N ratio EOC is likely to dominate the input of organic matter relative to lower C:N ratio algal cells.

In one instance, the C:N ratio for the net production of TCO_2 and NH_4^+ was 110 and in another instance approached infinity. Whether or not the C:N ratio of the remineralised carbon and nitrogen reflects the C:N ratio of the organic matter undergoing remineralisation will depend on whether or not the population of bacterial cells remineralising the organic matter are in steady state. A pulsed input of labile organic matter to sediments has been shown to stimulate bacterial production, which resulted in an uptake of inorganic nitrogen by bacteria in order to synthesise their low C:N ratio biomass (van Duyl et al. 1993; Pedersen et al. 1999). Bacterial production may also be continuously stimulated by a ready supply of labile organic carbon derived from MPB as well as grazing within the sediment (Meyer-Reil and Faubel 1980; Kemp 1990; Epstein 1997; Coull 1999). Thus, a continuous uptake of

nitrogen and transfer into the microbial food web may take place (Blackburn 1988). The fact that a significant fraction of nitrogen within the sediments was bound up in microbial biomass (Figure 4.6) suggests this was likely to be occurring in these sediments. As such, it is suggested the generally high ratio of TCO_2:NH_4^+ production, was caused by the decomposition of labile high C:N ratio EOC which stimulated the reassimilation of NH_4^+ by actively growing bacteria resulting in only a very small release of nitrogen into the porewaters relative to carbon oxidised.

These findings are in general agreement with a study by Alongi (1991), who also observed a lack of nutrient production within the pore-waters of a tropical mudflat. He similarly ascribed this observation to a reassimilation of nutrients by bacteria, which ultimately sequestered nutrients within the sediments. Similar findings have been made in oligtrophic subtropical systems in Northern NSW Australia where it was also found that estuarine sediments generally acted as a sink for nutrients (Ferguson 2002). This suggests that in more oligotrophic systems, there will be a rapid incorporation of DIN into biomass and that there will be a tight cycling of nitrogen within the biotic nitrogen pools.

At this stage, the likelihood of the measured microbial nitrogen pool being derived from MPB should be considered. This is thought to be unlikely for two reasons. Firstly, microbial nitrogen was sampled from the 0.5–3.5 cm sediment horizon where one might expect the biomass of bacterial cells is likely to be well in excess of that of algal cells. Secondly, there was no significant relationship between chlorophyll *a* and microbial nitrogen or microbial nitrogen:total free nitrogen ratios.

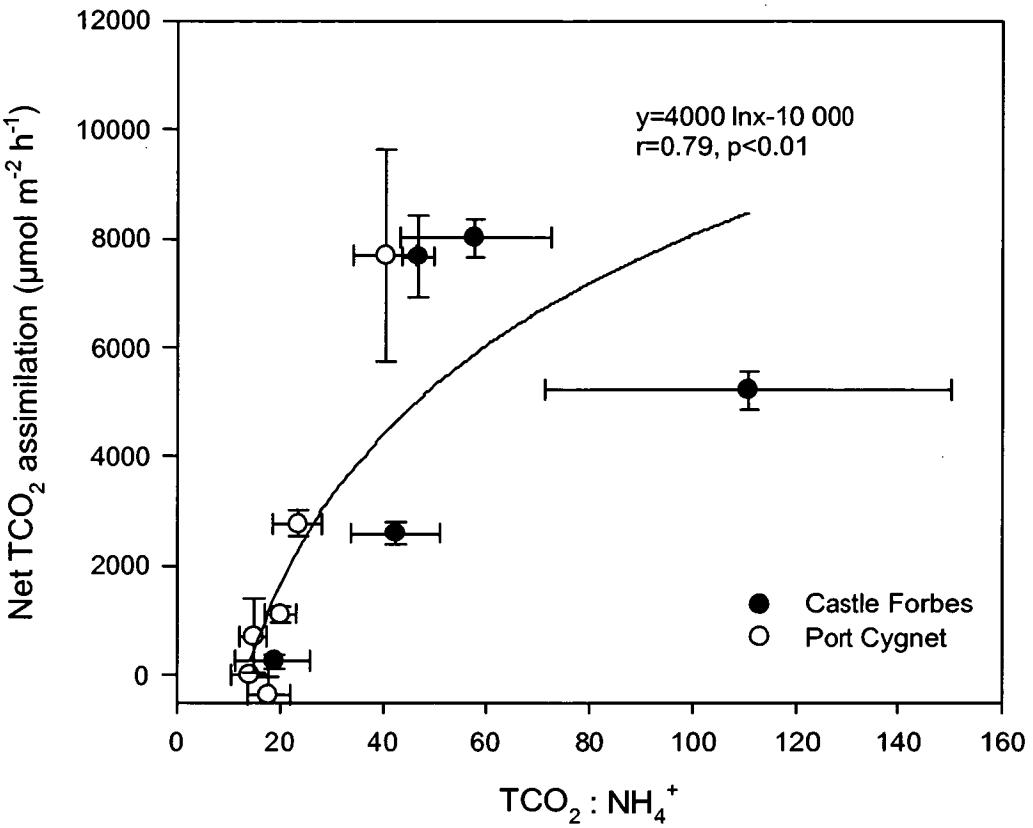


Figure 4.8. A plot of net primary production versus the ratio of TCO_2:NH_4^+ produced by the sediments at Castle Forbes Bay and Port Cygnet. Error bars represent the standard error of the means (n =3–4).

Dissolved organic nitrogen fluxes

The most significant DON fluxes were measured at Site CF upper, where the highest MPB biomass, productivity and respiration rates have been observed (Chapter 3). This is consistent with previous studies which have shown that DON effluxes may be significant where there are high inputs of fresh organic matter (Hansen and Blackburn 1992; Enoksson 1993; Blackburn et al. 1996; Pedersen et al. 1999; Tyler et al. 2001) including that derived from MPB (Eyre and Ferguson 2002; Ferguson 2002). The most significant DON effluxes were observed when sediment respiration rates were highest during late spring 2000. It is suggested that this high respiration rate reflected the breakdown of microalgal cells from an early spring bloom, as was observed in the following spring of 2001 (Chapter 3). During this period, the efflux of DON dominated over DIN fluxes. The low DIN effluxes observed at Site CF throughout the year support previous observations that DON will be the major dissolved species of nitrogen to be released to the water column from sediments with high organic matter inputs from MPB and macroalgae (Tyler et al. 2001; Eyre and Ferguson 2002). A possible mechanism for this is an uptake of DIN by MPB and subsequent release of DON by algal cells (Nagao and Miyazaki 2002). Grazing of MPB and subsequent release of DON to the water column through "sloppy feeding" is also possible (Eyre and Ferguson 2002). Alternatively, labile DON is lost in the early stages of decomposition, leaving a relatively refractory carbon pool (Blackburn et al. 1996). Bacteria degrading this pool then assimilate inorganic nitrogen to synthesise their own biomass (see nitrogen assimilation discussion) which results in a low efflux of inorganic N.

Of particular interest in terms of its bioavailability, is the identity of the DON lost from the sediment, especially during the period of high efflux seen during late spring. Here, I measured urea and TDPAs and found the fluxes of these species to be insignificant. Other studies which have investigated the composition of DON produced within the sediment have also found that urea was a minor component of the DON pool (Blackburn et al. 1996; Lomstein et al. 1998; Pedersen et al. 1999). Dissolved free amino acids (DFAA) have generally been found to make up <10% of the DON pool and fluxes in sediments (Lomstein et al. 1998; Pedersen et al. 1999; Landén and Hall 2000). The TDPA method used here will only determine primary amino acids and will, therefore, only determine a sub-fraction of the DFAA pool.

The low efflux of TDPAs seen here is consistent with DFAA measurements in other systems. Total hydrolysable amino acids (THAAs) have been identified as a significant proportion of DON pools and fluxes within sediments making up 26% of DON in shallow water sediments (Lomstein et al. 1998). Pedersen et al. (1999) found that THAAs constituted ~17% of DON flux in the first 7 days following Eelgrass addition to sediments. The bulk fraction of DON released from sediments remains to be identified. In general it has been observed that DON released from the sediments will have a low C:N ratio (~<6), suggesting that DON released from sediments is not refractory (Blackburn et al. 1996; Burdige and Zheng 1998; Pedersen et al. 1999). It has been suggested that a large fraction of DON efflux may be composed of compounds such as RNA and amines (Lomstein et al. 1998; Pedersen et al. 1999). Uptake of DON was also observed at Site CF and are discussed in further detail in Chapter 6.

Denitrification

Measuring denitrification in intertidal sediments only during inundation imposes a degree of artificiality on the measured rates of denitrification as the sediments will be periodically exposed. This may lead to changes at the sediment surface potentially affecting the activity of nitrifying and denitrifying bacteria. In particular, the lack of a supply of NO_3^- from the water column during exposure will mean that D_h will be the only denitrification pathway. A recent study has, suggested that rates of D_h are unchanged during exposure (Ottosen et al. 2001). Given that D_{14} was generally fed by D_n in the Huon Estuary mudflats, it seems reasonable to assume that the denitrification rates presented here are representative of the system. Rates of denitrification were generally low, but comparable to those measured using the isotope pairing technique on other intertidal flats during times of low water column NO_3^- concentrations (Cabrita and Brotas 2000; Trimmer et al. 2000b). Similarly low rates have also been observed in shallow subtidal coastal sediments (Rysgaard et al. 1995; Jensen et al. 1996; Sundbäck et al. 2000). A number of reasons for the low rates of denitrification are possible. Low concentrations of NO_3^- in the water column would have resulted in greatly reduced rates of diffusion of NO_3^- into the sediment, which resulted in the generally negligible rates of D_w. Denitrification rates may also be depressed in the intertidal zone possibly due to more extreme and fluctuating environmental conditions (see later discussion) (Ottosen et al. 2001).

Benthic microalgal production also had a negative impact on denitrification as indicated by the negative correlations between various productivity indicators and D_{14}. While both D_n and D_w were also both negatively correlated with productivity, they were less statistically significant than the correlation between D_{14}, suggesting the negative effect of MPB on D_{14} arose through a negative impact on both D_n and D_w. In Figure 4.9a it can be seen that D_w was always much less than the NO_3^- influx into the sediment, which suggests that assimilation of NO_3^- from the water column by MPB detracted from D_w. Potential negative effects of primary production on D_n include competition for NH_4^+ and CO_2 between photoautotrophs and nitrifying bacteria, and possibly increases in pH and O_2 (Henriksen and Kemp 1988; Risgaard-Pedersen et al. 1994). The strong correlation between NH_4^+ flux and D_n, in both the light and dark supports the contention that an availability of NH_4^+ was controlling D_n. This then suggests that assimilation of NH_4^+ by MPB would have a negative affect on D_n. Competition for NH_4^+ between nitrifiers and heterotrophic bacteria will also have a negative impact upon nitrification rates (Strauss and Lamberti 2000). The stimulation of heterotrophic bacterial activity by MPB (see earlier discussion) is, therefore, another means by which the presence of MPB will negatively impact on D_n.

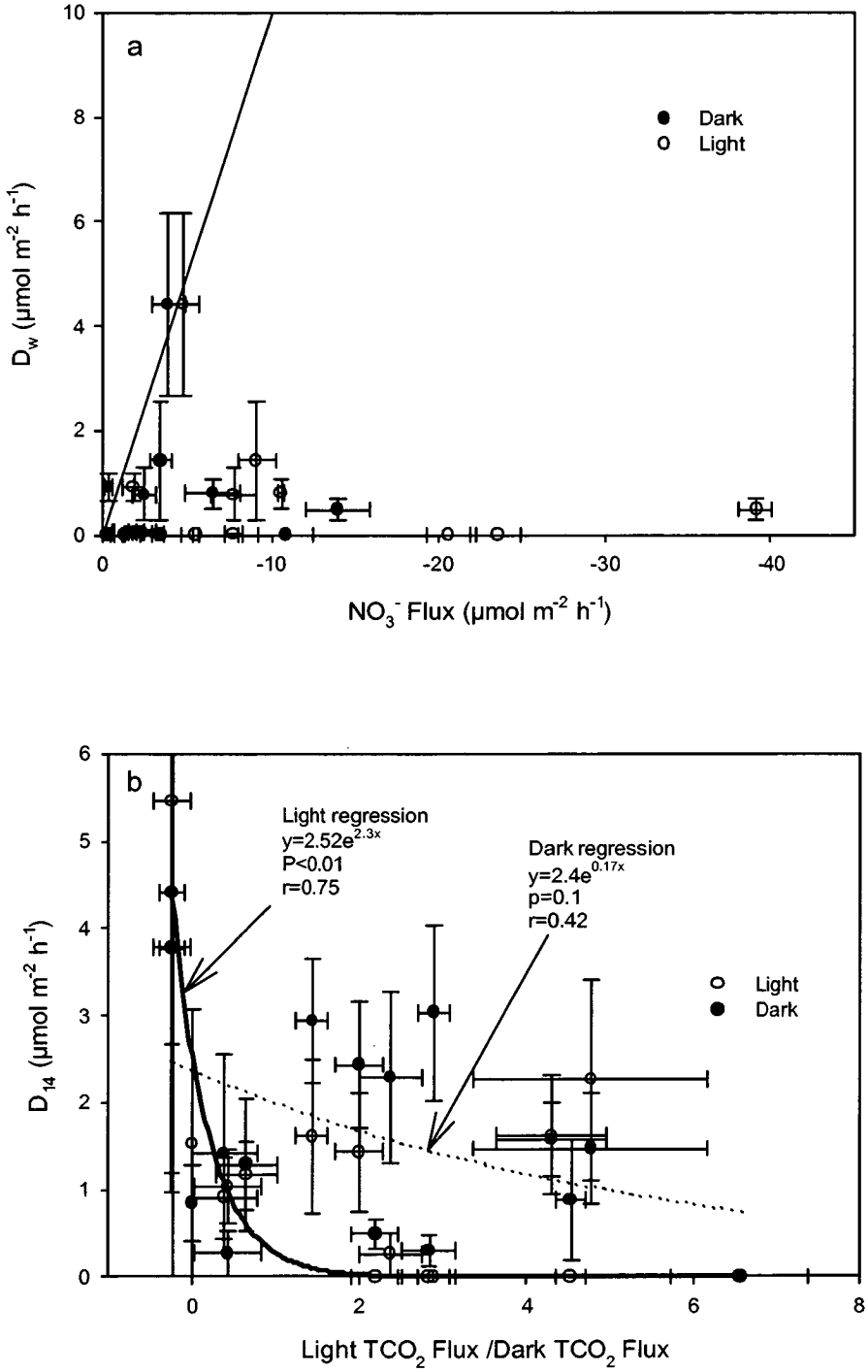


Figure 4.9. **(a)** Uncoupled denitrification (D_w) versus NO_3^- flux measured in the light and dark. Marked line shows 1:1 relationship between D_w and NO_3^- flux. **(b)** Total denitrification (D_{14}) versus light TCO_2 flux /dark TCO_2 flux. Marked lines are the regression lines for the light and dark data. Error bars represent the standard error of the mean (n=4).

At Site PC, denitrification rates were always significantly higher on the lower mudflat than the upper mudflat. Rates of primary production were not significantly different between the upper and the lower mudflat (Chapter 3), suggesting a factor other than primary production was important in controlling the rates of denitrification in this system. Oxygen penetration into the sediment was measured once during the year, it was found that the lower mudflat had a deeper O_2 penetration than the upper mudflat, reflecting the greater porosity on the lower mudflat. Small burrows were also observed in cores taken from the lower mudflat which were not observed on the upper mudflat. It, therefore, seems probable that higher denitrification rates on the lower mudflat arose from increased rates of nitrification associated with increased O_2 penetration caused by a greater sediment porosity. Higher rates of D_h, and possibly also D_w ensue from bioirrigation which has been shown to stimulate D_w by increasing the rate of NO_3^- diffusion into the sediment (Rysgaard et al. 1995).

Interactions between DIN fluxes and sediment metabolism

It has previously been shown that net benthic metabolism will exert a controlling influence upon DIN fluxes in coastal sediments (Eyre and Ferguson 2002; Ferguson 2002). In this instance, it is probably inappropriate to extrapolate the short term metabolism measurements made in this study over a diurnal cycle (see (Rizzo et al. 1996) for discussion and also Chapter 3). Here, the light/dark TCO_2 flux ratio without any correction for day length has been used as a metabolism index. Other authors have used light:dark metabolism ratios in a similar manner (Rizzo et al. 1996; Viaroli et al. 1996). Figure 4.10 shows a plot of DIN fluxes with respect to light:dark TCO_2 flux ratio. It can be seen that under light conditions sediments with a light:dark TCO_2 flux ratio of >1 were generally a sink for DIN. Under dark conditions sediments with a light:dark TCO_2 flux ratio of greater than two were generally a sink for DIN. Theoretically, sediments with a light:dark TCO_2 flux ratio of greater than one, produce more carbon than is respired in the light, and therefore, one would expect a net assimilation of DIN under light conditions. A light:dark TCO_2 flux ratio of less than one indicates more carbon is respired than produced, and therefore, one would expect a net release of DIN under light conditions. Assuming roughly equal length of day and night, one would expect sediments with a light:dark TCO_2 flux ratio of greater than 2 to produce more carbon than is respired over a diurnal cycle. The good agreement between the observed and theoretical impact of

the light:dark TCO_2 flux ratio on benthic DIN fluxes shows that the light:dark TCO_2 flux ratio of a system is a useful indicator of the ecological functioning of a system.

In this instance the light:dark TCO_2 flux ratio illustrates how sediments will become net sinks for DIN with increasing autotrophy. This is a consequence of assimilation of nitrogen by both photoautotrophs and heterotrophs. Under autotrophic conditions the supply of carbon to the sediments from MPB will continually stimulate bacterial production and, hence, assimilation of NH_4^+ (see earlier discussion). Assimilation of nitrogen by MPB under autotrophic conditions further drives the system to a net consumption of nitrogen. This results in an intense competition for nitrogen between the assimilatory pathways of heterotrophic bacteria (Ferguson 2002) as well as MPB and the energy yielding pathways of nitrifiers and denitrifiers (see earlier discussion). The ultimate effect of this competition is a negative impact upon denitrification under both light and dark conditions (Figure 4.9b). This illustrates how biological processes and competition between organisms in oligotrophic systems will act to conserve nitrogen within the biological pool.

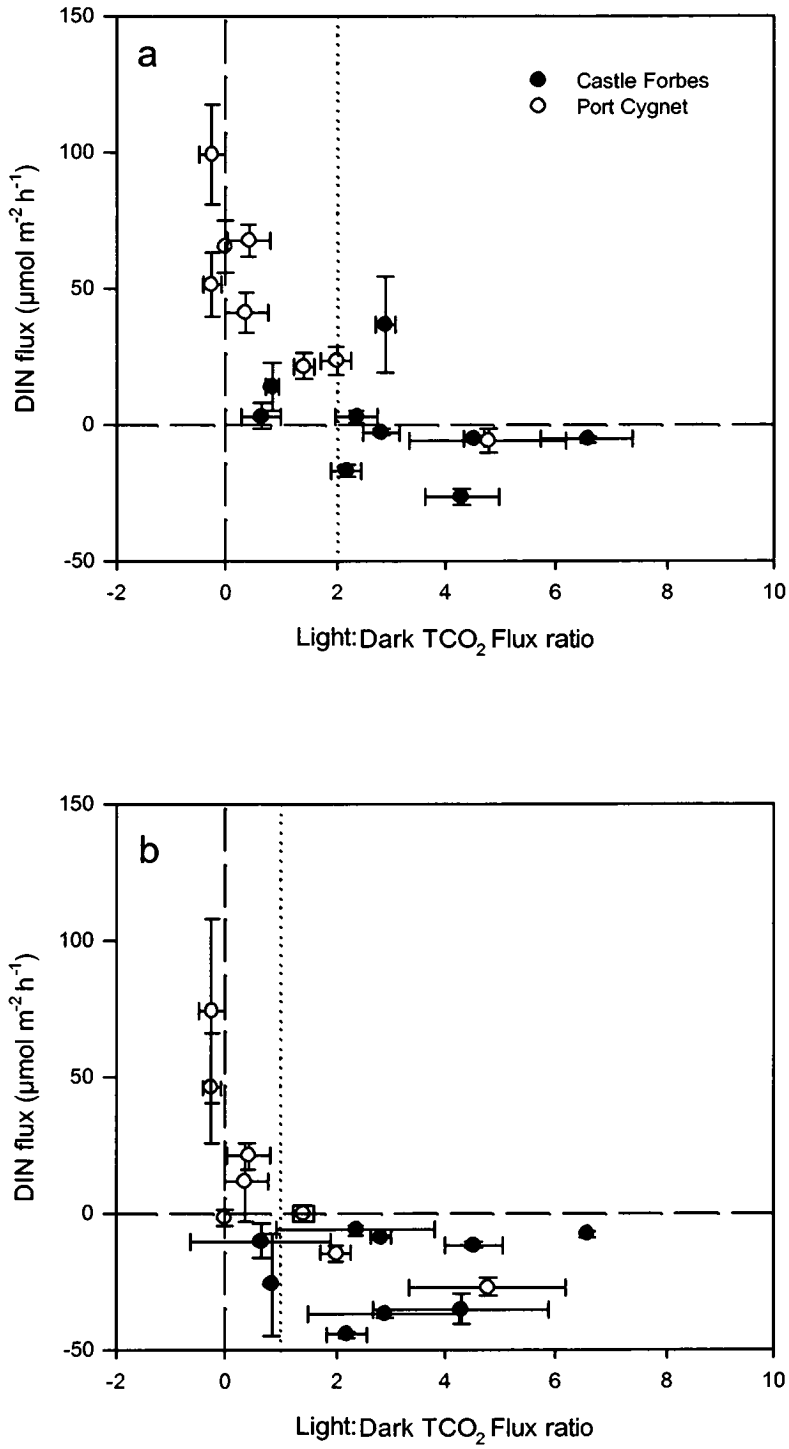


Figure 4.10. DIN flux ($NOx+NH_4^+$) versus light:dark TCO_2 flux ratio measured in the dark **(a)** and light **(b)** at Castle Forbes Bay and Port Cygnet. Dotted lines mark the light:dark TCO_2 flux ratios where one would theoretically see a transition between a release and an uptake of DIN (see text for further details). Error bars represent the standard error of the mean (n=4).

Chapter 5

Nitrogen assimilation by microphytobenthos on intertidal mudflats in a temperate Australian estuary

Abstract

The daily nitrogen demand of microphytobenthos (MPB) was calculated based upon the measured rates of primary production (TCO_2 assimilation) assuming balanced growth and algal cells with a molar C:N ratio of 10. Estimates of the actual uptake rate of nitrogen were also made from the measured fluxes of dissolved nitrogen between the sediment and the water column and the measured production rate of NH_4^+ within the sediment. At times of high primary production, the calculated demand for nitrogen was found to be well in excess of the measured uptake. Subsequent measurements of N_2 fixation using the acetylene reduction assay (calibrated using ^{15}N-N_2) showed that N_2 fixation could, at times, account for the observed deficit in nitrogen uptake. In general, however, N_2 fixation could not account for the deficit in dissolved nitrogen assimilation. It is suggested that a stoichiometric relationship between carbon and nitrogen assimilation reflecting the C:N ratio of algal cells will only occur during the initial development of the MPB biofilm. Once the MPB biofilm has become established the majority of carbon assimilation is directed into the production of extracellular organic carbon (EOC) such as extracellular polymeric substances (EPS), rather than cell growth. Under conditions of high EPS production carbon and nitrogen are not assimilated in a ratio reflecting that of algal cells (C:N~6–10).

Introduction

Production by microphytobenthos (MPB) is now recognised as a major contributor to primary production in estuaries and shallow coastal areas (Underwood and Kromkamp 1999). The high rates of primary production by MPB may be due in part to their ability to source nutrients from both the water column, as well as from within the sediment (MacIntyre et al. 1996; Underwood and Kromkamp 1999). Studies of MPB production have indeed found that nitrogen uptake from the water column was insufficient to meet the calculated nitrogen requirements of the MPB communities during summer when DIN concentrations in the water column were extremely low and primary production was highest (Kristensen 1993; Sundbäck et al. 2000). In these cases, it was suggested that regenerated nitrogen from within the sediment supplied most of the nitrogen demand by MPB.

Where much of the MPB consists of cyanobacteria such as in cyanobacterial mats, a significant fraction of the nitrogen demand of the community may be met by N_2 fixation (Stal 1995). These cyanobacterial communities generally form on sheltered sandflats, such as on the north sea in Germany and the barrier islands off the coast of Carolina (Stal et al. 1984; Bautista and Paerl 1985). These communities are commonly dominated by non-heterocystous cyanobacteria of the genera *Oscillatoria*, *Microcoleus* and *Lyngbya* (Paerl et al. 1991; Stal 1995; Paerl et al. 1996). While the importance of N_2 fixation in cyanobacterial mats (Pinckney et al. 1995; Stal 1995; Paerl et al. 1996) and coral reefs (Charpy-Roubaud et al. 2001, and references therein) has received widespread attention, the importance of N_2 fixation more generally by MPB, particularly in temperate areas, has received little attention. Given that cyanobacteria have been observed in temperate highly productive MPB (Kristensen 1993; Sundbäck et al. 2000 this study, Chapters 2 and 3), it seems highly likely that N_2 fixation may meet a significant fraction of the nitrogen requirements of MPB.

No studies of MPB to date have estimated the relative importance of nitrogen assimilated from the water column, regenerated within the sediment and N_2 fixation. In this chapter, modelled NH_4^+ production rates within the sediment, in combination with sediment-water nitrogen exchange rates and primary production measurements (presented in Chapters 3 and 4) are used to show the relative importance of nitrogen

derived from the sediment and the water column. The importance of N_2 fixation to MPB production is estimated from these results combined with a new data set presented here on N_2 fixation.

Materials and Methods

Nitrogen assimilation by microphytobenthos

The nitrogen demand by MPB was calculated using the gross primary production data (based on TCO_2 fluxes) presented in Chapter 3. The rates of nitrogen uptake by MPB were estimated using the dissolved nitrogen benthic fluxes, denitrification and sediment NH_4^+ production data presented in Chapter 4.

The daily effective photoperiod was the same as that used in Chapter 3. It was assumed production by MPB was saturated at the irradiance of 500 μE used in the laboratory and that algal production was also always saturated in the field during the effective photoperiod (see Chapter 3 for discussion).

Calculated nitrogen demand by MPB

Nitrogen demand by MPB was calculated from gross primary production data, assuming carbon and nitrogen were assimilated in proportion to their cellular C:N ratio, which was assumed to be 10. This was in the middle of the range of C:N ratios measured for MPB at these sites (Chapter 2, Table 2.1): it is also close to the value of 9 used by Sundbäck and Miles (2000). Such an approach is commonly used to estimate nitrogen assimilation by MPB (Dong et al. 2000; Sundbäck and Miles 2000; Thornton et al. 2002). The standard error due to spatial variation in gross production was propagated through this calculation as well as an uncertainty of 2 in the C:N ratio and an uncertainty of 1 h in the effective photoperiod.

Measured nitrogen uptake by MPB

If there was a flux of NH_4^+ into the sediment during illumination, then the assimilation by MPB of NH_4^+ produced within the sediment was calculated as follows:

$$A_{NH4}=P_{NH4}-J_D*D$$

Where

A_{NH4}=The daily assimilation of NH_4^+ by benthic microalgae

P_{NH4} = daily production rate of NH_4^+ within the sediment

J_D = dark efflux of NH_4^+

D = Daily dark period

If an efflux of NH_4^+ was measured from the sediment during illumination, it was assumed that NH_4^+ met nitrogen demand in excess of that measured for the uptake of dissolved nitrogen from the water column (see below).

For the spring budget calculation, the winter production rates of NH_4^+ within the sediment were used, as porewater profiles were not taken in spring. This seems reasonable given that the winter and spring temperatures were not greatly different and the calculated rates of NH_4^+ production within the sediment did not vary greatly between the three times at which this was measured. In some instances, a zone of NH_4^+ consumption was observed below the sediment surface (Chapter 4). For the purposes of this budget, it was assumed that all NH_4^+ assimilation near the sediment surface (0–3 cm) was due to assimilation by MPB (see Chapter 4 discussion).

Assimilation of water-column nitrogen species was calculated as follows

$$A_N = J_L * L + J_D * D$$

Where

A_N= assimilation of an individual nitrogen species

J_L= light uptake of nitrogen species

J_D= dark uptake of nitrogen species

L and D are the light and dark periods

The assimilation of NO_3^- from the water column by MPB was calculated after the uncoupled denitrification rate (D_w) had been subtracted from the NO_3^- influx to the sediment. Assimilation of NO_3^- produced within the sediment was assumed to be insignificant, as preliminary studies showed that NO_3^- was not detected in the surface porewaters.

The standard error due to spatial variability in NH_4^+ production rates, the light and dark fluxes of NH_4^+ as well as an uncertainty of one hour in the lengths of light and dark periods were propagated through the calculation. The final uncertainty in the calculated nitrogen demand and measured nitrogen uptake derived from the propagation of standard errors and estimated uncertainties has been termed the estimated experimental error.

N_2 fixation

N_2 fixation was determined using the acetylene reduction assay (ARA) according to Capone (1993) as follows. Eight cores were taken on the upper and lower mudflats at each site using perspex tubes (4.5 cm id × 15 cm) during February (summer), April (autumn), August (winter), September (spring) and October (spring) 2002. In each case, a plug of sediment approx 6 cm long was taken. The cores were then returned to the laboratory within one hour of collection and submerged in a water bath (40 l) of water collected from the site and incubated at in-situ water temperature. Cores were stirred and illuminated as described for the isotope pairing technique (Chapter 4). The following morning the water was drained from half of the cores taken from each position on the mudflat. The cores were then sealed with a perspex lid, which had a rubber septum, and 20% of the headspace replaced with acetylene that had been generated freshly from CaC_2. The cores were then left illuminated for 2 h to allow for an initial lag phase, which had been observed in ethylene production during preliminary experiments. A 5-ml sample of the headspace gas was taken using a 5-ml draw (6.5 ml volume) Vacutainer™ (Becton Dickinson). This was repeated twice over the course of the day. At dusk, the remaining cores which had also been illuminated throughout the day in the same water bath, were similarly drained and had their headspace replaced with 20% acetylene. The sampling procedure was then repeated with the cores in the dark. The daytime incubations generally lasted 6–8 h and the dark incubations lasted for up to 12 h.

In February, an experiment was undertaken to compare the rates of acetylene reduction in exposed and inundated cores. Eight cores were taken from the upper mudflat at Site CF. The cores were then illuminated the following day, at dusk four of the cores were completely drained. The remaining four cores had half of their overlying water drained and the stirrer bars, just submerged. The headspace of both

cores was then replaced with 20% acetylene and the cores sampled as described above.

Experiments were also undertaken to compare the rates of acetylene reduction in intact and slurried cores, and also to calibrate the ratio of N_2 reduced to acetylene reduced using ^{15}N-N_2. In February, cores were taken from the upper mudflat at Site CF, and the lower mudflat at Site PC. The cores were then illuminated for most of the following day. Four intact cores were incubated in the dark using the acetylene reduction assay as described above. For the remaining four or five cores, the top 0.5 cm of sediment was then taken and slurried with a small amount of site water. 3 ml of the slurry was then pipetted into eight 6.5-ml Vacutainers. The Vacutainers were sealed and 1 ml of air was withdrawn from four Vacutainers and replaced with 1 ml of acetylene. These containers were briefly shaken for a minute, before being allowed to incubate at 20 °C which was the same as in-situ temperature. Gas samples (50 µl) were then taken at 1, 5, 15 and 50-h intervals and analysed immediately for ethylene production. The Vacutainers were shaken before sampling. The remaining four Vacutainers were purged with a He/O_2 mixture before being shaken and purged again to remove all N_2 present in the slurry. 1 ml of gas was then withdrawn from the Vacutainers and replaced with 1 ml of $^{15}N_2$ (98% ^{15}N, Cambridge isotopes). They were shaken and allowed to incubate in parallel with those that had acetylene added. The Vacutainers were opened in a time series corresponding to the sampling times for those samples being incubated with acetylene (1, 5, 15, 50-h intervals). Upon opening, the slurry was immediately frozen for later analysis of ^{15}N.

Ethylene in the samples was determined the next day as follows. 50 µL of gas was injected into a Hewlett Packard 5890 gas chromatograph equipped with an Alltech AT Alumina column (30 m, 0.53 mm id) and a flame ionisation detector. Samples were quantified against standards prepared from the serial dilution of ethylene (98%, BOC, Chatswood, Australia). Sample concentrations were corrected for dilution, which occurred as a consequence of 5 ml of gas being drawn into a 6.5-ml Vacutainer. The detection limit was 1.5 ppm ethylene with a precision of ~5% at 63 ppm. The rate of ethylene production was calculated using linear regression of the concentration change in ethylene over time as follows.

$$flux = \alpha \times \frac{V}{A}$$

where

α= linear regression slope of ethylene concentration in sediment core (µmol/l/h)

V= headspace volume (l)

A= sediment surface area (m^{-2}).

For the slurried cores, the rates of acetylene production were calculated on an areal basis using the known surface area of sediment from which the slurry had been made (4–5 cores). Cores were also run without the addition of acetylene to ensure that no ethylene was naturally produced within these sediments. For cores incubated with overlying water, the concentration of ethylene in the gas phase was corrected using the Bunsen coefficient for ethylene as described in Capone (1993). It was assumed that all the ethylene diffused out of the sediment into the water/gas phase. Sediment samples for the determination of ^{15}N enrichment were dried in an oven at 60°C before being ground and weighed into tin cups (Elemental Microanalysis Ltd., UK). The $\delta^{15}N$ and %N in the samples were analysed using a Carlo Erba NA1500 CNS analyser interfaced *via* a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer operating in continuous flow mode. This instrument and standards were calibrated for natural abundance measurements of ^{15}N content, which is expressed in the $\delta^{15}N$ convention:

$$\delta^{15}N(‰) = \left[\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{atmosphere}} - 1\right] \times 1000$$

Where $(^{15}N/^{14}N)_{atmosphere}=0.003676$

The atom% ^{15}N in the sample was then calculated as follows (Montoya et al. 1996):

$$\text{Atom\%}^{15}N = 100 \times \frac{(10^{-3}\delta^{15}N + 1)(^{15}N/^{14}N)_{atmosphere}}{1 + (10^{-3}\delta^{15}N + 1)(^{15}N/^{14}N)_{atmosphere}}$$

Linear regression analysis of the amount of excess ^{15}N in the sediment versus time was used to calculate the rate of ^{15}N fixation. The actual rate of N_2 fixation was then calculated after correction for the enrichment in the ^{15}N-N_2, which was 98%.

Chlorophyll measurements

Chlorophyll was extracted as described in Chapter 2. Extracts were analysed using the HPLC method as described in Chapter 2, or spectrophotometrically using a GBC UV/Vis spectrophotometer, and the equations of Jeffrey and Humphrey (1975).

Statistical analysis.

Statistical analysis was carried out using Statistica Vers 6.0 (StatSoft Inc). 1-way and 2-way analysis of variance (ANOVA) were carried out on log-transformed data. Where log transformation failed to correct heteroscadisticity as indicated by a Cochrans C test, a non-parametric test was used. Correlation and multiple regression analysis were used to explore relations between variables.

Results

Algal nitrogen assimilation budget

The measured rates of nitrogen uptake compared to calculated nitrogen demand by MPB are presented in Figure 5.1. Calculated algal nitrogen demands by MPB ranged between 440 μmol N m^{-2} day^{-1} at Site CF lower during winter and 12500 μmol N m^{-2} day^{-1} at Site CF upper during spring. At Site CF, the calculated nitrogen demand by MPB was consistently much greater than the measured nitrogen assimilation, with the exception of during winter. During winter, low rates of primary production and relatively high water column concentrations of NO_3^- meant the demand for nitrogen by MPB could be met by supply from the sediment and the water column. At Site PC, the nitrogen demand by MPB was generally much less as a consequence of the much lower rates of primary production observed at this site (Chapter 3).

At Site CF, DON made up a significant fraction of nitrogen assimilated, particularly at CF lower, where DON generally dominated nitrogen uptake by the sediment during autumn and spring. As a consequence of the low rates of NH_4^+ production in the sediment at this site (Chapter 4), NH_4^+ produced within the sediments only appeared to be of minor importance to MPB at Site CF lower. At Site CF upper, NH_4^+ produced within the sediment made up a variable fraction of nitrogen

assimilated, during autumn over 90% of assimilated nitrogen was from NH_4^+ produced within the sediment, and during spring <30% of nitrogen assimilated came from within the sediment. NH_4^+ produced within the sediments was the most importance source of nitrogen to MPB at Site PC year round. No significant fluxes of DON were observed at Site PC. DIN from the water column generally only comprised very small fraction of measured nitrogen uptake by MPB except during winter, when concentrations of NO_3^- and NO_2^- were elevated in the water column.

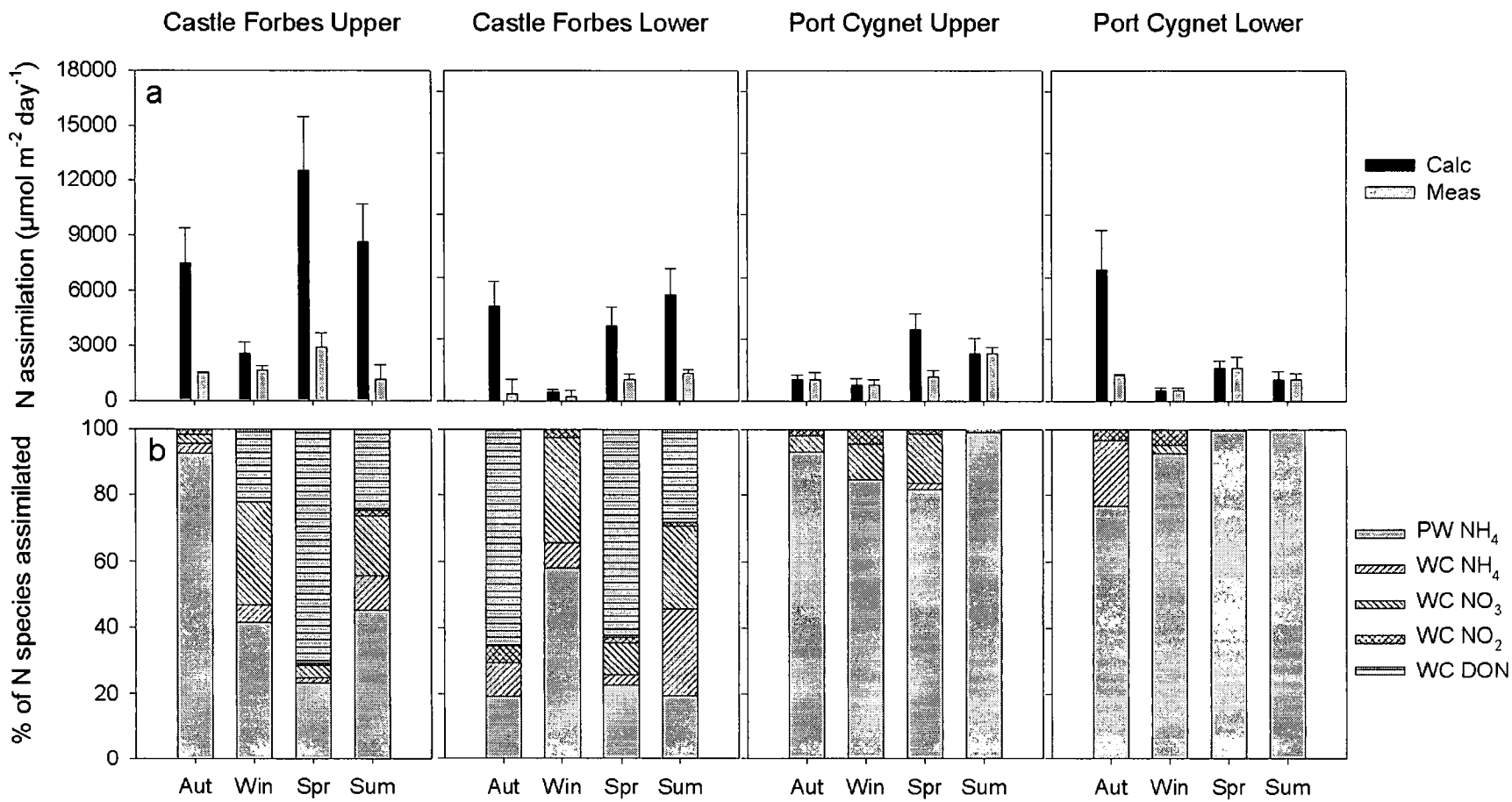


Figure 5.1. **(a)** The measured (meas) rates of assimilation of dissolved nitrogen by MPB compared to the calculated (calc) rates of assimilation using gross TCO2 uptake rates and assuming algal cell growth with a C:N ratio of 10 at each of the study sites during 2001. **(b)** The proportions of the various forms of nitrogen assimilated from the water column (WC) and porewater (PW) by MPB. Error bars represent the standard error of the mean (n=3–4).

N_2 fixation

Comparative experiments

Table 5.1 shows the results of experiments undertaken to compare rates of N_2 fixation measured in exposed, innundated and slurried cores as well as a comparison of the acetylene reduction assay (ARA) and measured rates of ^{15}N-N_2 uptake. Rates of N_2 fixation in the exposed and inundated cores were found to be not significantly different (t-test, $P>0.05$). Rates of N_2 fixation in the slurried and intact cores were, however, found to be significantly different at both Site PC and CF (t-test, $P<0.001$).

Calibration of the acetylene reduction assay

Figure 5.2 shows a plot of excess ^{15}N versus time at both Site PC and CF. It can be seen that at Sites CF and PC there was a linear increase in the ^{15}N enrichment within the sediment over time. At Site CF, the production of ethylene was markedly non-linear with rates of acetylene reduction increasing after about 20 h of incubation. At Site PC, the rate of acetylene reduction remained linear over the course of the incubation period. The rate of acetylene reduction was calculated using only data obtained within the first 20 h of incubation for both sites. The average ratio between acetylene reduced and ^{15}N fixed was 5.0 at Site CF and 7 at Site PC (Table 5.1).

Table 5.1. Mean rates of acetylene reduction (μmol m^{-2} h^{-1}) measured in intact, inundated and exposed cores, as well as slurried cores. Also N_2 fixation (μmol m^{-2} h^{-1}) measured using ^{15}N-N_2 in a slurried core and the ratio between acetylene reduced and N_2 fixed in slurried cores. Values in brackets are the standard error of each mean (n=3–5). CF is Castle Forbes Bay, PC is Port Cygnet.

Site	Innundated intact Core	Exposed intact Core	Slurried Core	$^{15}N_2$ fixation rate	Acet:N_2 ratio
CF	500 (160)	520 (90)	87 (9)	17 (1)	5.0 (0.7)
PC	-	30 (8)	4.2 (0.7)	0.6 (0.1)	7 (2)

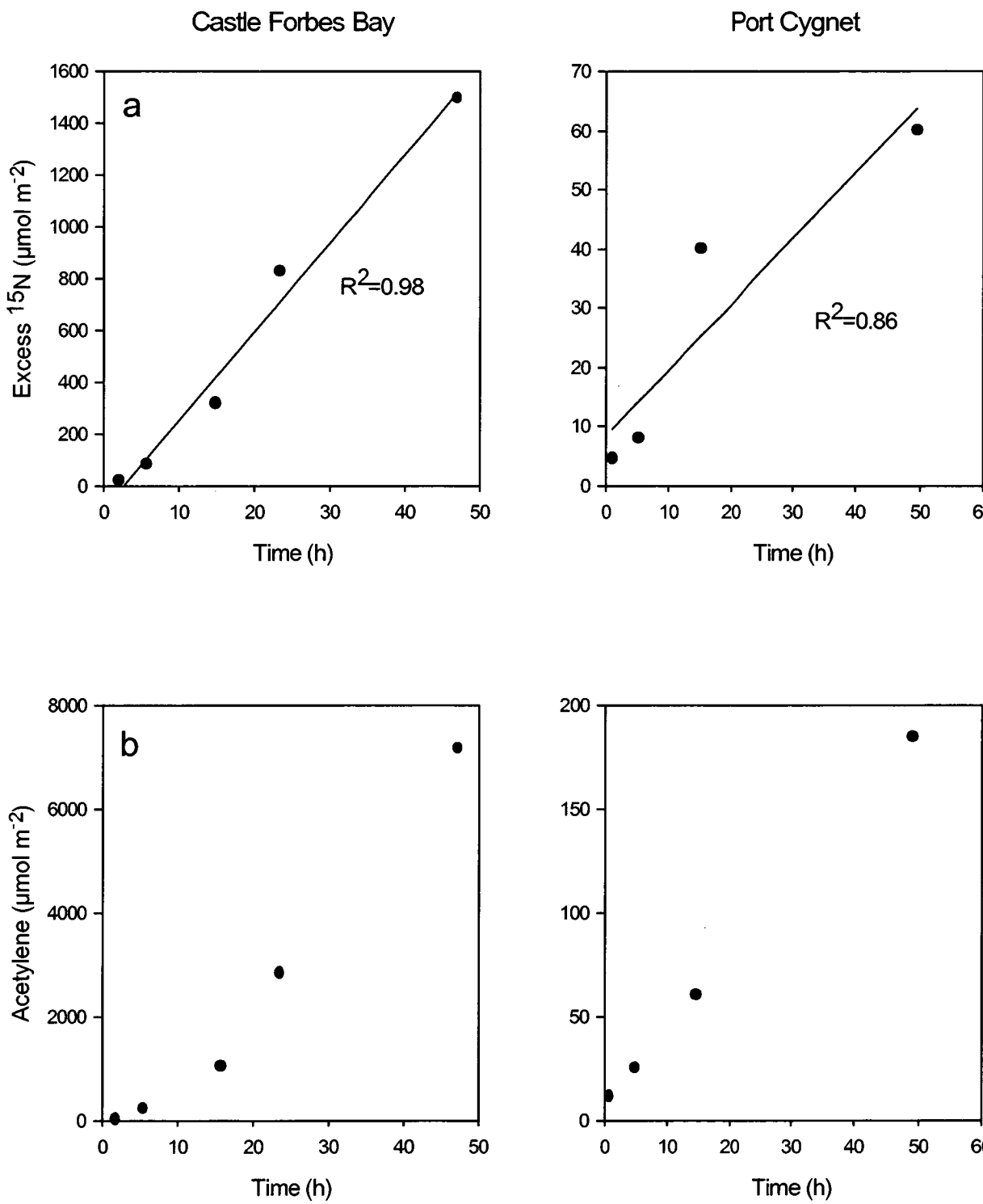


Figure 5.2. **(a)** A plot of excess ^{15}N versus time in sediment slurries taken from both study sites, measured during February 2002 and **(b)**, a plot of acetylene reduced versus time run in parallel incubations of the same slurry.

N_2 fixation rates in the field

The rates of N_2 fixation measured using the ARA are shown in Figure 5.3. Data taken for the intact core and slurry comparison is also presented and, therefore, two samples are shown for summer at these sites. Rates of N_2 fixation measured by acetylene reduction were highly variable, ranging from undetectable up to 250 μmol m^{-2} h^{-1}. The highest rates of N_2 fixation were observed on the upper mudflat at Site CF during summer. It was also at this site that the greatest deficits in measured nitrogen uptake compared to calculated requirements were observed (Figure 5.1). The highest rates of primary production and algal biomass were also observed at this site during 2001 (see Chapters 2 and 3). N_2 fixation rates were generally highest in the dark at Site CF upper and CF lower. At Site PC, by contrast, the rates of N_2 fixation tended to be greater in the light. Dark rates of N_2 fixation were significantly greater at Site CF than at Site PC on an annual basis (Kolmogorov-Smirnov test, $p<0.025$). There was no significant difference between the light N_2 fixation rates between Sites PC and CF on an annual basis.

Chlorophyll a

Chlorophyll *a* (chl *a*) concentrations were generally highest at Site CF upper, with concentrations of up to 400 mg m^2 being recorded. Chl *a* concentrations were significantly greater during 2002 than during 2001 (data presented in Chapter 3) (1-way ANOVA, $p<0.01$). N_2 fixation rates in the light were weakly but significantly related to chl *a* ($r = 0.51$, $p = 0.05$).

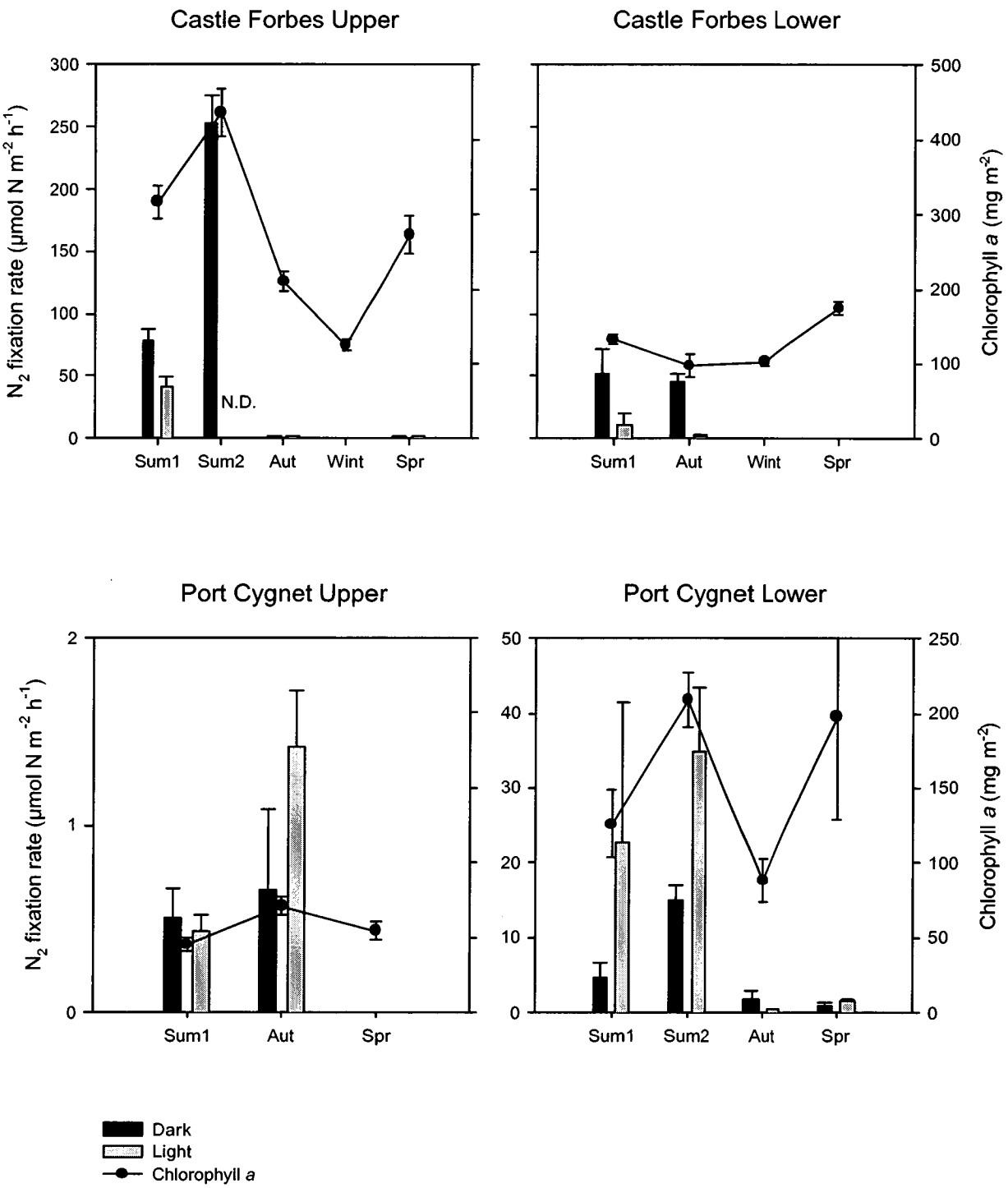


Figure 5.3. The measured rates of nitrogen fixation using the acetylene reduction assay under light and dark conditions and sediment chlorophyll *a* concentrations at each of the study sites during summer (Sum) autumn (Aut) winter (Win) and spring (Spr) 2002. Sum 1 and Sum 2 refer to samples taken on 3-Feb and 13-Feb, respectively at Site CF upper and 6-Feb and 10-Feb at Site PC lower, respectively. Rates of nitrogen fixation were calculated using an acetylene to N_2 ratio of 4 (see text for details). N.D. = not determined. Error bars represent the standard error of the mean (n=3–4).

Discussion

Ratio of acetylene reduced to N_2 fixed.

The process of N_2 fixation can be summarised by the following equation:

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$$

A theoretical conversion ratio between C_2H_2 and N_2 reduced of 3:1 is often employed. This is based on the fact that the reduction of N_2 to NH_3 requires 6 electrons, while the reduction of C_2H_2 to C_2H_4 requires 2 electrons. This equation does not take into account the hydrogenase reaction, which results in the evolution of H_2 under N_2 fixing conditions. The addition of C_2H_2 will inhibit the hydrogenase reaction, which means that 8 mol of reductant will be used to reduce 4 mol of C_2H_2 to 4 mol of C_2H_4. Under natural conditions, 8 mol of reductant would be used to reduce 1 mol of N_2 to 2 mol of NH_3 and 1 mol of H_2. A conversion ratio of 4:1 is, therefore, likely to be more appropriate (Postgate 1982). Recent studies have generally shown good agreement with the theoretical ratio of 4:1 in pelagic cyanobacteria (Montoya et al. 1996) and references therein. Here, the ratios of C_2H_2 reduced to N_2 fixed were found to be 5.0 at Site CF and 7 at Site PC. Calibration of the acetylene N_2 ratio has rarely been performed on benthic cyanobacterial samples (Potts et al. 1978; Charpy-Roubaud et al. 2001). The ratios found here are close to the range found by Potts et al. (1978) of 4.7–5.7, but were much higher than the range of values found by Charpy-Roubaud et al. (2001), which were generally less than 4:1. The acetylene:N_2 ratios determined here, were within two of their respective standard errors of the theoretical acetylene:N_2 ratio. It was, therefore, assumed that the measured ratio was not significantly different to the theoretical ratio of 4:1.

Comparison of measured N_2 fixation rates with other systems

The rates of N_2 fixation measured in this study, were comparable to those reported in other temperate intertidal sediments. In an intertidal cyanobacterial mat community, Paerl et al. (1996) reported acetylene reduction rates which if converted to N_2 fixation rates (using an acetylene to N_2 ratio of 4), ranged from less than 5 μmol N m^{-2} h^{-1} up to 200 μmol N m^{-2} h^{-1}. As with this study, the highest rates were observed at night during summer. In a Massachusetts salt marsh, N_2 fixation rates by

cyanobacteria ranging from essentially undetectable up to ~700 μmol N m^{-2} h^{-1}, with values generally falling in the range of 30–60 μmol N m^{-2} h^{-1} were observed (Carpenter et al. 1978). It was found that light and an availability of fixed nitrogen were the primary factors controlling N_2 fixation in this system. The low winter rates of N_2 fixation in my study, and the others cited above, are most likely a consequence of suppressed nitrogenase activity due to low light availability, low temperatures and higher concentrations of dissolved inorganic nitrogen within the water column.

In comparison with temperate seagrass beds, the rates of N_2 fixation measured in this study were generally high. Rates of N_2 fixation in temperate *Zostera* beds have been found to be consistently in the range of 1–20 μmol N m^{-2} h^{-1} by a number of studies in Europe (Welsh et al. 1996a; McGlathery et al. 1998; Welsh et al. 2000). The rates of N_2 fixation within these systems (McGlathery et al. 1998; Welsh et al. 2000), however, appear to be maintained more consistently throughout the year compared to those observed here.

While cyanobacterial mats exhibit high rates of N_2 fixation, their importance to coastal systems is often limited due to their restricted areal distribution. The sediments studied here could not be described as a cyanobacterial mat in the conventional sense (See Chapter 2). Nevertheless, high rates of N_2 fixation were observed within these sediments, including those on the lower mudflats, which were submerged for greater than 99% of the time. A recent study also reported N_2 fixation rates of up to 300 μmol m^{-2} h^{-1} in "unvegetated" subtidal sediments, which had high rates of primary production stimulated by organic carbon additions (Newell et al. 2002). This may suggest that intertidal and shallow euphotic sediments may be capable of comparable rates of N_2 fixation to those observed in cyanobacterial mats, and in excess of rates observed in temperate seagrass communities. Seagrass communities have been suggested as important sites of N_2 fixation in coastal systems (Moriarty and O'Donohue 1993). The potential importance of unvegetated sediments that have a mixed MPB community, including cyanobacteria, should not be overlooked when considering sources of newly fixed nitrogen within coastal systems.

Intertidal sediments only make up about 5% of the upper Huon Estuary and Port Cygnet, so nitrogen fixation by MPB on the intertidal mudflats is likely to be of limited importance in the upper estuary and Port Cygnet. While only intertidal sediments were considered in this study, the lower mudflats were inundated for >99% of the time and may, therefore, be representative of shallow sediments more generally. Approximately a third to a half of the upper Huon Estuary has an average water depth of less than one meter (Butler et al. 2000). It is, therefore, likely that the rates of nitrogen fixation measured at Site CF lower were representative of up to half of the upper estuary. This being the case, it seems likely that nitrogen fixation would provide an important input of bioavailable nitrogen to the benthic and estuarine foodwebs.

Core treatment

As stressed by Capone (1993), I considered it imperative to maintain the intact sediment structure during the acetylene reduction assays. In the Huon Estuary mudflats, a large fraction of the inorganic nitrogen assimilated by MPB was supplied by a flux of remineralised nitrogen from within the sediment (Figure 5.1). Destroying the sediment structure either by slurrying the cores or separating the MPB from the sediments was considered likely to alter the rates of N_2 fixation. The effect of slurrying the cores was illustrated in Table 5.1, where it can be seen that rates of N_2 fixation were 6–7 times lower than those measured in intact cores. It is suggested that this occurred as a consequence of the N_2-fixing cyanobacteria at the sediments surface (see discussion section, N_2-fixing organisms) being exposed to high concentrations of NH_4^+ from within the porewaters, thus, inhibiting nitrogenase activity (Carpenter et al. 1978; Welsh et al. 1996b). The rapid inhibition of nitrogenase activity after the addition of inorganic nutrients has previously been demonstrated in benthic systems, including seagrass and cyanobacterial mat communities (Joye and Paerl 1993; Welsh et al. 1997). Alternatively, this difference may have been caused by a physical disturbance of the microbial community, which exposed nitrogenase to O_2, thus inhibiting its activity. Damage to algal cells by shaking the slurry is also a possible reason for the lower N_2 fixation rates observed in the slurries.

It has previously been observed that tidal inundation and exposure may affect rates of N_2 fixation (Currin and Paerl 1998). The possibility of tidal inundation and

exposure was investigated here by conducting incubations in exposed and inundated cores. No significant difference was found in N_2 fixation rates measured in emersed and inundated cores run in the dark at Site CF upper. The tidal cycle was, therefore, assumed not to have a significant affect on N_2 fixation in this study.

N_2-fixing organisms

The high rates of N_2 fixation observed at Site CF, were most likely carried out by cyanobacteria, including *Oscillatoria* spp., which were observed on the surface of the mudflat at this site. Pigment analysis also showed that cyanobacteria made up a significant fraction of the MPB community at both Sites CF and PC. There was also a significant variation in N_2 fixation rates in response to light. Rates of light N_2 fixation were significantly related to levels of chl *a* in the sediment surface ($r=0.51$, $p=0.05$), supporting the contention that cyanobacteria were responsible for N_2 fixation. The significantly greater rates of N_2 fixation seen in the dark at Site CF are consistent with previous studies of sediments colonised by non-heterocystous cyanobacteria (Stal 1995). This nighttime maximum in N_2 fixation most likely arises from oxygen inhibition of the nitrogenase enzyme during daytime photosynthesis. At Site PC, significantly greater rates of N_2 fixation were observed during the day. No microscopic examination of the sediment was undertaken at this time of high N_2 fixation, and so the identity of the dominant cyanobacterium is unknown. It does, however, seem likely that that this community was dominated by a heterocystous species of cyanobacteria which can show daytime maxima in N_2 fixation (Stal 1995).

The assay performed here only measured N_2 fixation at the sediment surface because no acetylene was injected into the sediment. A large number of anoxic sediment bacteria are, however, also capable of N_2 fixation within the sediment (Herbert 1999). Nevertheless, it was assumed that there was unlikely to be any significant nitrogenase activity within these sediments for two reasons. Firstly, high NH_4^+ concentrations were observed within the porewaters which have been shown to be inhibitory to nitrogenase activity (Carpenter et al. 1978; Welsh et al. 1996b). Secondly, N_2 fixation is also an energy-intensive process and high rates of N_2 fixation below the sediment surface are generally associated with the release of labile organic carbon from rooted macrophytes (Welsh et al. 1997; McGlathery et al. 1998; Herbert 1999). As a consequence, reported rates of heterotrophic N_2 fixation within unvegetated sediments are generally low compared with the rates measured here.

For example, Nedwell and Abdul Aziz (1980) cite annual rates of heterotrophic N_2 fixation equivalent to <1 μmol m^{-2} h^{-1}, which is insignificant compared to the higher rates measured here at the sediment surface. Previous experiments on mudflats have also demonstrated that nitrogenase activity is insignificant below a depth of 0.5 cm (Stal et al. 1984).

Nitrogen assimilation budget approach

Two approaches were used to calculate the assimilation of NH_4^+ from the porewaters by MPB. MPB have been shown to be capable of assimilating NH_4^+ in the dark (Rysgaard et al. 1993), therefore, calculating the assimilation of NH_4^+ by MPB as the difference between the light and the dark fluxes of NH_4^+ will underestimate the true uptake rates of NH_4^+ derived from within the porewaters. This is exemplified in Figure 4.3 in Chapter 4, where it can be seen that there was an uptake of NH_4^+ by the sediment in the dark at Site CF upper. In these circumstances, the difference between the measured NH_4^+ flux and the calculated rate of NH_4^+ production within the sediment provide a good means of estimating the assimilation of NH_4^+ by MPB. However, at times of low MPB activity, only a small amount of NH_4^+ produced within the sediment will be assimilated. Under such conditions, the magnitude of the measured efflux of NH_4^+ may approach, or even exceed the calculated production rate within the sediments, as was the case at Site PC lower during winter and summer. This will arise as a consequence of spatial and temporal variability, as well as the enhancement of sediment fluxes by bioirrigation and macrofaunal excretion, which were not taken into account in the modelled production rates of NH_4^+ within the sediment. At times of low productivity then, the use of modelled NH_4^+ production rates was an unsuitable means to obtain an estimate of NH_4^+ assimilation rates by MPB. At these times an efflux of NH_4^+ was generally observed from the sediment during periods of illumination, particularly at Site PC. Under such conditions it seems reasonable to assume that the MPB would be able to derive all their nitrogen requirements from NH_4^+ being released from the sediment. Therefore, any deficit in the calculated nitrogen requirements of MPB after nitrogen uptake from the water column had been accounted for was assumed to have been filled by NH_4^+ derived from the sediment.

Dissolved organic nitrogen assimilation

Uptake of DON by organisms in the sediment was observed on a number of occasions under both light and dark conditions at Site CF (Figure 4.3, Chapter 4). In these instances this uptake of water-column DON was well in excess of that for DIN, suggesting that MPB were heavily reliant upon DON as a nitrogen source. The vast majority of dissolved nitrogen in the Huon Estuary is in the form of DON, which is delivered by the Huon River in the form of terrestrially derived humic substances (Butler et al. 2000). Recent studies have suggested that DON in such a form may be bioavailable to microalgae, with up to 23% of DON from forested catchments being utilised by algal cultures (Carlsson et al. 1999; Seitzinger et al. 2002). The peak in DON uptake occurred at Site CF in spring, coinciding with the time of highest productivity at the site. This suggests that MPB may be capable of assimilating significant quantities of DON to meet their nitrogen requirements. During spring at Site CF upper, the uptake of DON from the water column met approximately 17% of the total calculated nitrogen requirement for the MPB. It should be noted at this point that no previous studies have demonstrated the uptake of DON by MPB. A recent study has shown that the urease activity of marine algae may be highly species specific (Fan et al 2003) and therefore the uptake of DON MPB remains speculative. The possibility that bacteria within the sediment were using DON from the water column should also be considered. It has previously been suggested (see Chapter 4) that bacteria within the surface sediments are actively consuming nitrogen, and therefore, the consumption of DON by bacteria cannot be ruled out. It has been shown that marine bacterioplankton are also capable of assimilating DON derived from riverine sources (Stepanauskas et al. 1999). The dark uptake of DON may further point to bacterial activity, although the enhanced uptake in the light does suggest that uptake by MPB is at least partially responsible for the uptake of DON. While I am unable to categorically ascribe a particular sink for the uptake and release of DON from the sediments these results highlight the importance of measuring DON fluxes at sites where MPB are present. For the purposes of this budget, it was assumed that DON was being utilised exclusively by MPB.

The discrepancy between measured nitrogen uptake and calculated demand

At Site CF, the calculated amount of nitrogen assimilated was always in excess of that measured during autumn, spring and summer, suggesting that a source of

nitrogen other than dissolved nitrogen was being assimilated. Uncertainties in the C:N ratio of MPB, the true effective photo-period and natural variability will all lead to errors in the estimates of the calculated and measured nitrogen uptake rates. Some of the difference between the measured and calculated nitrogen uptake rates may simply arise from experimental error. In order to obtain a conservative estimate in the difference between the measured and calculated nitrogen uptake, the following approach was used. One estimated experimental error (see methods section) was added to the measured nitrogen assimilation rates and one estimated experimental error was subtracted from the calculated nitrogen assimilation rates. The difference between these two rates was, thus, used as a conservative measure of "missing" nitrogen. Using this approach, there was still a significant deficit in the nitrogen assimilation budget on an annual basis at Site CF upper and lower, as well as Site PC lower. Expressed as an annual average on a daily basis, this amounted to 3600, 1500 and 360 $\mu mol.m^{-2}\ day^{-1}$ at Sites CF upper, CF Lower and PC lower, respectively.

In this study, a comparison of the rates of N_2 fixation and measured deficits in nitrogen assimilation compared with nitrogen uptake estimated from the C:N ratio and TCO_2 assimilation is complicated by the fact that the N_2 fixation and nutrient / TCO_2 uptake measurements were made in different years. Interannual variability in rates of primary production and nitrogen availability may confound the interpretation of these results. The temporal variations of dissolved nitrogen in the Huon Estuary have been well characterised by the recently completed Huon Estuary study (Butler et al. 2000). The three-year study showed that the concentration of dissolved inorganic nitrogen (DIN) in the surface waters of the estuary followed a well defined annual cycle with maximum concentrations (~7 μM) observed between April and October. During the warmer months between October and April, DIN concentrations in the surface waters were always lower and generally <1 μM. Based on these data, it seems reasonable to assume that the rates of nitrogen supply from the water column to the MPB are not likely to differ significantly between 2001 and 2002. The rates of nitrogen supply from within the sediment were also assumed not to vary between 2001 and 2002. Chl *a* concentrations measured during the 2002 N_2-fixation surveys were significantly greater than those measured during 2001 (see results section). Given greater chl *a* concentrations and the significant positive relationship between chl *a* and N_2 fixation it is highly likely that the measured rates

of N_2 fixation during 2002 would be greater than those occurring in 2001. The rates of N_2 fixation measured in 2002 could thus be considered a maximum estimate for 2001.

At certain times, the measured rates of N_2 fixation, such as those measured in February 2002 at Site CF upper, were able to account for the measured deficit in nitrogen uptake that occurred during December 2001 at this site. At other times, the measured rates of N_2 fixation fell well short of the measured deficit, such as those measured in October 2002 at Site CF upper compared to September 2001 at this site. Based on the measurements made here, it was estimated that the annual average N_2 fixation rates were 850, 430, 10 and 220 μmol m^{-2} day^{-1} at Sites CF upper, CF lower, PC upper and PC lower, respectively. These rates represented 24%, 29% and 147% of the conservatively estimated nitrogen deficits given earlier for Sites CF upper, CF lower and PC lower. Thus, at Site CF in particular, there was still a significant discrepancy between the measured and calculated nitrogen assimilation rates when measured rates of N_2 fixation were considered. Furthermore, a large deficit between calculated nitrogen demand and measured uptake, occurred at Site PC lower during autumn, when cyanobacteria only made up a very small fraction of the community (Chapter 2, Figure 2.9). Therefore, nitrogen fixation was unlikely to have made up the deficit in MPB nitrogen demand at this time.

A number of oceanic field studies have also shown that measured rates nitrogen assimilation fall short of those expected based on CO_2 assimilation and Redfield stoichiometry (Mulholland and Capone 2001; Engel et al. 2002 and references therein). Studies of phytoplankton cultures have shown that whether or not there is a stoichiometric relationship between carbon and nitrogen assimilation by phytoplankton depends heavily on their growth phase and nutrient availability. In a study of N_2 fixation and primary production in *Trichodesmium* sp. cultures, it was found that rates of N_2 fixation closely tracked CO_2 assimilation in the log phase of growth. After the log phase of culture growth, N_2 fixation declined relative to CO_2 fixation. This excess of carbon assimilation over nitrogen was not converted into algal biomass as the cumulative CO_2 assimilation rate also exceeded POC accumulation by over 50% (Mulholland and Capone 2001). It was suggested that the excess of carbon fixed over the build up of POC was respired, or possibly lost as

DOC. In a mesocosm study of diatoms, it was found that rates of gross production continued at similar rates after NO_3^- within the medium had been depleted, and hence, the assimilation of carbon in relation to nitrogen approached infinity (Engel et al. 2002). In that study, it was observed that at the onset of NO_3^- depletion, a rapid increase in the production of extracellular polymeric substances (EPS) occurred. Increased rates of EPS production appear to be a generally observed phenomenon during nutrient limitation in phytoplankton as well as benthic diatoms and cyanobacteria (Decho 1990; Stal 1995; Staats et al. 2000; Thornton 2002), and most likely occurs as a consequence of overflow metabolism (Stal 1995; Staats et al. 2000).

EPS may only account for a small fraction (~20%) of total extracellular organic compounds (EOC), which are made up of carbohydrates and simple sugars (Decho 1990; Underwood et al. 1995). During periods of low nutrient availability then, the bulk of carbon assimilation may be directed into the synthesis of EOC instead of cellular growth. MPB in particular, have been observed to have high rates of EOC production, with up to 73% of carbon fixed being converted into EOC, compared to 23% for phytoplankton (Goto et al. 1999). High rates of EOC production may thus provide a sink for the excess of carbon assimilated over nitrogen observed in this study.

As for the culture studies, the stage of maturity of the MPB community is likely to be of fundamental importance in determining whether or not uptake of carbon and nitrogen are observed in proportions close to the C:N ratios of the algal cells. Here, I have taken a previously proposed scheme for the development of a biofilm in cohesive intertidal sediments (Yallop et al. 2000) and modified it slightly based on the culture studies discussed above. This scheme is presented in Figure 5.4, and is used to help explain the observed patterns of carbon and nitrogen assimilation observed on the Huon Estuary mudflats. Four phases of growth have been denoted here. In phase 1 of growth, a "seed" population of MPB begin an initial period of rapid cell growth, where nitrogen and carbon are assimilated in proportion to the C:N ratio of the algal cells. Evidence for such a period of growth comes from the February surveys of Sites CF upper and PC lower (Figure 5.3), when algal biomass increased rapidly between the two dates sampled and rates of N_2 fixation were high.

In phase 2, cell growth rates are maximal and carbon and nitrogen assimilation rates attain their maximum values. In this stationary phase of cell growth, the rate of EPS and presumably EOC production begins to increase rapidly (Decho 1990; Underwood and Smith 1998). The samples taken on 13-Feb and 10-Feb at Sites CF upper and PC lower are most likely examples of samples taken during this phase of the MPB growth. In Phase 3, nutrients become limiting — where nitrogen assimilation is supported by N_2 fixation, phosphorus is likely to become the limiting nutrient — cell growth rates and nutrient assimilation begin to fall, maximum biomass of the community is reached. High rates of primary production continue, with the bulk of production being converted into EOC. The samples taken during spring at Sites CF upper and lower, as well as Site PC lower, represent this stage of the MPB growth cycle. In Phase 4, the MPB biofilm begins to degrade. The survey taken during autumn at Site CF upper most likely captures this phase of the cycle, when biomass is decreasing and rates of N_2 fixation are also low. Under such a scenario the rate of carbon assimilation will be a poor indicator of cell growth, rather rates of N_2 fixation will provide a better measure of cell growth as suggested by Mulholland and Capone (2001).

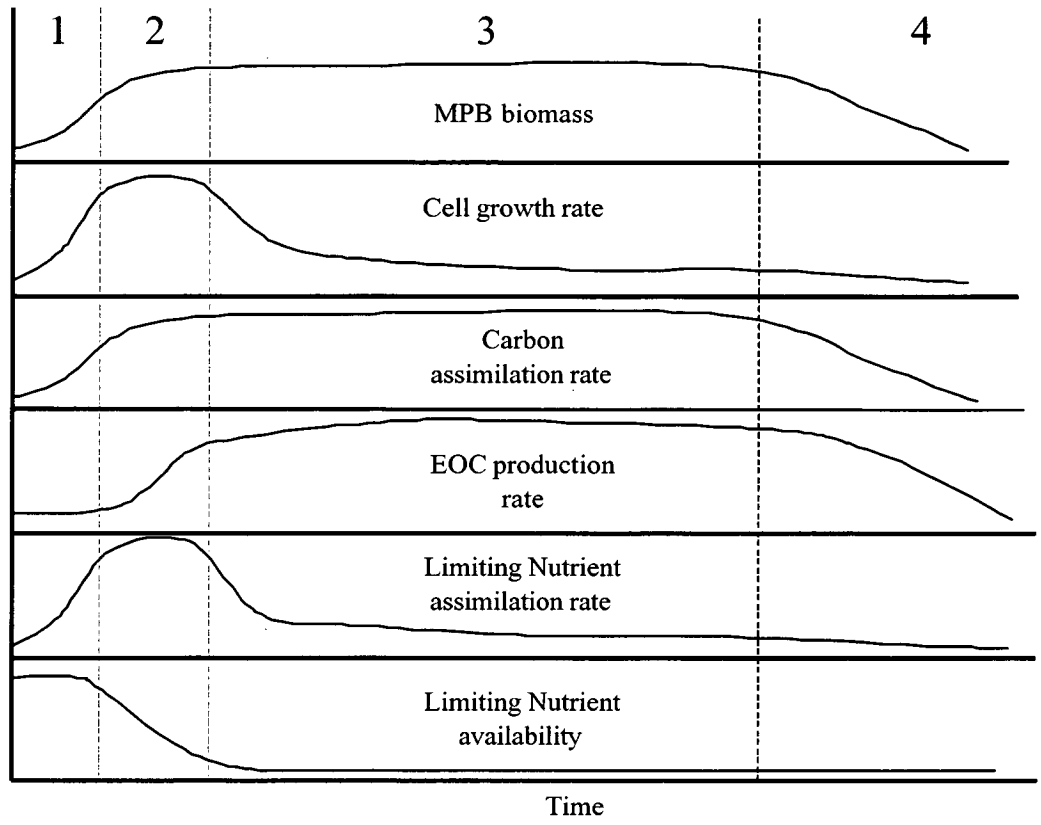


Figure 5.4. A proposed scheme for the changes in a range of variables over four phases in the development of a biofilm in intertidal sediments (modified from Yallop et al. (2000)). Phase 1 – colonisation and active growth of microphytobenthos (MPB). Phase 2 – cell growth rates slow as nutrients become limiting, rates of carbon assimilation attain maximum values. Phase 3 – cell growth rates and limiting nutrient assimilation rates slow as the biofilm matures, high rates of carbon assimilation persist, and are directed into the synthesis of extracellular organic carbon (EOC). Phase 4 – period of biofilm degradation. (See text for further discussion)

While no direct measurements of EOC production rates were made in this study, strong evidence for high rates of production come from two sources. Firstly, the average production quotient (PQ) measured here was 1.06 (Chapter 3), which is below that expected for the synthesis of a typical mixture of cellular organic compounds (Laws 1991). The synthesis of carbohydrates has an expected PQ of 1.0 (Laws 1991), and therefore, high rates of carbohydrate synthesis are consistent with the lower than expected PQ measured. Secondly, high rates of EOC production are consistent with the hypothesis that respiration within the sediment is driven by an input of EOC to the sediments (in particular at Site CF) developed in Chapters 2, 3 and 4. It was further suggested in Chapter 4, that the input of labile, but high C:N ratio EOC resulted in the observed high ratio of TCO_2:NH_4^+ produced within the sediment. In support of this hypothesis, there was a significant positive relationship between the measured C:N ratio of carbon and dissolved nitrogen assimilated by MPB, and the C:N ratio of TCO_2 and NH_4^+ produced within the sediment (Figure 5.5). Thus, metabolism in this system occurs through the production and remineralisation of labile, but high C:N ratio organic matter. The outlying data point in Figure 5.5 occurred when there was a high C:N ratio of carbon and nitrogen remineralised compared to that assimilated. This was most likely caused by a reassimilation of NH_4^+ by bacteria within the sediment (See Chapter 4).

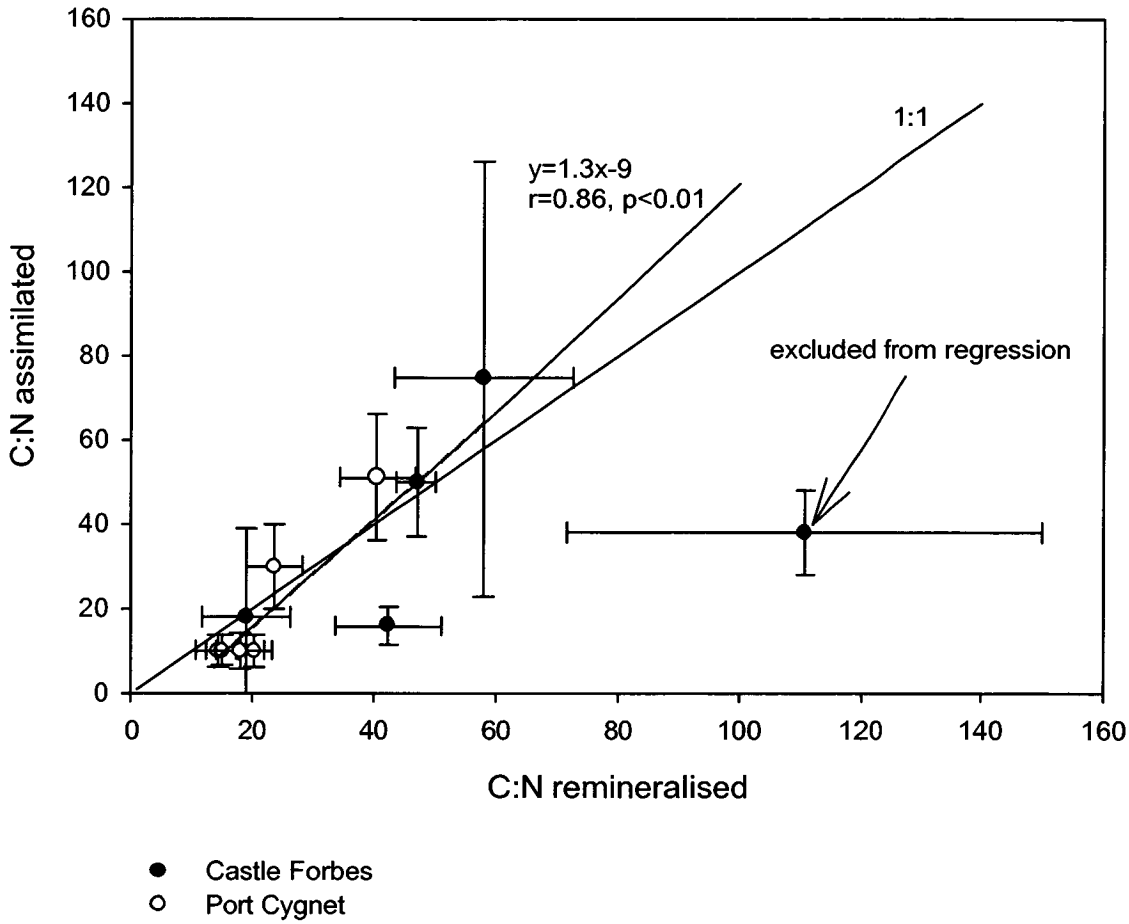


Figure 5.5. A plot of the measured C:N ratio of carbon and dissolved nitrogen assimilated by microphytobenthos (see materials and methods section for details) versus the C:N ratio of TCO_2 and NH_4^+ produced within the sediments (data presented in Chapter 4). Error bars represent the standard error of the mean (n=3–4). The outlying data point most likely arose a consequence of bacterial reassimilation of NH_4^+ within the sediment.

The importance of N_2 fixation as a source of nitrogen to the MPB in the initial growth phase of the MPB (Figure 5.4) is illustrated by the high rates of N_2 fixation observed in February at Sites CF and PC (Figure 5.3). This is also consistent with the fact that the MPB community at Site CF had a high proportion of cyanobacteria. The $\delta^{15}N$ values of the MPB and sediments at Site CF were also very low, particularly during summer, compared to at Site PC, where benthic diatoms had a high $\delta^{15}N$ (Chapter 2). The relatively low $\delta^{15}N$ in MPB at Site CF is consistent with the MPB at this site deriving the majority of their nitrogen requirements from N_2 fixation (Capone et al. 1998; MacGregor et al. 2001). The increase in the $\delta^{15}N$ of the surface sediment during winter and spring (Figure 3.1, Chapter 3) is also consistent with this hypothesis. During winter, N_2 fixation rates were lower (Figure 5.3), because algae were able to derive their nitrogen requirements solely from the water column (Figure 5.1). As a consequence, the $\delta^{15}N$ of algae would have increased as their reliance on NO_3^-, most likely derived from the southern ocean, which has a $\delta^{15}N$ in excess of 6‰ (Lourey et al. submitted), increased. During the summer and autumn, the reliance on nitrogen derived from atmospheric N_2 fixation increased, which resulted in a decrease in the $\delta^{15}N$ of the sediment surface, concomitant with a decrease in the $\delta^{15}N$ of the MPB detritus entering the sediment.

In conclusion, the measured rates of carbon assimilation by MPB on the intertidal mudflats in the Huon Estuary could not be reconciled with measured uptake rates of dissolved nitrogen assuming the growth of cells with a C:N ratio of 10 at times of high productivity. High rates of N_2 fixation were measured at times and were capable of making up this deficit. However, on many occasions, the measured rates of N_2 fixation could not account for this deficit. It is proposed that at these times, much of the carbon assimilated was used in the synthesis of EOC rather than cell growth. As such, the assimilation of carbon and nitrogen do not occur in the same proportions as the C:N ratios expected for algal cells.

Chapter 6

System budget and conceptual functioning

Construction of the System Budget

A conceptual diagram and budget of carbon and nitrogen cycling is shown for each site in Figures 6.1 and 6.2. The budget for each site was constructed based on the data presented in each of the preceding chapters. Primary production and respiration rates were presented in Chapter 3. Sediment-water nutrient exchange, and denitrification rates were calculated on a daily basis using light and dark fluxes presented in Chapter 4, and the effective daily photoperiod as calculated in Chapter 2. Assimilation of dissolved nitrogen by microphytobenthos (MPB) was calculated as outlined in the budget presented in Chapter 5. Rates of nitrogen fixation were calculated using the light and dark N_2 fixation rates presented in chapter 5 and the effective daily photoperiod. For Site CF upper, the preliminary sampling taken in November 2000 was excluded from the budget because sediment NH_4^+ production rates and denitrification were not measured. The gross rates of NH_4^+ production were calculated assuming that the organic matter undergoing remineralisation was derived from MPB. The C:N ratio of MPB-derived organic matter entering the sediment was estimated from the measured ratio of TCO_2 to nitrogen assimilated (including N_2 fixation). The net rate of NH_4^+ production within the sediment was calculated using porewater profiles as described in Chapter 4. Reassimilation of nitrogen by bacteria was estimated from the difference between gross and net remineralisation rates of NH_4^+. The rates presented here are an average of the seasonal measurements made over the course of the year.

Scaling up the hourly measurements made here, to an estimate of what is actually occurring in the field, is difficult because of the influence of the tidal inundation and exposure cycles. The presence of MPB adds further complexity to the exchange of nutrients over inundation and exposure cycles (Thornton et al. 1999). The lower mudflats at each site were estimated to have been exposed for less than 1% of the time, and therefore, all measurements made on the lower mudflats were scaled up without any correction for tidal exposure. However, the upper mudflats were exposed for 28% of the time at Site CF and 43% of the time at Site PC, consequently, to obtain a realistic estimate of the relative importance of the various processes, some correction should be applied at these sites. The uptake of nitrogen by the sediment will not occur during exposure, and thus rates of dissolved nitrogen uptake by the sediment have been corrected for exposure time on the upper mudflats.

This was done by taking the annual average uptake for each nitrogen species and multiplying by the proportion of the time the mudflat was inundated during 2001. Fluxes corrected in this way included uncoupled denitrification (D_w), NOx, NH_4^+ and DON assimilation. The measured effluxes of NH_4^+ were scaled up without any correction for exposure time. This is justifiable if it is assumed that the NH_4^+ produced during tidal exposure, will build up in the porewaters and is then released upon immersion (Caetano et al. 1997). The experiments conducted here were performed on sediments that had been submerged for at least 15 hours before flux measurements commenced. As such, the fluxes measured here most likely represented steady-state production rates, which were extrapolated to the entire day. This assumption may not be generally valid, especially when and where MPB are present. They will draw down NH_4^+ within the porewater, which may dramatically reduce the efflux of NH_4^+ upon inundation (Thornton et al. 1999). The exact effects of this are hard to predict, but it may mean the effluxes of NH_4^+ presented here have been slightly overestimated.

Many of the measurements of the rates of processes have been made independently of one another. As a result, budgets do not balance exactly because of methodological, instrumental, spatial and temporal uncertainties. For instance, the estimated rate of NH_4^+ production at Site PC lower is less than the sum of the measured sediment efflux, the calculated assimilation by MPB and the loss via denitrification. In this regard, the budget presented here should be regarded as a first-order estimate of the relative importance of the various carbon and nitrogen recycling processes active on the mudflat ecosystems.

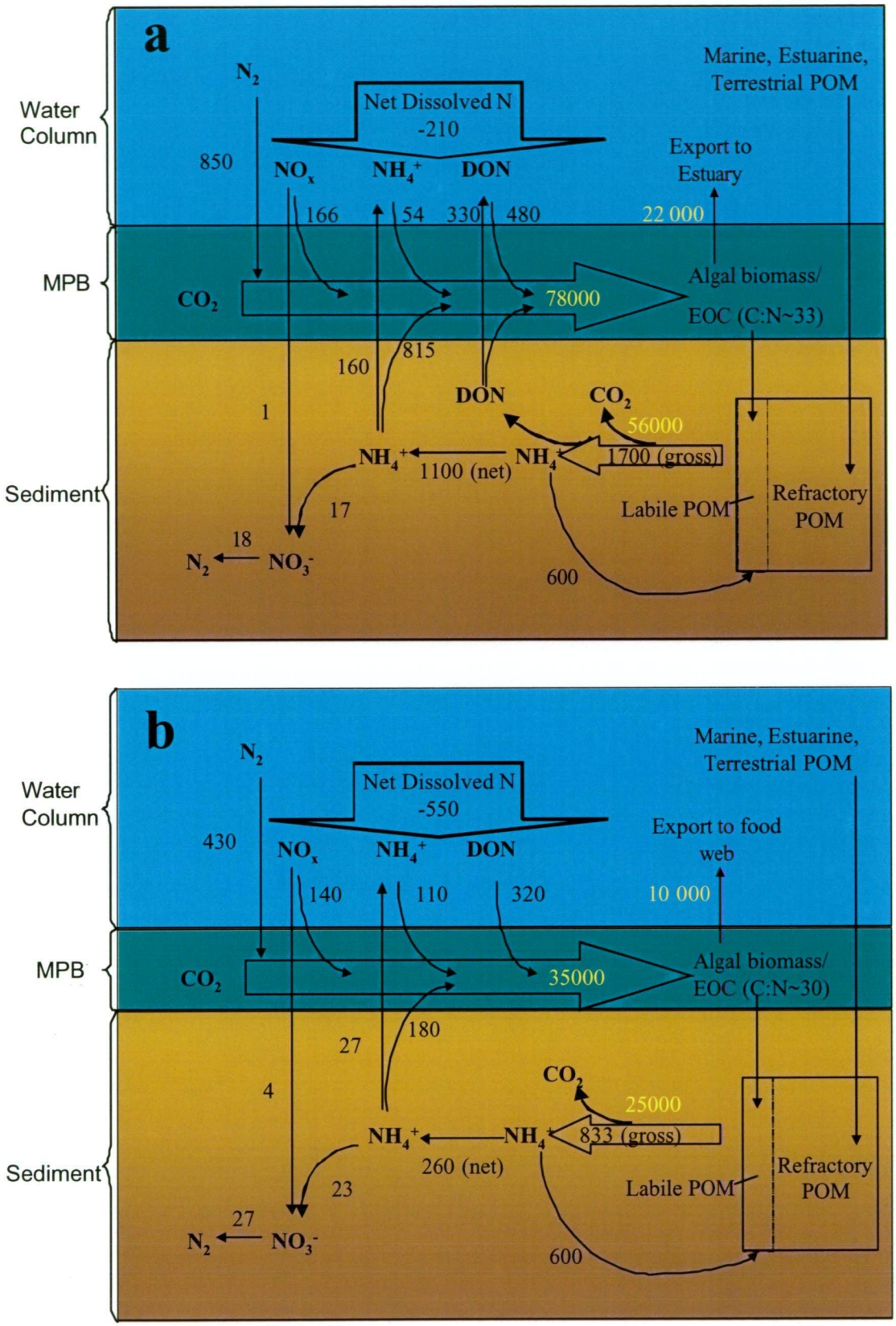


Figure 6.1. A budget of carbon and nitrogen cycling on the upper mudflat **(a)**, and the lower mudflat **(b)** at Castle Forbes Bay during 2001. Values are the annual average of data taken from four seasons expressed as µmol m^2 day^{-1}. Values in yellow represent carbon cycling rates and values in black represent nitrogen cycling rates. The thickness of the green band gives a relative approximation of the algal biomass present.

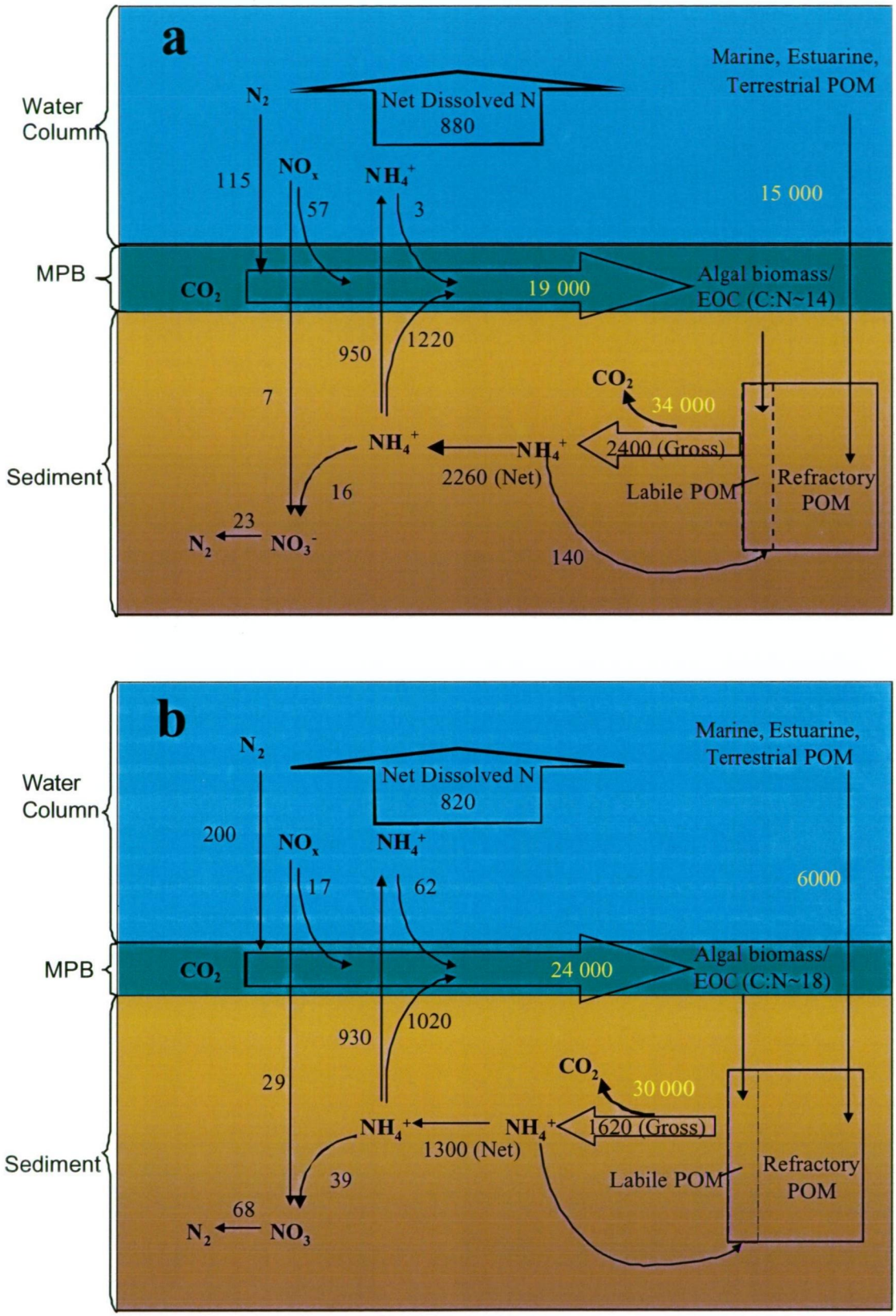


Figure 6.2. A budget of carbon and nitrogen cycling on the upper mudflat **(a)**, and the lower mudflat **(b)** at Port Cygnet during 2001. Values are the annual average of data taken from four seasons expressed as µmol m^{-2} day^{-1}. Values in yellow represent carbon cycling rates and values in black represent nitrogen cycling rates. The thickness of the green band gives a relative approximation of the algal biomass present.

System Budget and Conceptual Functioning

The following relates to Figures 6.1 and 6.2. Rates of primary production were greater at Site CF compared to Site PC. It is proposed that this arose as a consequence of greater exposure to wave energy at Site PC, as indicated by the larger sediment grain size measured at this site. The corollary of this was that the mudflat at Site CF was net autotrophic over the course of the year, with a net export of carbon from the mudflat. By contrast, the mudflat at Site PC was net heterotrophic, exhibiting a net import of carbon. This had profound consequences for the functioning of the mudflats in terms of their nitrogen flows. At Site CF there was a net import of nitrogen from the water column as well as from the atmosphere via nitrogen fixation, driven by an assimilative demand for nitrogen. At Site PC there was a net export of dissolved nitrogen to the water column and much lower rates of nitrogen fixation as a consequence of the net remineralisation of organic matter.

At Site CF, the assimilation of nitrogen in forms other than DIN made up a significant fraction of total nitrogen assimilated. N_2 fixation accounted for 36% of nitrogen assimilated on both the upper and lower mudflats, while DON accounted for 20 and 27% of nitrogen assimilated on the upper and lower mudflats respectively. Further evidence for the importance of nitrogen fixation to MPB at Site CF comes from the light $\delta^{15}N$ values of the MPB taken from this site. At Site PC, no organic nitrogen assimilation was observed and N_2 fixation made up a much smaller fraction of total nitrogen assimilated, being 8 and 15% on the upper and lower mudflats, respectively.

The measured ratio of carbon to total nitrogen assimilated by MPB was very high and well in excess of the measured C:N ratios of MPB, particularly when rates of primary production were highest. It is suggested that once an MPB biofilm has become established and nutrients become limiting, the assimilation of carbon by MPB will be directed into the synthesis of high C:N ratio extracellular organic carbon (EOC) rather than cellular growth. The input of this labile, but high C:N ratio, organic matter drives production by bacteria, which in turn assimilate nitrogen in order to synthesise their low C:N ratio biomass. The C:N ratio of the algal material entering the sediments was highest at Site CF, where the algal biofilm had a

greater biomass and high rates of primary production were most likely directed into the synthesis of extracellular organic carbon. As a consequence, a high ratio of TCO_2 to net NH_4^+ produced within the sediments is observed, particularly at Site CF. This finding is significant as it suggests that the use of simple stoichiometric models often used to calculate nitrogen assimilation by MPB (Cabrita and Brotas 2000; Dong et al. 2000; Sundbäck and Miles 2000) and remineralisation (Berelson et al. 1998) within the sediment, may not always be appropriate. This is particularly likely where macronutrients, such as nitrogen and phosphorus, have become limiting.

Rates of denitrification were low at both sites, and this process was not significant in terms of the overall nitrogen cycling processes. The low rates of denitrification most likely result from several factors, including 1) high rates of primary production (particularly at Site CF), which created competition for nitrate and NH_4^+ between nitrifying/denitrifying bacteria and MPB as well as other sediment bacteria; 2) low NO_3^- concentrations in the water column, which meant that rates of uncoupled denitrification (D_w) were extremely low; 3) fluctuating environmental conditions in the upper intertidal zone may also have had a negative impact upon denitrification.

The major effect of the inundation gradient on carbon and nitrogen cycling processes was indirect; it arose as a consequence of the availability of light to the sediment surface. This effect only manifested itself at Site CF, where inputs of highly coloured water from the Huon River meant that light penetration was diminished over the relatively shallow inundation gradient. A greater light availability on the upper mudflat meant that rates of primary production and respiration were significantly greater in this zone compared to the lower mudflat. Effluxes of DON were also greater on the upper mudflat, most likely as a consequence of greater rates of deposition of labile organic matter derived from MPB, or alternatively direct release from MPB itself. At Site PC, where the water was less affected by the presence of coloured organic matter, significant differences in primary production and respiration rates along the inundation gradient were not observed, with the exception of denitrification. Rates of denitrification on the lower mudflat at Site PC were significantly greater than on the upper mudflat, most likely as a consequence of significantly deeper oxygen penetration on the lower mudflat and possibly also the presence of bioturbating infauna. The regular exposure of sediments may also have

had a negative impact on denitrification caused by periodic exposure to extremes of salinity and temperatures.

This study found a number of important differences between the functioning of these mudflats and their more studied European counterparts. In European systems much higher rates of denitrification are often observed (Cabrita and Brotas 2000; Dong et al. 2000). This is most likely attributable to the low NO_3^- concentrations within the Huon Estuary, which are as much as three orders of magnitude below those found in many eutrophied European estuaries. High NO_3^- concentrations in these systems will drive high rates of denitrification via uncoupled denitrification (Dong et al. 2000; Trimmer et al. 2000a). Another consequence of greater inorganic nitrogen concentrations is that there will be a reduced competition between the assimilative demand for nitrogen—both by MPB (Nedwell et al. 1999) and heterotrophic bacteria (Strauss and Lamberti 2000)—and the respiratory demand for nitrogen by nitrifying and denitrifying bacteria. Thus, in estuaries with higher nitrogen loadings, the importance of nitrification and denitrification within the system budget will be greater than in systems with low nitrogen loadings.

On a more general level of basic sediment function, the results of this study are very similar to those of a recent similar study of sub-tropical Australian systems (Ferguson 2002). This study similarly found that the sediments generally a net sink for DIN, that the activity of MPB had a distinct effect on nitrogen fluxes and that there were at times significant fluxes of DON. Perhaps most significantly there was a large deficit in DIN regenerated within the sediments relative to respiration rates. Rates of denitrification were also low in these sub-tropical systems, and too low to account for the observed stoichiometric deficit in DIN remineralised. Ferguson (2002) proposed that this deficit was caused by an intense competition between heterotrophs and autotrophs which led to an immobilisation of nitrogen into microbial biomass. The hypothesis that the remineralisation of extracellular organic material derived from diatoms presented in this thesis could also be used to explain the deficit in DIN remineralised observed by Ferguson 2002.

Whether or this hypothesis is correct, the key point is that both studies have shown that stoichiometric models of organic matter remineralisation are not applicable to

Australian systems. This most likely arises as a consequence of Australian Estuaries experiencing higher light levels and more oligotrophic conditions than their more widely studied North American and European counterparts. High light levels lead to conditions conducive to high rates of benthic productivity. Without a ready supply of nutrients to synthesise cellular material MPB produce large amounts of extracellular organic compounds due to overflow metabolism. This labile but high C:N ratio organic material stimulates bacterial activity which further stimulates the uptake of DIN by heterotrophic bacteria. This competition results in a tight conservation of nitrogen within the biological pool, rather than being lost through processes such as nitrification and denitrification. The corollary of this hypothesis is that a much greater fraction of nitrogen within Australian systems will be within the biotic component compared to the dissolved organic and inorganic components. As a consequence future studies of nitrogen dynamics in Australian estuaries should pay particular attention to nitrogen in the form of biomass, perhaps through food chain studies, including 'pulse chase' experiments using stable isotopes.

The community composition of the MPB, within this system was also generally much different to that observed in European estuarine mudflats. Diatoms have generally been observed to dominate the community in these systems (Cariou-Le Gall and Blanchard 1995; Barranguet et al. 1997; Brotas and Plante-Cuny 1998; Lucas and Holligan 1999). In this study, green algae and cyanobacteria were also found to make up an important component of the MPB. I propose that this is a consequence of the low nitrogen loadings in the Huon Estuary. The conditions promote the growth of cyanobacteria, which are able to fix nitrogen in order to meet their metabolic requirements and possibly other MPB species to a certain extent. While the importance of nitrogen fixation as a nitrogen source for MPB has been demonstrated in the Huon Estuary, it is unlikely that nitrogen fixation by MPB in eutrophied systems is significant because of high nitrogen loadings. The contribution of nitrogen fixation in estuaries is likely to decrease with increased nitrogen loadings (Nedwell et al. 1999).

In conclusion, the presence of MPB on the intertidal flats profoundly influenced the cycling of nitrogen within these systems. This occurred through:

- The delivery of labile, but high C:N ratio organic matter to the sediments, which stimulated sediment respiration rates and resulted in the production of high C:N ratio remineralisation products.

- The modulation of nutrient efflux from the sediments via the uptake of nitrogen remineralised within the sediment.

- The inhibition of denitrification through competition for NO_3^-, NH_4^+ and through the increase in O_2 penetration during photosynthesis.

- High rates of nitrogen fixation during periods of high primary production.

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