Benthic Respiration and Nutrient Cycling in the Huon Estuary (Southern Tasmania)

By

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Declaration of originality

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Abstract

Sediment biogeochemistry was studied in the Huon estuary, which is located in Southern Tasmania, Australia. The sources of organic matter and rates of decomposition were investigated as well as fluxes of nutrients, which are liberated during organic matter decomposition. The study aimed to develop a conceptual understanding of benthic respiration and nutrient cycling in the Huon estuary and the influence of organic carbon on these processes. This study also sought to evaluate the ecological significance of nutrient inputs from sediments compared with other nutrient sources to the estuary.

Sediments were studied over one year, including sampling in March, July and November in 2004 and then in April 2005. To represent the terrestrial and marine end members of the estuary, locations were situated in the upper and lower reaches respectively.

Employing a variety of organic – geochemical approaches, this study showed that sediments were dominated (i.e. >50%) by allochthonous, river-supplied terrestrial organic matter. Spatial differences were observed between the upper and lower locations with the upper estuary receiving most of its source of organic matter from terrestrial sources (approx. 75%). In contrast, the lower location had a greater variety of organic matter sources, with approximately 50% coming from terrestrial sources, approximately 20% from phytoplankton and about 15% from bacteria. No discernable differences were found between sampling stations within each location.

Organic matter source markers for some inputs, in particular markers for terrestrial organic matter, were higher in winter, most likely due to an increase in input from catchment runoff. Increased river flows were observed in July during this study, carrying higher concentrations of particulate organic matter from catchment runoff, which is likely to increase the terrestrial detritus load at the seafloor as indicated by the increase in terrestrial biomarkers during this period.

Oxygen and CO_2 fluxes indicated that aerobic respiration was one of the main pathway for carbon degradation in Huon estuary sediments, although anaerobic

respiration was also present. The low CO_2:O_2 and Alkalinity:O_2 flux ratios was evidence of aerobic respiration. Additionally modelled oxygen consumption profiles showed that the majority of oxygen was consumed near the surface, most likely due to aerobic heterotrophic bacteria. A small oxygen consumption peak was also observed at the anoxic/oxic interface most likely due to sulphide oxidation.

Spatial and temporal differences in respiration occurred with carbon contents and temperature considered the main drivers respectively for this variability. Differences also occurred between total versus diffusive oxygen uptake rates, and was likely due to the presence of benthic fauna. Respiration in Huon estuary sediments compared well with other deep coastal sediments whereby rates of TCO_2 and O_2 fluxes were very similar to Monterey Bay in California and the Southern Kattergat in the Baltic Sea

Benthic fluxes of nutrients were low in the Huon estuary. Average fluxes of ammonia, nitrate, phosphate and silicate were 1.3, 10.1, 1.6 and 32.5 $\mu mol\ m^{-2}\ h^{-1}$ respectively. An extrapolation of these measurements to the whole estuary revealed that the sediments were only a minor source of nutrients, providing approximately 96 tonnes of inorganic nitrogen, 32 tonnes of phosphate and 586 tonnes of silicate.

On all occasions, the DIN flux was dominated by nitrate, which was always released from the sediment to the overlying water. The effluxes of nitrate and influxes of nitrite and ammonium are most likely associated with intensive nitrification, stimulated by the presence of relatively deep oxygenated zones. Peaks of nitrate in the oxic zone observed from nitrate pore water profiles also provided additional evidence of nitrification. The net efflux of nitrate from the sediments, suggests that they act as net regenerators of nitrogen as opposed to nitrogen assimilators.

The benthic effluxes of nitrogen however were smaller then expected from carbon oxidation rates. The low N:P ratio of benthic fluxes (approx. 3:1) indicates that processes such as denitrification and anaerobic ammonium oxidation (ANAMMOX) may be important nitrogen elimination process. However another potential pathway for nitrogen released during organic matter remineralisation is for the decomposing

bacteria to reassimilate some of the ammonium due to the low nitrogen content of the organic matter been decomposed.

Due to increasing levels of chlorophyll *a*, largely due to the growing aquaculture industry, a laboratory experiment was conducted to observe the response of Huon estuary sediments to increasing loads of labile organic carbon. The addition of *Spirulina*, produced a dramatic increase in the flux rates of all analytes. The change in fluxes for most analytes correlated well with increasing carbon loading however the rate change occurred in two distinct stages for some of the analytes, including oxygen and ammonium. Results showed rapid change in oxygen and ammonium flux rates and oxygen penetration with increasing carbon load size. However when the carbon load became >20.2 g C m^{-2} the change in flux rates and decrease in oxygen penetration slowed significantly for these analytes.

The point at which the rate change of fluxes slowed possibly indicates that the biogeochemical system was switching from one that was dominated by aerobic respiration to one dominated by anaerobic respiration. A number of trends in the data indicate this including increasing CO_2:O_2 ratio, increasing alkalinity fluxes out of the sediments, most likely due to sulphate reduction: an anaerobic metabolic process, and declining oxygen penetration depths. DIN fluxes also became dominated by ammonium rather then nitrate as sediments became more anaerobic. Nitrate effluxes, evidence of nitrification, rapidly became nitrate fluxes into the sediment, suggesting denitrifiers could no longer obtain nitrate *in situ* due to the reduction in nitrification, and thus the breakdown of coupled nitrification/denitrification. As the sediments became anaerobic, denitrification became dominated by direct denitrification whereby denitrifiers obtain their nitrate requirements from the water column, as evidenced by uptake of nitrate by the sediments. Dissimilatory nitrate reduction to ammonium (DNRA) may also have contributed to the large efflux of ammonium as sediments became more anaerobic.

This study has provided insights into how sediments function in relatively pristine coastal environments, in particular what the sediments are not doing at the moment (such as releasing large amounts of ammonium), but what they could be doing if labile organic carbon is increased. The findings have provided a good contrast to

sediments that exist in environments exposed to increasing anthropogenic influence and provide a snapshot of how heavily polluted systems may once have functioned. This study has therefore added to the growing literature of how coastal sediments recycle carbon and nitrogen and their importance to local and global carbon and nitrogen cycles.

Finally this thesis has shown that it is difficult to interpret specific processes such as nitrification and denitrification from core incubations and the inferred stoichiometric relationships of the carbon, nitrogen and phosphate fluxes due to the complex nature of biogeochemical processes occurring in the sediments at any time. To build on the foundations laid by this study, the next step would be to carry out a set of carefully designed experiments in regards to both benthic respiration and nutrient cycling. In particular, an experiment that simultaneously measures bacterial nitrogen assimilation, ANAMMOX, denitrification and nitrification would be useful to gain a better understanding of the nitrogen cycle. These experiments should be carried out under both unimpacted and impacted sediments as while I have shown that organic carbon loading alters sediment metabolism which is consistent with the literature, at this stage it is still unknown as to exactly how benthic metabolism would be controlled if farming does greatly increase. Furthermore, how these individual processes (i.e. nitrification, ANAMMOX, denitrification and DNRA) collectively influence nutrient fluxes from the sediment under unimpacted and impacted conditions needs to be elucidated.

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Chapter 1

Introduction

Chapter 1. Introduction

Anthropogenic pollution of estuarine environments continues to increase around the world due to rapid urbanisation. Human activities including land clearing, agriculture, effluent disposal, aquaculture and combustion of fossil fuels are all impacting on the coastal environment leading to nutrient enrichment and increased sedimentation (Gray et al., 2002; Nixon, 1995; Holmer, 1991). Understanding how estuarine ecosystems function prior to and after anthropogenic perturbation is required to ensure their sustainability for future generations.

1.1 Sediment Biogeochemistry

Estuaries act as an important link between the land and the sea and therefore play a crucial role in coastal ecosystems. High sedimentation rates in estuaries mean that they play a crucial role in recycling carbon and nutrients to the broader ecosystem. Depending on the lability of the organic matter depositing onto the seafloor, the sediments will either rapidly remineralise the organic carbon releasing it back into the water column as carbon dioxide (CO_2) or the carbon will be sequestered within the sediments (Kristensen, 2000).

Particulate organic nitrogen (PON) is also remineralised during organic matter decomposition. The remineralised nitrogen can be released back into the overlying water column, as dissolved organic nitrogen (DON), ammonium or nitrate, which can be readily available for uptake by phytoplankton. However it can also be converted to di-nitrogen (N_2) gas via denitrification or the ANAMMOX reaction and therefore no longer available for phytoplankton uptake. Alternatively, ammonium can be reassimilated by heterotrophic bacteria, during organic matter decomposition within the sediment (Kristensen, 2000; Lomstein et al., 1998).

1.1.1 Benthic Metabolism

Sedimentary organic matter can be remineralised either through aerobic or anaerobic pathways. Organic matter can generally be characterised as having the following chemical composition:

$$(CH_2O)a\ (NH_3)b\ (H_3PO_4)c \qquad (1)$$

The amount of *a*, *b* and *c* largely depends on the source of the organic matter and stage of decomposition.

When organic matter is initially deposited onto the sea floor, organisms such as benthic fauna, bacteria and fungi (Kristensen, 2000) aerobically decompose the organic matter according to the following stoichiometry:

$$(CH_2O)a\ (NH_3)b\ (H_3PO_4)c + dO_2 \rightarrow aCO_2 + bNH_3 + cH_3PO_4 + dH_2O \qquad (2)$$

Because aerobic respiration occurs in a relatively thin oxidised upper layer in coastal sediments, a large fraction of the organic matter can be buried into anoxic layers.

Anaerobic metabolism proceeds in a sequence of metabolic processes that generally change with depth. The following order in which anaerobic metabolism occurs will depend on the availability of electron acceptors (Kristensen, 2000):

- Mn^{4+} Manganese oxide reduction
- NO_3^- Denitrification
- Fe^{3+} Iron oxide reduction
- SO_4^{2-} Sulphate reduction
- CO_2 Carbon dioxide reduction

In general, aerobic and anaerobic respiration contributes approximately 50% each to total respiration with sulphate reduction often the largest contributor within anaerobic respiration in marine sediments (Kristensen, 2000), although recent studies have shown dissimilatory iron and manganese reduction to be important in some environments (Arnosti and Holmer, 2003).

Benthic metabolism can be influenced by a number of factors. These factors include:

- Organic enrichment (Holmer and Kristensen, 1992; Caffrey et al., 1993; Christensen et al., 2000; Grenz et al., 2000; Gray et al., 2002);
- Bioturbation/irrigation (Aller, 1994; Kristensen, 2000; Berg et al., 2001; Glud et al., 2003; Nielsen et al., 2004);
- Temperature (Klump and Martens, 1989; Vidal et al., 1997; Thamdrup et al., 1998; Westrich and Berner, 1998); and
- Oxygen concentration in the water column (Rasmussen and Jorgensen, 1992).

Benthic respiration is directly influenced by the source and supply of labile organic matter. In sediments that undergo organic enrichment either during phytoplankton blooms or via anthropogenic inputs, sediments may become anaerobic due to the depletion of oxygen within the sediments. The oxygen penetration depth is directly influenced by the supply of labile organic carbon, with shallow oxic zones related to high respiration rates due to increased organic loading (Kristensen, 2000).

Benthic remineralisation of carbon is strongly related to the sedimentation rate of carbon to the sea floor with the rate being strongly attenuated by the depth of the water column (Devol and Christensen, 1993). Many studies have shown that carbon sedimentation rates decrease with depth (Devol and Christensen, 1993), and that the relationship between sediment carbon oxidation rates and depth is best described by a power function (Christensen, 1989; Devol and Christensen, 1993). That is, carbon oxidation rates rapidly decline with increasing depth of the water column. Glud et al. (2000) found that carbon oxidation rates in Young Sound, Greenland, declined exponentially with depth because organic carbon and nutrients were being recycled in the upper water column before they reached the sea floor. Hulth et al. (1994) also found carbon oxidation rates to be strongly correlated with depth in the arctic sediments of Svalbard. In both these studies it was found that the amount and quality of organic carbon reaching the seafloor declined with depth, and therefore carbon oxidation rates also declined. This is not surprising given that the quantity and quality of organic carbon is a major driver of benthic mineralisation rates (Holmer and Kristensen, 1992; Caffrey et al., 1993; Christensen et al., 2000; Grenz et al., 2000; Gray et al., 2002).

The effect of temperature has been shown to affect both anaerobic and aerobic respiration. A study by Thamdrup et al. (1998) showed that aerobic respiration in marine sediments increased by a factor of 2-3 for a temperature increase of 10^{o}C. Likewise, studies have shown that sulphate reduction increases by a factor of 3 for a temperature increase of 10^{o}C (Westrich and Berner, 1988 and references therein)

Benthic respiration can also be influenced by a variety of benthic faunal processes including bioturbation and irrigation. Bioturbation can enhance the movement of particles within sediments, which leads to greater substrate exposure, and hence an increase in organic matter decomposition. Sediment particles may also be transported between anaerobic and aerobic environments resulting in increased reoxidation and possibly increased remineralization (Aller, 1994). Irrigation and ventilation within and around worm burrows can also enhance benthic respiration by supplying oxygen and other oxidized compounds (electron acceptors) at depth in the sediments (Kristensen, 2000).

1.1.2 Nutrient Recycling

Due to the remineralisation of organic matter, inorganic nutrients are released as shown by the simplified stoichiometric equation for aerobic respiration (equation 2) above. Of particular interest in Australian coastal environments is the nitrogen cycle as this is generally the limiting nutrient for phytoplankton growth.

After the remineralisation of organic matter, the ammonium produced may then be assimilated by bacteria, released from the sediment, become adsorbed onto sediment particles or oxidised to NO_3^- (Blackburn and Henriksen, 1983; Klump and Martens, 1983).

Nitrogen Cycling

Nitrification

Nitrification is the process whereby ammonium is oxidised to nitrite (nitrosofying bacteria such as *Nitrosomonas* sp.) and thence to nitrate (nitrifying bacteria such as *Nitrospina* sp.) (Ward, 2000). This can be represented by the following stoichiometry:

$$NH_3 + O_2 \rightarrow NO_2- + 3H+ + 2e- \quad (3)$$

$$NO_2- + H_2O \rightarrow NO_3- + 2H+ + 2e- \quad (4)$$

Nitrification is typically an aerobic process, and is generally confined to the oxic zone within sediments. However recent evidence suggests nitrification may also be coupled to manganese IV reduction (Hulth et al., 1999) within the anaerobic zone. Nitrification 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 the metabolism of bacteria, benthic algae and macrofauna.

It has been found that nitrification can be significantly inhibited by the activity of benthic diatoms (Henriksen and Kemp, 1988) and heterotrophic bacteria (Strauss and Lamberti, 2000) possibly through competition for NH_4^+. Rates of nitrification are generally highest around macrofauna burrows, due to the occurrence of relatively high concentrations of NH_4^+ within an oxic environment (Henriksen and Kemp, 1988). Nitrification is important in the nitrogen cycling pathway as it is the precursor to denitrification.

Denitrification

Denitrification is the reduction of NO_3^- to N_2 mediated by bacteria (Hulth et al., 2005). The process of denitrification requires the availability of NO_3^- (or NO_2^-), organic carbon and anoxic conditions. Denitrification is represented by the following stoichiometry:

$$106(CH_2O)16(NH_3)(H_3PO_4) + 94.4HNO_3 \rightarrow 106CO_2 + 55.2N_2 + H_3PO_4 + 177.2H_2O \quad (5)$$

The bacteria involved in denitrification can obtain nitrate from either the water column or from nitrification in sediments. When nitrate for denitrification is obtained from nitrification, the process is called coupled nitrification – denitrification (Capone, 2000). A number of factors may influence denitrification including oxygen, NO_3^-, and organic matter concentrations (Seitzinger, 1990). High rates of denitrification are often found in systems with high NO_3^- concentrations in the water column (Trimmer

et al., 2000a), while denitrification rates in systems with low concentrations of NO_3^-, depend entirely on nitrification occurring within the sediment.

ANAMMOX

An alternative pathway to denitrification is anaerobic ammonium oxidation (ANAMMOX) whereby ANAMMOX bacteria convert nitrite and ammonium to N_2 (Hulth et al., 2005). However the ANAMMOX reaction can only take place in the presence of both ammonium and nitrite in an anoxic environment. The ANAMMOX reaction is strongly inhibited by the presence of dissolved oxygen (Hulth et al., 2005). Nedwell et al. (1999) suggested that the ANAMMOX reaction is most likely to occur in hyper-nutrified environments. Since that study however, Kuypers et al. (2005) discovered the occurrence of ANAMMOX in the oxygen minimum zone of the Benguela upwelling off the coast of Namibia. Therefore the occurrence of ANAMMOX could be more widespread in a range of ecological systems then first thought.

Dissimilatory Nitrate Reduction to Ammonium (DNRA)

This process is mediated by bacteria that couple the oxidation of organic carbon or reduced Fe^- and S to the reduction of nitrate via nitrite to ammonium (Hulth et al., 2005) and appears to be most important in organic rich sediments with low NO_3^- concentrations (Nedwell et al., 1999). In a recent study, sediments enriched by fish farm effluent were shown to have DNRA rates 7 times higher than denitrification, while sediments unaffected by the fish farm effluent had insignificant rates of DRNA (Christensen et al., 2000). This is important for nutrient cycling as it can lead to large supplies of ammonium to the water column, where otherwise it may have been removed from the environment via denitrification or ANAMMOX.

Phosphorus cycling

The release of phosphate from sediments maybe important in environments which are considered to be phosphate limited. The release of phosphate from sediments into the water column is mainly regulated by the decomposition of organic matter (Neilsen et al., 2001). Additionally the efflux of remineralised phosphate is controlled in the surface oxic layer by sorption to iron oxyhydroxides (Hopkinson et al., 2001) and is

only released when iron (III) is reduced to iron (II) (Butler et al., 2000). Assimilation by benthic algae may also influence the cycling of phosphorus in sediments (Neilsen et al., 2001).

Silicate cycling

Sediment silicate fluxes are related to the availability of biogenic silica (Forja et al., 1994). The main source of biogenic silica to the seafloor in marine environments is mainly in the form of skeletons or skeletal fragments of silica – secreting micro-organisms e.g. diatoms (Zabel et al., 1997). Up to 80% of biogenic silica may be recycled by digenetic processes in surficial sediments (Zabel et al., 1997). Silicate fluxes can be influenced by irrigation rates and seasonality (Berelson et al., 2003), with temperature and the occurrence of diatoms the likely drivers of differences between seasons.

Benthic regeneration of silicate can have important consequences on the broader coastal ecosystem. In some systems such as the Black sea (Friedl et al., 1998) benthic regeneration may be the most important source of silicate including that supplied by river discharge. This can be important in terms of phytoplankton growth. While silica is not a limiting nutrient to overall primary production, changes in the relative abundances of Si to N and P can potentially alter the phytoplankton community structure (Giblin et al., 1997). A decrease in this ratio can lead to shift from siliceous diatoms to flagellates and coccolithophores (Friedl et al., 1998), which can lead to an increase in toxic algal blooms (Giblin et al., 1997).

1.2 The Study Context and Research Objectives

The Huon Estuary (Figure 1-3) is located in southern Tasmania, Australia. Southern Tasmania is classified as cold temperate and is largely under developed with primary industries like farming and forestry the main industries (Butler et al., 2000). The Huon estuary provides a good study site because it has been the focus of several related studies including the Huon estuary study (HES) in the late 1990s and a study by Cook (2002) on nitrogen and carbon cycling on intertidal mudflats. Thus detailed

knowledge already exists on water quality, sediment characteristics and physical processes such as flow rates and salinity gradients. The HES was commissioned in the late 1990's to evaluate the environmental quality and understand the working of the Huon estuary as a system. This need emerged because of the rapidly expanding farming of finfish in these waters and the desire to manage the industry in a sustainable manner.

One of the main aims of that study was to identify the sources of nutrients into the Huon estuary and to develop overall system nutrient budgets. One of the key findings to emerge from the HES was that marine sources clearly dominated nitrogen inputs to the system annually. However uncertainty remained as to the source of nitrogen loads in bottom waters in summer. The authors concluded that this had potentially major implications for the estuarine assimilation capacity for fish-farm effluents as well as catchment loads.

The lack of understanding of how nutrients were cycled in the bottom waters of the estuary emerged as a key knowledge gap. The HES recommended that this gap should be addressed with specific process studies focused on nutrient regeneration in bottom waters and sediments of the lower Huon Estuary. Therefore one of the key aims of the current study was to address this knowledge gap by measuring benthic nutrient fluxes and nutrient porewater concentrations and thus infer key processes. A second aim was then to evaluate the importance of benthic nutrient regeneration to the broader Huon estuary system. Additional to these aims was to evaluate the role organic matter plays in driving respiration and nutrient cycling in Huon estuary sediments.

Most previous studies on sediment respiration and nutrient cycling have been undertaken in European and North American estuaries which have been heavily influenced by high loads of nutrients and labile organic carbon driven by high population densities and intensive agricultural land use (Middelburg et al., 1996a; Nedwell et al., 1999). In contrast, sediments in Australian estuaries are mostly exposed to relatively low levels of nutrients and labile organic carbon due to lower population densities and more intact catchments (Harris, 2001). Therefore the models that have been developed from northern hemisphere studies may not be applicable to the 'relatively pristine' conditions that exist in Southern Tasmania. While the general

metabolic reactions are well known for carbon and nutrient cycling, how these reactions interrelate is less well known for unimpacted sediments in Australian environments. Studies on estuarine systems that are relatively pristine are important to advancing our understanding of global carbon and nutrient cycles.

In Summary, the overall aim of this study was to identify and understand the important biogeochemical processes occurring in sediments of a near pristine Australian temperate estuary. Of particular interest were the interactions between carbon inputs, benthic respiration and nutrient cycling and how these interactions control sediment-water exchange of nutrients. To achieve this, a field study was designed to identify the sources of organic carbon, benthic respiration and nutrient recycling processes. The first three research chapters are structured around the field study. Chapter 2 identifies the sources of organic carbon in the sediments using a variety of organic geochemistry techniques. Identifying the sources of organic carbon is important because the biodegradability of the carbon will affect the ability and thence rate that bacteria can remineralise the carbon. In general, organic carbon from terrestrial sources is refractory, or hard to breakdown, while marine phytoplankton sources of organic carbon are generally labile and thus easy to breakdown. Therefore the portion of labile carbon is important for driving benthic respiration and thence nutrient cycling.

Chapter 3 examines benthic respiration and carbon cycling. This was examined using measurements of CO_2 and O_2 fluxes in core incubations and employment of oxygen microelectrodes to obtain sediment oxygen microprofiles. Chapter 4 uses nutrient flux measurements from core incubations and porewater profiles to identify the key nutrient cycling processes such as nitrification and denitrification. These three research chapters are then drawn together in the final discussion chapter to develop conceptual models of respiration and nutrient cycling and to discern the importance of organic carbon on these processes. Chapter 5, the fourth research chapter, involved enriching sediments from the Huon estuary with organic carbon to see how the carbon and nutrient cycling processes change. This was done because the Huon estuary has an expanding aquaculture industry, which has been responsible for increased nutrient and chlorophyll levels in the water column. The potential for increased phytoplankton blooms can have a direct effect on sediment ecological processes such as nutrient

cycling. Therefore understanding how sediments in the Huon estuary respond to organic enrichment is critical to understanding the overall assimilation capacity of the estuary to nutrient enrichment from fish farms and other sources of anthropogenic pollution.

In summary, four research chapters and the general discussion are presented in this thesis which address the following questions

Chapter 2 – Sources of Organic Matter

- What are the major sources of organic carbon?
- How does the source and quantity of organic carbon change both temporally and spatially?

Chapter 3 – Benthic Respiration

- What are the rates of benthic respiration and how do they change temporally and spatially?
- What are some of the major factors controlling respiration?
- How does benthic respiration in the Huon estuary compare with other coastal systems?

Chapter 4 – Benthic Nutrient Cycling

- What are the major recycling pathways for nutrients within the sediments?
- How do these processes vary temporally and spatially?
- How does benthic nutrient cycling in a mesotrophic system contrast with more enriched systems?

Chapter 5 – The effect of Organic Enrichment on Sediment Respiration and Nutrient Recycling Pathways

- What is the response of benthic respiration to organic enrichment?
- How does organic enrichment impact on the nutrient cycling processes?

Chapter 6 – General Discussion

- Construction of Conceptual models and budgets for carbon and nutrient cycling
- What is the influence of ‘natural’ organic carbon inputs on benthic respiration

- How does organic carbon enrichment change the key processes in the conceptual models
- Evaluates the ecological significance of benthic nutrient cycling in the broader estuarine environment

1.3 Experimental approach

1.3.1 Sources of Organic Matter

C/N Ratio

The carbon to nitrogen (C/N) ratio can be used as a proxy to measure the quality of organic matter. A C/N ratio close to that of the Redfield ratio (6.625) is indicative of organic matter derived from marine phytoplankton while organic matter derived from terrestrial sources can have a C/N ratio of 20 or more (Bordovskiy, 1965).

This approach is, however, very approximate as degradation may significantly alter these ratios (Thornton and McManus, 1994). Low C/N ratios tend to increase during degradation because nitrogen is lost from organic matter at a greater rate then produced by decomposing bacteria. In contrast, high C/N ratios tend to decrease during degradation as bacterial nitrogen production occurs at a faster rate then nitrogen loss from the decomposing organic matter (Thornton & McManus, 1994). Therefore, as new material is constantly arriving at the seafloor and in different degradation states the surface sediment contains a mixture of 'fresh' and degraded material, and the longer organic matter has been deposited on the seafloor the more difficult it becomes to distinguish between the two sources using C/N ratios.

Stable Isotopes

Ratios of $^{12}C/^{13}C$ isotopes provide a good estimate of the relative contribution of terrestrial and marine sources to sedimentary organic matter (Fry and Sherr, 1984). Terrestrial organic matter will generally have a $\delta^{13}C$ value of -26 to -30‰ and organic matter with a marine origin will generally have a $\delta^{13}C$ of -19‰ to -23‰ depending on the particular organisms present (Heip et al., 1995). The relative proportion of marine and terrestrial carbon in a sample can then be estimated by linear additions of 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 two well-defined end-members. Furthermore, this technique gives little information about the type of marine or terrestrial organic matter in question.

Molecular Biomarkers

While C/N ratios and stable isotopes can tell us the relative contributions of terrestrial and marine organic matter, molecular biomarkers such as sterols, fatty acids and alcohols allow us to distinguish between the various sources of organic matter and can therefore provide very specific information. However, many lipid biomarkers originate from more than one source, making it difficult to isolate the source of the lipids when based on one compound only. Thus, the use of several lipid biomarkers can be used in combination to identify the source of organic matter within the sediments.

Alkanes and alcohols can be used to identify terrestrially derived organic matter. Alkanes from cuticular waxes of higher lands typically range between C23-35 with a distinct predominance of odd-carbon chain lengths and a concentration maximum at C27, C29 or C31. In regards to alcohols, C22 to C28 are generally characteristic of plant waxes from higher plants (Birgel et al., 2004). Alkanes are hydrocarbons that contain only single bonds whereas alcohols are hydrocarbon derivatives in which one or more hydrogens of a parent hydrocarbon have been replaced by a hydroxyl or alcohol functional group as depicted in figure 1.1.

Alkane Alcohol

Figure 1.1 Basic structures of alkanes and alcohols

Sterol biomarkers have been used to characterise organic matter in many studies. Most sterols are found in cell membranes and lipoproteins and have various functions including controlling membrane fluidity and permeability. In some plants, they function in cell proliferation, signal transduction and act as modulators of the activity

of membrane-bound enzymes (Volkman, 2003). Sterols generally make good biomarkers as they are relatively stable and have a long geological record, and they possess structural features (e.g. position of double bonds), which are restricted to a few groups of organisms (Volkman, 1986). However, some sterols are widely dispersed in many organisms, making it difficult to assign those particuliar sterols to a particuliar source of organic matter. For example, Cholest-5-en-3β-ol (Cholesterol) is generally associated with zooplankton or other marine fauna as it is the major sterol of marine animals. However, it also widely dispersed in marine phytoplankton, particularly in dinoflagellates and some diatoms (Volkman, 1986).

Some of the main sterols of interest in marine sediments include 24-methylcholesta-5,22E-dien-3β-ol (Diatomsterol), Cholest-5-en-3β-ol (Cholesterol), 24-ethylcholesta-5,22E-dien-3β-ol (Stigmasterol), 24-ethylcholest-5-en-3β-ol (Sitosterol) and 24-methylcholest-5-en-3β-ol (Campesterol), which are commonly used as markers for diatoms, marine animals and higher plants respectively (Volkman, 1986). The structure of these sterols is given below in Figure 1-2. A significant feature of all these compounds is the double bond at C-5.

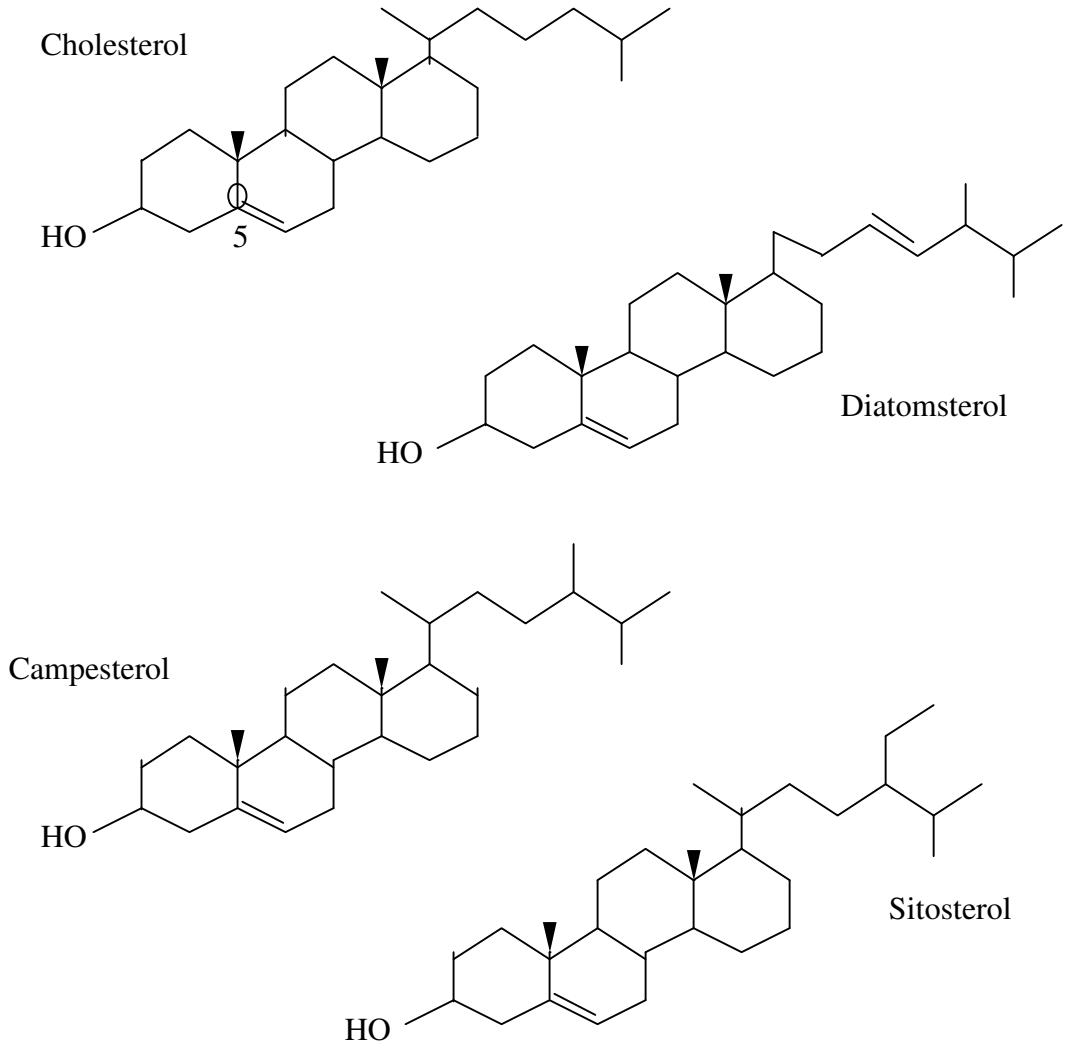


Figure 1-2 The structural formula of cholesterol, diatomsterol, campesterol and sitosterol.

Fatty acids are generally the most abundant lipid type in surface sediments (Volkman et al., 1998) and fulfill a range 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 found in various biological classes and provide a range of useful markers for phytoplankton, macroalgae, bacteria, seagrasses and terrestrial plants in the marine environment (Volkman et al., 1980, Meziane et al., 1997, Volkman et al., 1998, Kharlamenko et al., 2001).

Phytoplankton are a major source of fatty acids in marine sediments and the contribution of different algal classes can often be discerned from characteristic

differences between classes (Smith et al., 1983; Dunstan et al., 1992; Viso and Marty, 1993; Volkman et al., 1989; Volkman et al., 1998; Zukhova and Aizdaicher, 1995). Table 1-1 provides a summary of the major fatty acids found in different algal classes. The table highlights that fatty acid compounds are often abundant in more then one algal class. However of more importance is the combination of fatty acid compounds within a class. For example 20:5n-3 is abundant in many algal classes but the combination of high abundances of 18:4n-3, 20:5n-3 and 22:6n-3 is unique to the algal class *Dinophyceae*.

Table 1-1. Characteristic fatty acids in phytoplankton classes (information source: Viso and Marty (1993))

Algal Class	Characteristic fatty acids
Bacillariophyceae	16:1n-7, 16:0, 20:5n-3 and 14:0
Dinophyceae	16:0, 18:4n-3, 20:5n-3 and 22:6n-3
Prymnesiophyceae	14:0, 16:0 and 18:1n-9
Chlorophyceae and Prasinophyceae	16:0, 18:3n-3 and 16:4n-3
Cryptophyceae	16:0, 18:1n-9, 18:4n-3 and 18:2n-6
Raphidophyceae	16:0, 16:1n-7, 20:5n-3 and 18:4n-3
Xanthophyceae	16:1n-7, 16:0 and 20:5n-3
Rhodophyceae	16:0, 20:4n-6 and 20:5n-3
Cyanophyceae	18:3n-3, 16:1n-7, 16:2n-6 and 16:1n-9

Bacteria are also associated with distinctive fatty acids, in particular *iso*-15:0 and *antesio*-15:0 branched fatty acids. Vaccenic acid (18:1n-7) is also present in many bacteria (Volkman et al., 1980; Perry, 1979). Perry et al. (1979) found that vaccenic acid accounted for 10.5% of fatty acids in aerobic heterotrophs and 17.3% in anaerobic heterotrophs. However, vaccenic acid has also been reported in many other organism including plankton, phytoplankton, molluscs and fish (Perry et al., 1979). Therefore fatty acids should be analysed in combination with other molecular biomarkers before conclusions about the origin of the organic matter can be drawn. In this study a variety of geochemical approaches including C/N ratios, stable isotopes and molecular biomarkers were used to characterise the sources of organic matter at the study locations. Used as stand alone techniques, they each have both strengths and weaknesses as discussed above. However combining the different approaches provides a powerful tool for identifying the sources of organic matter in sediments.

1.3.2 Benthic Fluxes

A number of different approaches have been used to study benthic respiration and the exchange of nutrients at the sediment – water interface. The most common techniques employed include sediment core incubations, in situ benthic chambers and the calculation of fluxes from porewater concentration profiles (Hammond et al., 1985). Briefly, each of these methods and their limitations are discussed below.

Core incubations
Sediment cores are usually collected either by divers or remote coring devices such as box corers from which sub-cores are taken. The cores can then incubated at in situ temperature onboard a ship or taken back to a land-based laboratory. The change in the concentration of the solutes of interest in the overlying water, are then measured in the dark and or light.

The limitations of this technique include the possible underestimation of benthic exchange in sediments that have abundant macrofauna due to burrows being disturbed and damaged during the coring process (Hammond et al., 2004). The process of obtaining cores can also be quite destructive and can lead to excessive disturbance of the sediment structure, particularly the surface (top 1 cm) if care is not taken retrieving the cores. Disruption of the sediment can significantly alter the biochemical structure within the cores and therefore seriously affect the processes occurring within. Retrieving cores from deep-sea sediments may also seriously affect bacterial metabolism that might be pressure dependent. Hammond et al. (2004) found that nitrate uptake in core incubations were significantly lower than in situ uptake, with the difference largely attributed to decreased denitrification rates in recovered cores in response to altered temperature and pressure. On the other hand, Miller-way et al. (1994) found no discernable difference between core incubations and in situ measurements in a shallow station on the Louisiana continental shelf.

Benthic chambers
To reduce the effect of altered pressure, exclusion of large benthic fauna and the destruction of benthic fauna habitats such as worm burrows on sediment – water exchange rates, benthic chambers can be deployed at the sediment surface. However, the chambers are difficult and expensive to deploy in deep waters (Hammond et al.,

1985). Another complication of this technique is that turbulence within the chamber may be different to that of normal conditions (Hammond et al., 1985). For instance, some authors have shown that the effect of stirring rate on benthic fluxes can be large due to the disturbance of the diffusive boundary layer (DBL) (Hammond et al., 1985).

The DBL is a thin laminar flow between the sediment and water interface and controls the rate of diffusion of solutes between the sediment - water interface and is dependent on the appropriate level of turbulence. Therefore turbulence levels different to natural conditions will greatly affect the flux of solutes through this layer (Hammond et al., 1985; Gundersen and Jorgensen, 1990; Jorgensen and Des Marais, 1990). Other limitations of chambers include the difficulty of measuring small fluxes, and that long deployments may lead to the production of anoxic conditions (Hammond et al., 1985).

Solute porewater profiles

The final technique discussed is the calculation of fluxes from pore water concentrations. The production of solutes within the sediment and diffusion rates across the sediment-water interface can be modeled from porewater profiles within the sediment and at the sediment-water interface (Berner, 1980). Berg et al. (1998) developed a model, 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 and includes the non-diffusive transport mechanisms of bioturbation and bioirrigation.

A number of techniques have evolved over time to determine porewater solute concentration profiles. In soft sediments, porewater solutes 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. Unfortunately, these techniques have limited resolution (approximately 2mm) and can therefore affect the accuracy of predicting diffusive fluxes of solutes from profiles. However, the advent of microelectrodes, has allowed measurement of 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.

In this study, core incubations were used to measure integrated fluxes of CO_2, O_2, NH_4^+, NO_3^-, NO_2^-, SiO_2, PO_4^{3-} and alkalinity while gradients of oxygen, NH_4^+, and NO_3^- were measured in the porewaters. Oxygen gradients were measured using microelectrodes while NH_4^+ and NO_3^- porewater profiles were measured by the core slicing and centrifuging technique. 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 O_2 consumption and NH_4^+ production within the sediments were calculated using the porewater modeling routine of Berg et al. (1998). Oxygen consumption measured in core incubations and modeled from porewater profiles were compared to infer the influence of macrofauna on respiration rates.

1.4 Experimental design

1.4.1 General Study Area

The Huon estuary is considered to be mesotrophic. Total nitrogen is high (10 - 20µm) mostly in the form of Dissolved organic nitrogen (DON), which enters the estuary from the forested catchments in the upper reaches. Dissolved inorganic nitrogen is low and dominated by nitrate. Nitrate tends to peak between March and September (>3µm) before been depleted in spring by phytoplankton blooms (<0.5 µm). Ammonia is almost always below 1µm. Nitrogen is considered to be the limiting nutrient in this system. The estuary is generally well oxygenated with 80 – 100% DO saturation typical throughout the year (Butler et al., 2000).

The high quality of water is due to the upper catchment of the estuary been near pristine, which is characterised by native forest. In the lower catchment, the main industry is agriculture, in the form of horticulture and livestock grazing. The vegetation of the catchments is characterised by alpine (austral-montane), temperate rain forest and Sclerophyll forests (Eucalyptus spp.) with only 5.6% of the catchment currently cleared. Coloured dissolved organic matter (CDOM) is rapidly leached from some vegetation types e.g. buttongrass moors (*Gymnoschoenus sphaerocephalus*), plant species (e.g. tea –tree *Leptospermum spp*.) and also soils (e.g. peats) leaving the estuary water column strongly coloured (Butler et al., 2000).

Apart from agriculture, no other major industries apart from a saw mill operating between the early 1900's to 1929 at Hospital bay, and a neutral sulphite semi-chemical pulp mill operating between 1962 – 82 and 1986 – 1991 at the same site as the saw mill existed. More recently marine farming has been introduced since the mid 1980's mainly in the form of salmonid culture and some shellfish culture. Three sewage treatment plants with some secondary treatment service the region, which is populated with about 13,000 people living in a number of small towns within the valley (Butler et al., 2000).

The main physical attributes of the Huon Estuary include a salt-wedge that penetrates into the upper reaches and relatively high flushing rates. Annual median flows are $41m^3 s^{-1}$ and the average flushing period is approximately 7 days. The estuary is 39km in length, with an area of $83km^2$ and a mean depth of 16.6m. The estuary is also characterised by a shallow brackish zone in the upper half of the estuary and a deeper marine lower estuary with the presence of a mixing zone between the brackish and marine sections located at the 'elbow' of the estuary (Butler et al., 2000).

1.4.2 Study Locations

Two locations exhibiting different sediment characteristics were chosen for this study. The main consideration was to select study locations that were potentially influenced by different sources of organic matter. This was done so that we could assess the impact of different types of organic matter on sediment respiration and nutrient cycling. Care was taken so that other potential drivers of respiration and nutrient cycling such as water depth, sediment grain size and water quality were similar at both sites. The final position of the two locations was based on the Huon estuary report (Butler et al., 2000), which investigated the sources of organic matter within the sediments.

The first location was positioned in the mid to upper reaches of the estuary, at the terrestrial end of the estuary while the second location was positioned at the mouth of the estuary at the marine end of the estuary (Figure 1-3). At both locations, the sediments were predominantly silt-clays, while depths ranged between 10 – 20 metres at the upper estuary location and 25 – 35 metres at the lower location.

1.4.3 Sampling Design

At both locations, a transect consisting of 3 sites from east to west was set up to study benthic respiration and nutrient cycling (Figure 1-3). At the upper location, the sites were called upper east, upper centre and upper west and will be referred to as UE, UC and UW respectively throughout the rest of the thesis. Likewise, the sites at the lower location were called lower east, lower centre and lower west and will be referred to as LE, LC and LW respectively throughout the rest of this thesis.

At each site triplicate cores were taken to measure sediment-water exchange of nutrients and respiration, duplicate cores were taken to measure oxygen and nutrient micro-profiles while sediment samples were also obtained for bulk carbon and nitrogen analysis, sediment porosity and grain size as well as stable isotopes $\delta^{13}C$ and $\delta^{15}N$ and lipid molecular biomarkers. Water samples were also taken to measure basic water quality parameters and background nutrient concentrations

The study locations were visited three times in 2004, including March, July and November, using a small research vessel. The two locations were sampled on separate occasions, generally 3 days to a week apart, due to resource and labour limitations. Data from an additional sampling trip in April 2005 from Hideaway bay (P3) and Garden Island (P4) (Figure 1-3) have also been used in this thesis and will serve as a comparative study of the benthic processes with the upper and lower locations of the main study.

The rationale behind selecting the upper and lower locations was to elucidate the impact of different sources of organic carbon on benthic respiration and nutrient cycling and to examine the spatial variability within a sampling location (i.e. variation between cores at site UW), between sites within the same location (i.e. UW v. UE) and between sampling locations (i.e. Upper estuary v. Lower estuary). Samples from both locations were also taken at different times during the year to determine if there was any temporal variability associated with the sources of organic matter, benthic respiration and nutrient cycling. Measuring algal sedimentation was not undertaken during this study due to time and technical constraints of the fieldwork. It is noted

however that such measurements would have being desirable and would have added valuable information to the thesis.

A separate study (chapter 5) was also done to assess the impact of organic enrichment on sediment biogeochemical processes. A detailed description of the methodology and experimental design can be found in chapter 5. Briefly however, sediment core samples were collected in June 2005, homogenised, reconstituted into cores and then loaded with different concentrations of organic carbon after a re-equilibrium period. To test the efficacy of using re-homogenised cores, sediment exchange of oxygen and nutrients were measured prior to carbon loading and compared with results from the 2004 field study.

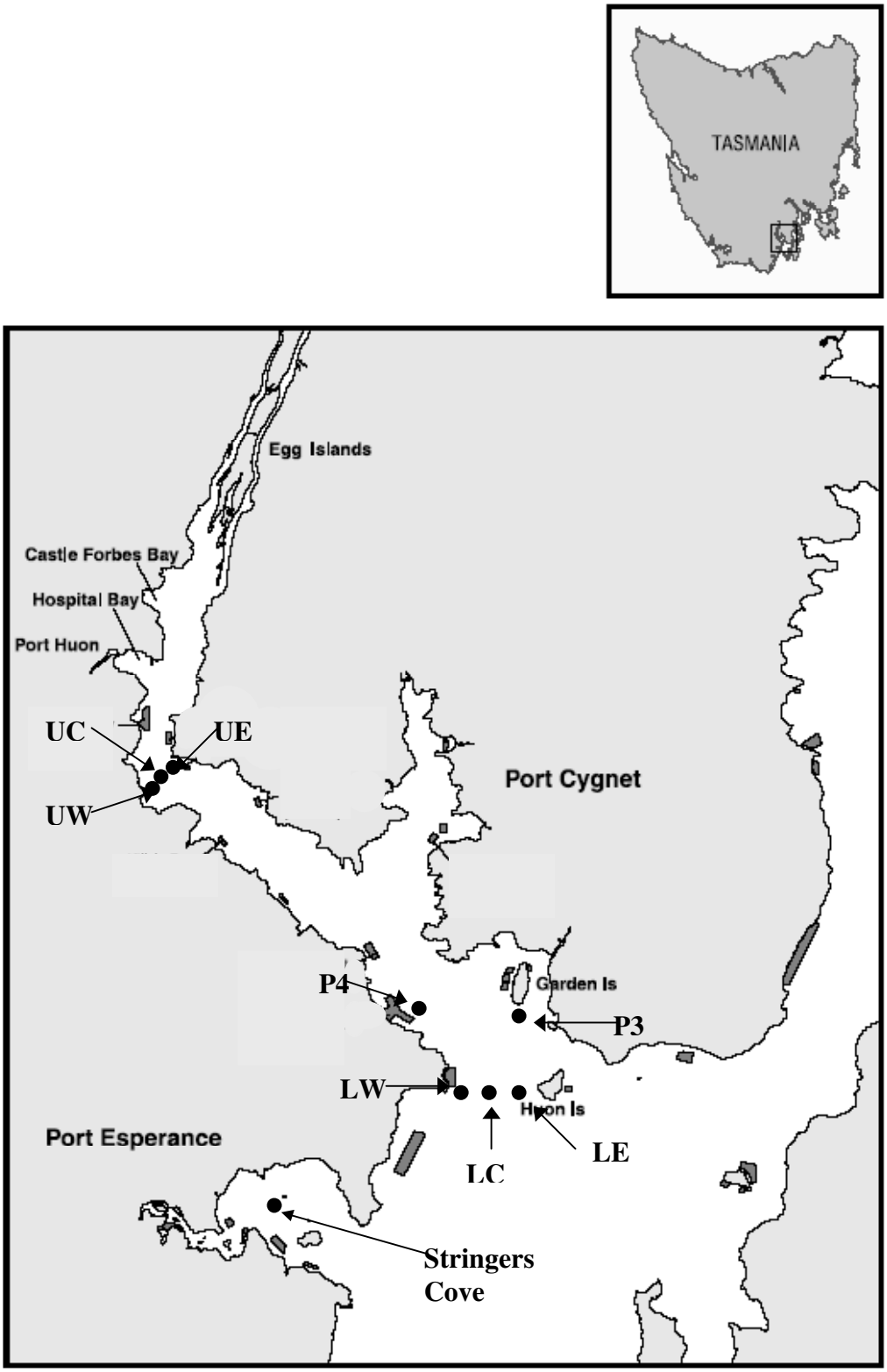


Figure 1-3 The sampling sites for the Huon Estuary Study. The sampling locations include the upper estuary stations UE, UC & UW, the lower estuary stations LE, LC & LW, and additional sampling stations at Hideaway bay (P4) and Garden island (P3). Also included is sampling station at Stingers cove, which was used in a different study, but is referred to in this study for comparative purposes.

Chapter Two

Sources of Organic matter in the Huon Estuary

Chapter 2. Sources of Organic Matter

2.1 Introduction

Sediments play a vital role in the ecological functioning of an estuary by retaining much of the organic matter and minerals supplied naturally by rivers, catchment run-off and inputs from the water column. Much of the organic matter is remineralised by the microbial and faunal populations present, liberating nutrients and consuming oxygen, but more refractory material is buried in the sediments (Herbert, 1999).

The quality of the organic matter reaching the seafloor will heavily influence the rates and recycling pathways of carbon and nitrogen in sediments (Herbert, 1999). The carbon to nitrogen (C/N) ratio can be used as a proxy to measure the quality of the organic matter. A C/N ratio close to that of the Redfield ratio (6.625) is indicative of organic matter derived from microalgae while organic matter derived from terrestrial sources can have a C/N ratio of 20 or more (Bordovskiy, 1965).

Where the original organic matter undergoing decomposition has a high C/N ratio, much of the nitrogen remineralised may be reassimilated into microbial biomass (Schlesinger, 1997). Organic matter that is more labile and has a lower C/N ratio will stimulate rapid remineralisation rates and a release of nitrogen from the sediment (Hansen and Blackburn, 1992). Note, however, that as degradation proceeds the C/N ratio usually increases as nitrogen-rich labile organic matter is consumed (Thornton and McManus, 1994).

Ratios of $^{12}C/^{13}C$ stable isotopes provide a good estimate of the relative contribution of terrestrial and marine sources to sedimentary organic matter (Fry and Sherr, 1984). Terrestrial organic matter will generally have a $\delta^{13}C$ value of -26 to -30‰ and organic matter with a marine origin will generally have a $\delta^{13}C$ of -19‰ to -23‰ depending on the particular organisms present (Heip et al., 1995). As a first approximation, the relative proportion of marine and terrestrial carbon in a sample can be estimated by linear additions of 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 two well-defined end-members.

Furthermore, this technique gives little information about the type of marine or terrestrial organic matter in question.

Various other proxies have been used to estimate the sources of organic matter in sediments and from this inferences about the amount of labile organic matter present can be made. For example, biochemical's such as carbohydrates, proteins and lipids are rapidly degraded in sediments and so measures of their abundance provide an estimate of the labile organic matter present (Misic and Fabiano, 1996; Fabiano and Pusceddu, 1998; Pusceddu et al., 1999; Danovaro et al., 2001). Alternatively, lipid and pigment biomarkers can allow the various sources of various sub-fractions of organic matter to be identified.

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 such as α- and β-amyrins, lupeol, taraxasterol, betulin etc. are widely used as markers for higher plants (e.g. Volkman et al., 1987; Volkman, 2000), even though some of these have additional minor sources (Volkman, 2005). Hopanoid alcohols are excellent markers for cyanobacteria and other prokaryotes (Summons et al., 1999).

The aim of the work described in this chapter was to determine the source and quality of sedimentary organic matter at the two study locations to help understand how organic carbon and nitrogen is recycled in the Huon estuary. Furthermore, very few sediment ecological studies have been undertaken in cold temperate Southern hemisphere estuaries and thus these data provide a good comparison with more heavily degraded Northern hemisphere estuaries. In order to get a comprehensive picture of sedimentary organic matter a variety of organic geochemical techniques were employed including analysing the sediments for total organic carbon and nitrogen contents, $\delta^{13}C$ and $\delta^{15}N$ isotopes and lipid biomarkers.

2.2 Methods

2.2.1 Total Carbon and Nitrogen concentrations and Stable Isotope $\delta^{15}N$ and $\delta^{13}C$ Analysis

Sediment sub-samples, taken from the same samples used for the analysis of lipid biomarkers, were dried in an oven overnight at 60°C, before being ground with a mortar and pestle. For the analysis of total organic carbon, sediments were weighed into smooth-walled tin cups and acidified with sulphurous acid to remove any inorganic carbon. 10 µl of 8% sulphurous acid was carefully added to each sample and then placed into an oven heated to 60°C until dry. This process was then repeated until effervescence was no longer detected. Sediment samples for nitrogen analysis were weighed into aluminium cups with no further processing required.

Samples were then analysed for nitrogen and carbon contents, and stable isotopes $\delta^{15}N$ 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 continuous flow mode. Combustion and oxidation were achieved at 1090°C and reduction at 650°C. Samples were analysed in duplicate. Results are presented in standard δ notation:

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

Where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon is Vienna PDB limestone and the standard for nitrogen is atmospheric N_2.

2.2.2 Lipid Biomarkers

2.2.2.1 Collection and extraction of sediment

Sediment samples were collected from the Huon estuary using a box corer. Representative sub-samples of the sediment surface were taken from two grabs at each site, were stored in glass jars and frozen until analysed. A 20–22 g aliquot of wet sediment was extracted in a 250 ml separation funnel using a modified Bligh and Dyer methanol/CH_2Cl_2/water mixture (2:1:0.8 by vol.). The samples were shaken

every hour for 3 hours and then left overnight to extract. Phases were separated the next day by addition of CH_2Cl_2 and water to bring the final mixture ratio to 1:1:0.9 methanol/CH_2Cl_2/water by volume. The total solvent extract was obtained by rotary evaporation of the organic solvent phase. This was then stored in glass vials, refrigerated ready for saponification.

2.2.2.2 Fatty acids and sterols

A 50% aliquot of the total solvent extract was saponified with potassium hydroxide in methanol, 5% wt./vol, under nitrogen for 2 hours at 80°C. Non-saponified neutrals were than extracted into hexane/CH_2Cl_2 (4:1 by vol, 3*3 ml) and transferred to sample vials ready for analysis by gas chromatography (GC) and gas chromatography mass spectrometry (GCMS) after derivatisation with 100 µl of BSTFA solution at 60°C for 1 hour. After acidification of the remaining aqueous layer using hydrochloric acid down to pH=2, total fatty acids were obtained and methylated to form their fatty acid methyl esters using methanol/hydrochloric acid/CH_2Cl_2 (10:1:1 by volume) at 80°C for 2 hours. A C_{23} straight-chain fatty acid methyl ester internal standard was then added before analysis using GC and GCMS.

2.2.2.3 Instrumentation

Gas chromatography

GC was performed on a Varian 3410 gas chromatograph fitted with and HP5 ultra capillary column (50 m; 0.32 i.d.; 0.17 µm film thickness), with an FID detector and SPI programmable injector. Samples were injected at 45°C; after 1 minute the oven temperature was raised at 30°C min^{-1} to 140°C and then at 3°C min^{-1} to 300°C where it was held for 5 minutes.

Mass spectrometry

GC–MS analysis of the FAME and neutral lipid fractions were performed with a Finnigan GCQ Plus GC-MS system fitted with on-column injection set at 45°C. Samples are injected using an AS2000 auto sampler into a retention gap attached to a HP5 Ultra2 50 m, 0.32 mm i.d, and 0.17 µm film thickness column using helium for

the carrier gas. Typical mass spectrometer operating conditions were as follows: EV, 70 eV; Emission current 250, transfer line 310^{o}C. Source temperature 240^{o}C, 0.8 scans sec^{-1} and mass range 40-650 Dalton.

2.2.3 Sediment Grain Size

Sediment samples were taken from the same box cores that were analysed for respiration, nutrient fluxes and lipid biomarkers. Three sub-samples were taken, of which two were weighed wet and then dried at 60^{o}C in an oven overnight and then reweighed to determine the % water content of the samples. The third sample was weighed wet and then washed through a series of Endecott (London UK) metal sieves including 500 μm, 250 μm, 125 μm and 63 μm meshes. The sieves were then dried overnight. The dry sediment was then reweighed and converted back to wet weight using the % water content as the conversion factor to determine the wet weight of each grain size fraction at each sampling site. The difference between initial wet weight and final wet weight was assumed to have been the sediment particles that passed through the 63 μm sieve. The amount of sediment in each grain size fraction was then expressed as a percentage of the total sample.

2.2.4 Porosity

The porosity of the sediment was determined by subtracting the dry weight of the sample from the wet weight and then dividing by the volume of the sample (wet weight/sediment density) (Dalsgaard et al, 2000)

2.3 Results

2.3.1 Sediment grain size, Porosity, Carbon and Nitrogen contents and Stable Isotope analysis

The sediment was dominated at both locations by fine silts, comprising 43–64.3% of the sediment mass, and sediment porosity ranged between 0.73 and 0.87 (Table 2-1). A clear difference was seen in organic carbon contents in sediments from the upper and lower estuary locations. The organic carbon contents averaged 8.1% and 4.1% at the upper and lower locations respectively. At the upper estuary stations values ranged between 7.7 and 8.8%, while at the lower end they ranged between 3.4 and 4.7%. Nitrogen content was generally low at all stations and ranged between 0.3 and 0.6%. The C/N molar ratio averaged 17.2 and 11.1 at the upper and lower estuary locations respectively (Table 2-1).

The $\delta^{13}C$ values at all stations ranged between -22.4 and -19.7‰ over the study period. The most isotopically enriched station was recorded in July at LW. Generally the lower estuary stations were more enriched (-19.7 to -21.7‰) compared to the upper estuary stations (-21.7 to -22.4). The $\delta^{15}N$ values ranged between 7.3 and 8.3‰ at the lower estuary stations while at the upper estuary stations they ranged between 4.1 and 5.9‰.

No clear temporal patterns emerged over the course of the study period at any of the stations. Organic carbon and C/N ratio increased from west to east at the upper stations, while the $\delta^{15}N$ values decreased. In contrast, the $\delta^{13}C$ values remained stable across the three stations at the upper location.

Table 2-1 Organic carbon and nitrogen as % dry wt. (%C_{org} and %N_{org}), stable isotope values $\delta^{13}C$ and $\delta^{15}N$, molar carbon:nitrogen ratios in organic matter (C% / N%), silt/clay fraction and porosity in surface sediments of the study locations

	%C_{org}	%N_{org}	C/N	$\delta^{13}C$	$\delta^{15}N$	%<63 µm	Porosity
LW							
March	4.5	0.5	10.5	-21.7	7.7	-	
July	3.8	0.4	11.1	-19.7	7.6	51.5	
November	3.6	0.4	10.5	-20.3	8.3	-	0.73
LC							
March	4.7	0.5	11.0	-21.3	7.4	-	
July	4.6	0.5	10.7	-20.3	7.4	43.7	
November	4.5	0.5	10.5	-19.9	8.1	-	0.81
LE							
March	3.4	0.3	13.2	-20.0	7.3	-	
July	3.9	0.4	11.4	-21.1	7.4	53.3	
November	3.8	0.4	11.1	-21.0	8.0	-	0.80
UW							
March	7.7	0.6	14.9	-22.3	5.2	-	
July	7.8	0.6	15.2	-22.3	5.1	60.1	
November	7.7	0.5	18.0	-22.2	5.9	-	0.87
UC							
March	8.1	0.6	15.8	-21.7	4.6	-	
July	8.2	0.6	16.0	-22.3	4.7	64.3	
November	8.0	0.5	18.7	-22.3	5.3	-	0.83
UE							
March	8.2	0.5	19.1	-22.4	4.2	-	
July	8.8	0.6	17.2	-21.9	4.1	57.0	
November	8.7	0.5	20.3	-22.1	4.8	-	0.77
Average Lower Stations	4.1	0.4	11.1	-20.6	7.7	49.5	0.78
Average Upper Stations	8.1	0.6	17.2	-22.2	4.9	60.5	0.82

2.3.2 Fatty Acids

A total of 31 fatty acids were identified from the surficial sediments (top 1 cm) including polyunsaturated (PUFA), monounsaturated (MUFA), saturated and branched fatty acids (Figure 2-1). Absolute concentrations ranged between 5.7 and 16.3 µg g^{-1} dry sediment. Saturated fatty acids were the most abundant form of fatty acid accounting for 50.6–72.9% of total fatty acids. The most abundant saturated fatty acid was 16:0, which is often abundant in marine sediments. MUFAs comprised 14.6

– 30.6% of total fatty acids, while PUFAs only accounted for 2.7–6.1% (Table 2-2). The only PUFAs detected were 20:4(n-6), 20:5(n-3) and 22:6(n-3), although minor amounts of C_{18} PUFA may have been present. The branched fatty acids, principally *iso*- and *anteiso*-odd chained compounds were a relatively minor component of fatty acids ranging between 0.8–1.1 μg g^{-1} dry sediment and contributing 8.0 and 16.9% of the total fatty acids. The main branched fatty acids present were the bacterially derived fatty acids *iso*- and *anteiso*-15:0.

Fatty acid distributions varied little between the upper and lower locations; however absolute amounts of long-chain fatty acids were more abundant in the upper locations. At both locations fatty acid profiles were dominated by terrestrial markers (long-chain saturated fatty acids) and to a lesser extent bacterial markers (branched fatty acids).

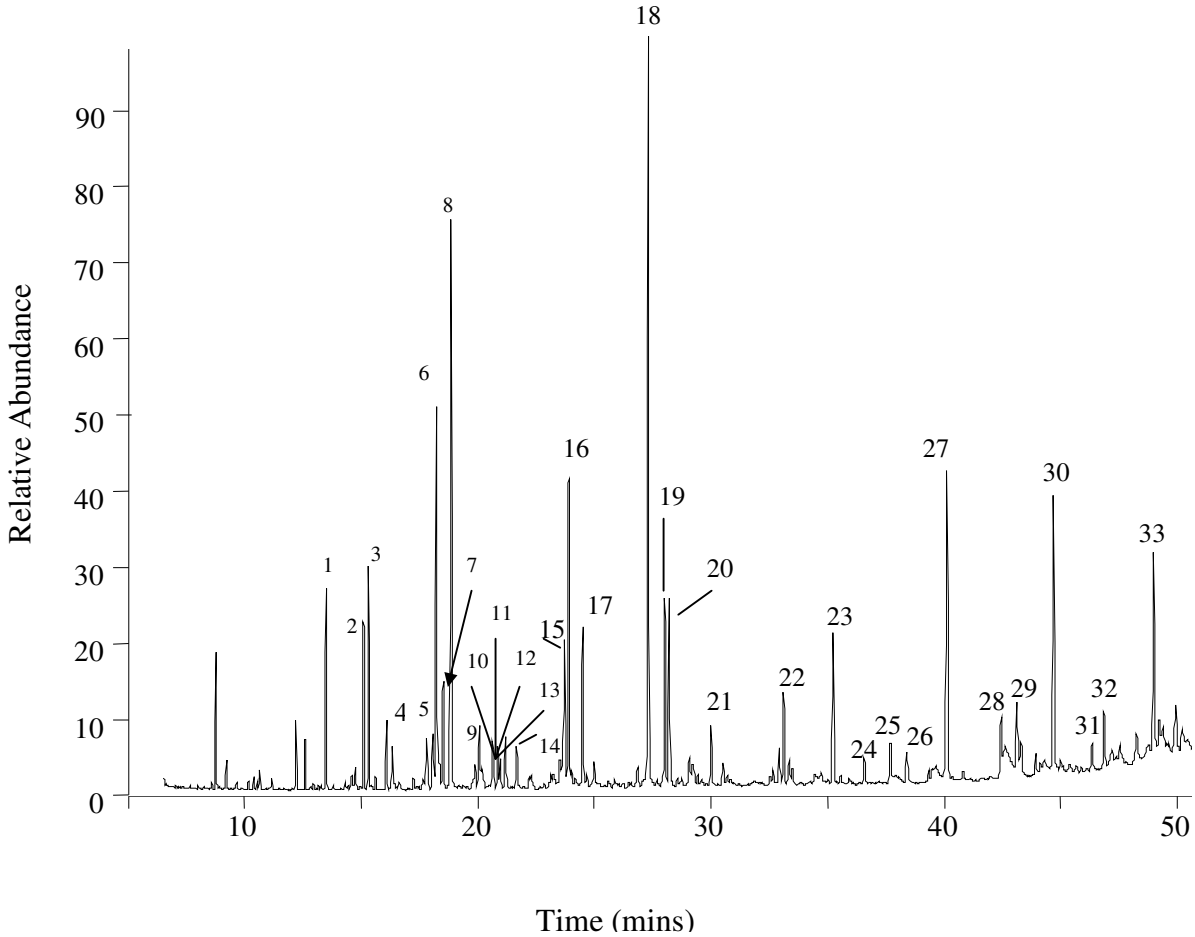


Figure 2-1 Fatty acid chromatogram of sediment at the Lower estuary. Peak numbers refer to table 2-2 below.

Table 2-2 Fatty acids from a representative sediment sample

No.	Fatty Acid	No.	Fatty acid
1	14:0	18	Internal Standard (19:0)
2	i15:0	19	20:4(n-6)
3	ai15:0	20	20:5(n-3)
4	15:0	21	20:0
5	16:1(n-9)	22	22:6(n-3)
6	16:1(n-7)	23	22:0
7	16:1(n-5)	24	Unknown
8	16:0	25	23:0
9	Unknown	26	Unknown
10	i17:0	27	24:0
11	a17:0	28	25:0
12	17:1(n-8)	29	Unknown
13	17:1(n-6)	30	26:0
14	17:0	31	Unknown
15	18:1(n-9)	32	27:0
16	18:1(n-7)	33	28:0
17	18:0		

Table 2-3 Fatty acid composition (µg g^{-1} dry weight) at the lower and upper locations. 'tr', <0.05 µg g^{-1} dry weight '-' not detected

	LE			LC			LW			UE			UC			UW		
	Mar	Jul	Nov	Mar	Jul	Nov	Mar	Jul	Nov	Mar	Jul	Nov	Mar	Jul	Nov	Mar	Jul	Nov
Saturated Acids <(n-C_{20})																		
12:0	tr	tr	tr	tr	0.1	tr	tr	Tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
14:0	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.4	0.4	0.4	0.4	0.5	0.4	0.6	0.4	0.4
15:0	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:0	1.0	1.2	0.9	1.1	1.1	0.9	1.5	1.4	0.9	1.2	1.6	1.4	1.4	1.8	1.3	1.8	1.4	1.2
17:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
18:0	0.2	0.3	0.2	0.3	0.3	0.2	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.5	0.3	0.4	0.4	0.3
20:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2
Sum	**1.8**	**2.3**	**1.6**	**2.1**	**2.2**	**1.6**	**2.7**	**2.4**	**1.7**	**2.3**	**3.0**	**2.6**	**2.6**	**3.4**	**2.5**	**3.4**	**2.7**	**2.3**
Saturated Acids >(n-C_{20})																		
22:0	0.2	0.3	0.2	0.3	0.3	0.2	0.2	0.3	0.2	0.7	1.2	0.9	0.8	1.2	0.8	0.8	0.9	0.6
23:0	0.1	0.1	tr	0.1	0.1	tr	0.1	0.1	Tr	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.2
24:0	0.3	0.5	0.3	0.5	0.5	0.3	0.5	0.5	0.3	1.1	1.8	1.3	1.3	2.0	1.4	1.3	1.4	1.1
25:0	0.1	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.8	1.2	0.9	0.9	1.3	0.8	0.9	0.9	0.6
26:0	0.3	0.4	0.3	0.4	0.4	0.3	0.4	0.5	0.3	0.8	1.3	0.9	0.9	1.5	1.0	0.9	1.1	0.8
27:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.4	0.3	0.3	0.5	0.3	0.3	0.4	0.2
28:0	0.2	0.3	0.3	0.3	0.4	0.2	0.3	0.4	0.3	0.4	0.9	0.6	0.6	1.1	0.7	0.6	0.8	0.6
30:0	0.2	0.3	0.2	0.3	0.3	0.2	0.3	0.3	0.2	0.1	0.3	0.3	0.2	0.4	0.2	0.2	0.3	0.2
Sum	**1.5**	**2.2**	**1.6**	**2.0**	**2.3**	**1.5**	**2.1**	**2.3**	**1.5**	**4.4**	**7.5**	**5.6**	**5.3**	**8.3**	**5.4**	**5.3**	**6.0**	**4.5**
Branched Saturated Acids																		
i14:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
i15:0	0.3	0.4	0.2	0.3	0.3	0.2	0.4	0.4	0.2	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.3
a15:0	0.4	0.4	0.3	0.4	0.4	0.3	0.4	0.4	0.2	0.4	0.4	0.4	0.4	0.5	0.4	0.5	0.4	0.4
i16:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
i17:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
a17:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Sum	**1.0**	**1.2**	**0.8**	**1.0**	**1.1**	**0.7**	**1.3**	**1.1**	**0.7**	**0.9**	**1.1**	**0.9**	**1.1**	**1.3**	**1.1**	**1.4**	**1.1**	**1.0**
Monounsaturates																		
16:1(n-9)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
16:1(n-7)	0.6	0.7	0.6	0.6	0.7	0.6	0.8	0.7	0.6	0.5	0.7	0.6	0.6	0.8	0.7	0.9	0.7	0.6
16:1(n-5)	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
17:1(n-8)	tr	0.1	tr	tr	0.1	tr	0.1	0.1	tr	tr	tr	tr	tr	tr	tr	0.1	tr	Tr
17:1(n-6)	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1
18:1(n-9)	0.3	0.4	0.2	0.3	0.3	0.2	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.5	0.3	0.4	0.4	0.3
18:1(n-7)	0.5	0.8	0.5	0.5	0.6	0.4	0.8	0.8	0.5	0.4	0.6	0.5	0.5	0.7	0.6	0.8	0.7	0.6
Sum	**1.8**	**2.4**	**1.7**	**1.9**	**2.1**	**1.6**	**2.5**	**2.5**	**1.6**	**1.5**	**2.1**	**1.8**	**1.8**	**2.5**	**2.1**	**2.7**	**2.1**	**1.9**
Polyunsaturates																		
20:4(n-6)	tr	0.1	tr	tr	0.1	tr	tr	0.1	tr	0.3	0.4	0.3	0.3	0.4	0.3	0.3	0.3	0.2
20:5(n-3)	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.3	0.1	0.2
22:6(n-3)	0.1	0.1	tr	tr	0.1	tr	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1
Sum	**0.3**	**0.3**	**0.4**	**0.3**	**0.3**	**0.3**	**0.6**	**0.3**	**0.3**	**0.5**	**0.7**	**0.7**	**0.6**	**0.8**	**0.7**	**0.8**	**0.6**	**0.6**
Total Fatty Acids	**6.5**	**8.4**	**6.1**	**7.1**	**8.0**	**5.7**	**9.2**	**8.6**	**5.8**	**9.6**	**14.4**	**11.7**	**11.4**	**16.3**	**11.7**	**13.5**	**12.6**	**10.2**
% Saturated Acids <(n-C_{20})	28.0	26.7	28.0	28.9	27.3	28.4	29.8	28.4	29.1	24.1	21.0	22.6	22.5	20.7	21.3	24.9	21.4	22.4
% Saturated Acids >(n-C_{20})	24.4	26.0	26.0	28.1	28.8	25.7	23.4	26.6	25.1	45.3	51.9	47.7	46.5	51.1	46.4	39.1	48.1	43.9
% Branched Saturated Acids	15.3	14.5	12.9	13.4	13.2	12.4	13.7	12.9	12.2	9.5	7.7	8.0	9.8	8.1	9.0	10.5	8.9	9.6
%Monounsaturates	28.1	28.8	27.9	26.0	26.7	28.0	27.1	28.5	28.2	15.8	14.5	15.6	16.2	15.2	17.8	19.8	16.8	18.5
% Polyunsaturates	4.2	4.0	6.2	3.7	4.0	5.5	6.0	3.7	5.4	5.3	5.0	6.1	5.0	4.9	5.6	5.8	4.8	5.5
16:1/16:0	0.9	0.9	0.9	0.8	0.9	0.9	0.7	0.6	0.9	0.6	0.6	0.6	0.6	0.6	0.8	0.7	0.7	0.8

2.3.3 Alkanes

Odd and even numbered alkanes ranging between C_{19} and C_{31} were identified from the surficial sediments (top 1 cm) at the upper and lower locations (Figure 2-2). Sediments at both locations showed a preference of long-chain (>C_{25}) odd-numbered alkanes and these peaked at C_{27}. The total concentration of alkanes ranged between 142 and 343 ng g^{-1} at all sampling sites (Table 2-4). Long-chain odd-numbered alkanes (C_{25}, C_{27}, C_{29} & C_{31}) ranged between 45.9 and 61.3% with an average of 53.5% of total alkanes at the lower estuary. In comparison, the upper estuary ranged between 54.9 and 62.8% and an average of 59.0% across all sampling periods.

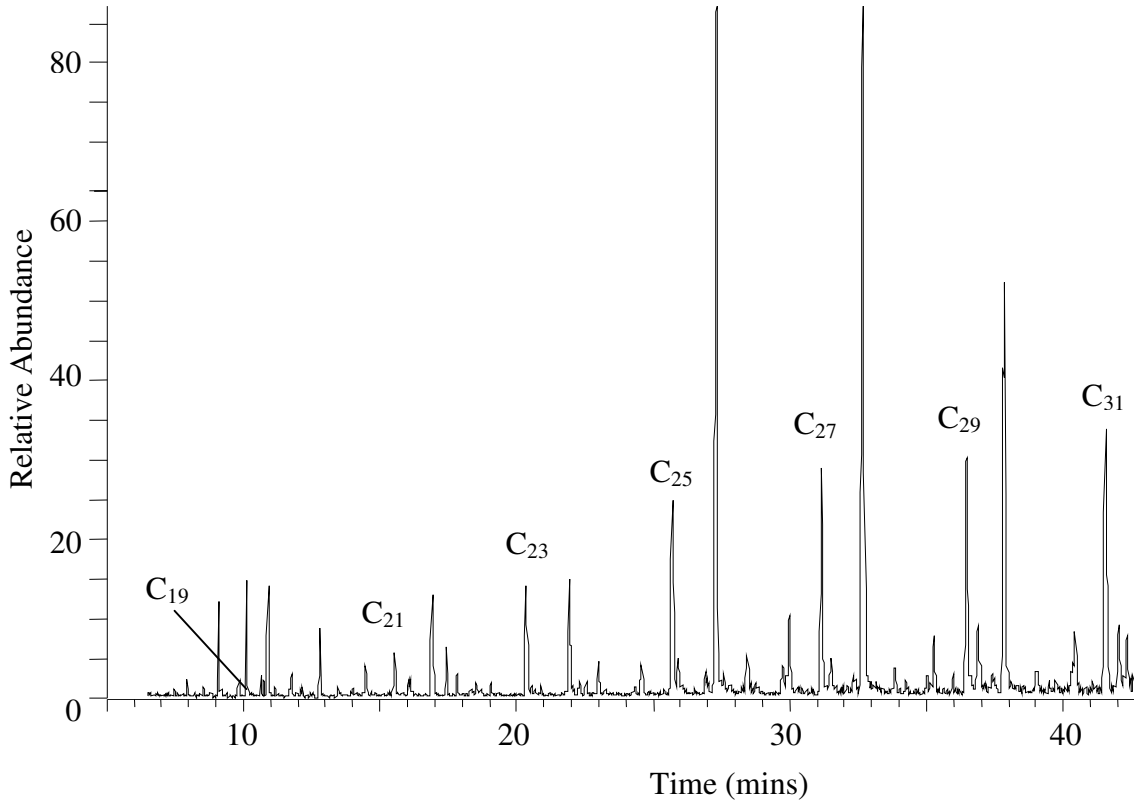


Figure 2-2 Gas chromatogram of alkanes in sediment at the Upper estuary. Peak numbers refer to carbon chain length of the alkanes. The major peaks in this chromatogram are alcohols.

Table 2-4 Distribution of odd-chain alkanes C_{19} – C_{31}, total sum of alkanes (including odd-numbered alkanes) and the percent contribution of odd-chained alkanes $C_{25\text{-}31}$, at both upper and lower locations sampled in March, July and November 2004.

	Alkane Concentration (ng g^{-1})							Total	$\sum C_{25,27,29,31}$ (%)
	C_{19}	C_{21}	C_{23}	C_{25}	C_{27}	C_{29}	C_{31}	ng g^{-1}	
LW									
March	12.5	19.8	19.4	17.4	70.7	18.1	6.8	199.7	56.5
July	12.4	17.6	19.7	16.4	69.1	22.9	8.0	193.4	61.3
November	8.1	13.5	13.7	12.7	39.9	13.2	5.2	129.3	54.5
LC									
March	11.4	22.4	15.0	8.3	41.7	15.7	6.4	157.1	45.9
July	10.5	19.0	17.6	13.6	53.2	18.0	6.4	170.6	53.7
November	5.0	14.5	12.8	7.6	37.2	11.6	6.2	118.4	53.0
LE									
March	11.9	13.1	12.1	13.0	46.8	13.1	7.0	154.0	51.9
July	10.8	16.3	16.4	12.7	56.3	18.8	7.1	173.8	54.6
November	6.5	13.4	14.9	11.2	40.5	11.8	7.1	142.0	50.5
UW									
March	13.8	22.1	50.3	51.2	119.3	32.5	6.4	343.3	61.1
July	13.2	19.9	38.8	31.6	66.1	36.7	8.6	255.2	56.1
November	9.8	15.7	29.0	25.8	51.8	22.7	8.3	197.9	54.9
UC									
March	12.2	22.1	51.3	57.4	101.8	30.1	23.7	339.1	62.8
July	13.8	23.0	48.1	43.0	80.0	46.6	9.3	309.2	57.9
November	8.4	19.2	38.3	34.1	59.1	27.2	9.6	238.0	54.6
UE									
March	8.5	16.5	44.1	48.5	83.6	25.5	21.9	287.8	62.3
July	11.3	18.9	42.6	40.0	71.3	42.5	10.3	270.2	60.8
November	9.7	21.5	46.9	38.3	73.9	41.1	31.0	304.7	60.4
Average Lower Stations	9.9	16.6	15.7	12.5	50.6	16.1	6.7	160.2	53.5
Average Upper Stations	11.2	19.9	43.3	41.1	78.7	33.9	14.3	283.0	59.0

2.3.4 Fatty Alcohols

The fatty alcohols detected (Figure 2-3) are predominately even numbered compounds ranging from C_{16} to C_{26}. C_{28} and C_{30} were detected but not quantified as they co-eluted with sterols. In all samples, the alcohols $>C_{22}$ are more abundant than those of short-chain homologues and typically maximised at C_{22} or C_{24}. Total concentrations of alcohols ranged between 1.1 and 1.8 µg g^{-1} at the lower estuary sites, and 2.3 and 4.3 µg g^{-1} at the upper estuary sites, an order of magnitude greater than total alkanes at the same sites (Table 2-5).

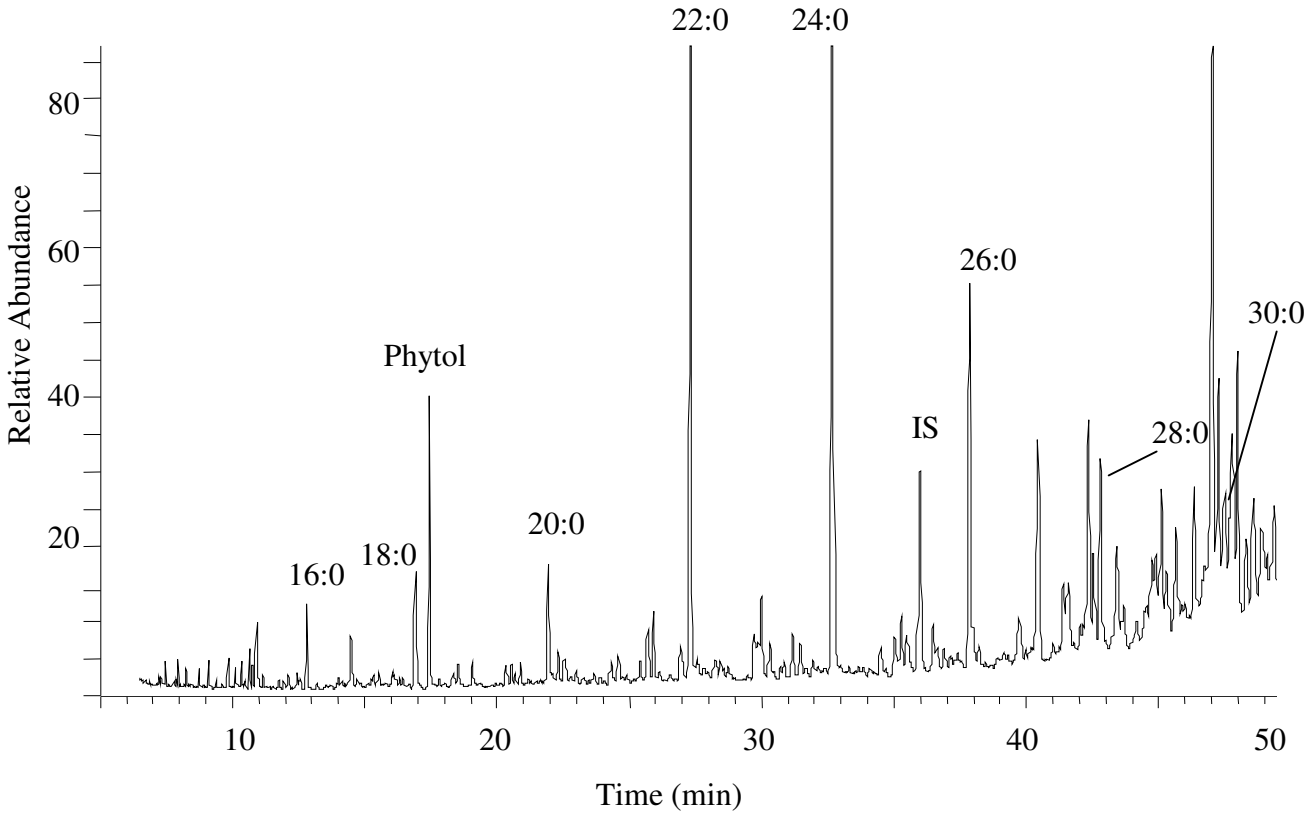


Figure 2-3 Gas chromatograms after saponification (as TMSi-ethers) of fatty alcohols. IS is the internal standard. Baseline rise in is due to column bleed at high temperatures.

Phytol was detected (Figure 2-3) in all samples. Absolute concentrations ranged between 0.2–0.4 µg g^{-1} dry sediment and averaging 0.3 µg g^{-1} at both locations across all sites and times in any particular location (Table 2-5). When normalised to TOC, phytol concentrations at the lower estuary sites were approximately double the

concentrations found at the Upper estuary sites. No temporal or spatial trends were found at either location.

Table 2-5 Fatty alcohol composition (µg g^{-1} dry weight) at the lower and upper locations (note, $C_{28:0}$ and $C_{30:0}$ were identified but not quantified as they co-eluted with sterols)

	Phytol	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	$C_{24:0}$	$C_{26:0}$	Total Alcohol	Phytol/TOC
				µg g^{-1} dw					µg g^{-1} TOC
LW									
March	0.4	tr	0.1	0.1	0.5	0.5	0.3	1.6	7.9
July	0.3	tr	tr	0.1	0.5	0.6	0.4	1.8	8.4
November	0.3	tr	tr	0.1	0.4	0.4	0.2	1.3	8.6
LC									
March	0.3	tr	0.1	0.1	0.4	0.5	0.3	1.5	7.2
July	0.3	tr	tr	0.1	0.5	0.5	0.3	1.6	6.7
November	0.3	tr	tr	0.1	0.4	0.4	0.2	1.1	6.4
LE									
March	0.3	tr	tr	0.1	0.4	0.4	0.2	1.2	8.5
July	0.3	tr	tr	0.1	0.5	0.5	0.3	1.6	7.9
November	0.3	tr	tr	0.1	0.4	0.4	0.2	1.1	7.9
UW									
March	0.4	0.1	0.1	0.1	1.0	1.0	0.5	2.9	4.7
July	0.4	0.1	0.1	0.2	1.2	1.2	0.6	3.5	4.9
November	0.3	tr	0.1	0.1	0.8	0.8	0.4	2.3	4.2
UC									
March	0.3	0.1	0.1	0.1	1.0	1.0	0.5	2.9	3.7
July	0.4	0.1	0.1	0.2	1.3	1.3	0.7	3.9	4.4
November	0.3	0.1	0.1	0.2	1.0	1.0	0.5	3.0	4.1
UE									
March	0.2	tr	0.1	0.1	0.9	0.9	0.4	2.6	2.5
July	0.3	0.1	0.1	0.2	1.3	1.4	0.7	4.1	3.1
November	0.3	0.1	0.2	0.2	1.4	1.4	0.7	4.3	3.6
Average Lower Stations	0.3	tr	tr	0.1	0.4	0.5	0.3	1.4	8.0
Average Upper Stations	0.3	0.1	0.1	0.2	1.1	1.1	0.6	3.3	3.9

2.3.5 Sterols

The concentrations of sterol biomarkers (Figure 2-4) were generally similar throughout the study period (Table 2-7). Sterol concentrations were consistent between the sites at each location; however the two locations differed for some sterols. Sitosterol, sitostanol and lupeol were generally 2 –3 times more abundant at the upper location suggesting greater influence of terrestrial sources of organic matter. On the other hand, epi-brassicasterol (diatom marker) and dinosterol concentrations were generally higher at the lower location suggesting greater influence of algal material. The C_{27} stanol:stenol ratio ranged between 0.1 and 0.2 across both locations and the C_{29} stanol:stenol ratio ranged between 0.2 and 0.3 across both locations.

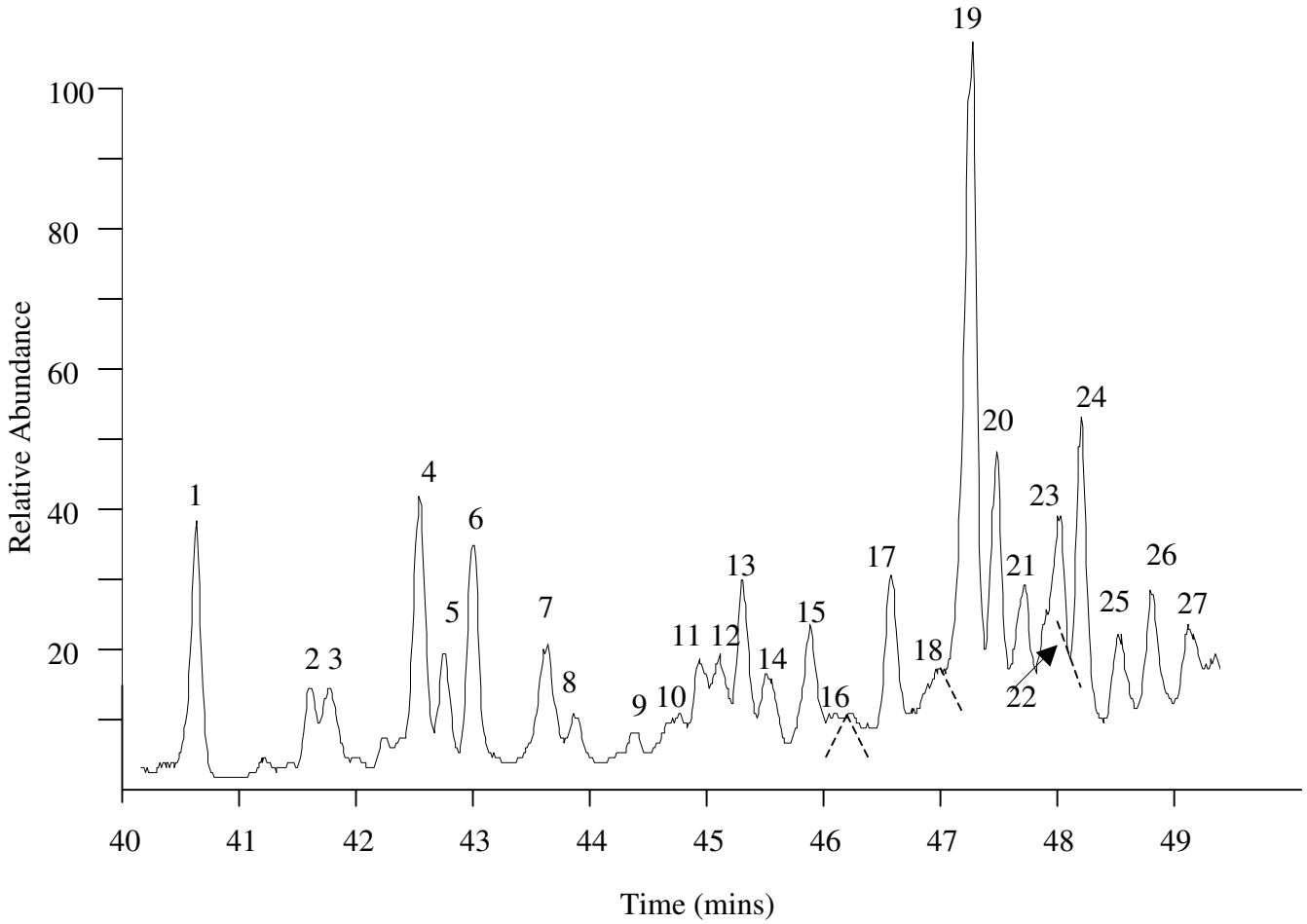


Figure 2-4 Gas chromatogram of sterols in sediment at the Upper estuary. Peak numbers refer to table 2-6 below.

Table 2-6 Sterols from Huon estuary sediment

No.	Sterol	No.	Sterol
1	Unknown	15	23,24-dimethylcholesta-5,22E-dien-3β-ol
2	Unknown	16	24- ethylcholesta-5,22E-dien-3β-ol (Stigmasterol)
3	Unknown	17	Friedoolean-14-en-3β-ol (Taraxerol)
4	Cholest-5-en-3β-ol (Cholesterol)	18	Olean-12-en- β-ol (β –amyrin)
5	5α-Cholestan-3β-ol	19	24-ethylcholest-5-en-3β-ol (Sitosterol)
6	C28:0 alcohol	20	24-ethyl-5α- Cholestan-3β-ol
7	24-methylcholesta-5,22E-dien-3β-ol (Diatomsterol)	21	C30:0 alcohol
8	24-methyl-5α-cholest-22E-en-3β-ol	22	Urs-12en-3β-ol (α-amyrin)
9	Unknown	23	4 α, 23,24-trimethyl-5α-cholest-22E-en-3β-ol (Dinosterol)
10	Unknown	24	Lup-20(29)-en-3β-ol (Lupeol)
11	24-methylcholesta-5,24(28)dien-3β-ol	25	Unknown
12	C28$\Delta^{24(28)}$	26	Unknown
13	24-methylcholest-5-en-3β-ol (Campesterol) + 24-methyl-5α-cholestan-3β-ol	27	Unknown
14	Hopanol		

Table 2-7 Sterol and stenol data (µg g^{-1} dry weight) at the lower and upper location. $C_{27}\Delta^5$ (Cholest-5-en-3β-ol (cholesterol)), $C_{27}\Delta^0$ (5α-cholestan-3α-ol (cholestanol)), Brac (24-methylcholesta-5,22E-dien-3β-ol (epi-brassicasterol)), Tara (Friedoolean-14-en-3β-ol (Taraxerol)), $C_{29}\Delta^5$ (24-ethycholest-5-en-3β-ol (sitosterol)), $C_{29}\Delta^0$ (24-ethyl-5α-cholestan-3α-ol (sitostanol)), Dino (4α-23,24-trimethyl-5α- cholest-22E-en-3β-ol (Dinosterol), Lup (Lup-20(29)-en-3β-ol (Lupeol))

	$C_{27}\Delta^5$	$C_{27}\Delta^0$	Bras	Tara	$C_{29}\Delta^5$	$C_{29}\Delta^0$	Dino	Lup	Total	Stanol/Stenol C_{27}	Stanol/Stenol C_{29}
LW											
March	0.6	0.1	0.2	0.1	0.4	0.1	0.4	tr	1.9	0.20	0.30
July	0.7	0.1	0.2	0.1	0.4	0.1	0.3	tr	1.9	0.18	0.24
November	0.5	0.1	0.1	0.1	0.3	0.1	0.3	tr	1.5	0.19	0.37
LC											
March	0.5	0.1	0.2	0.1	0.3	0.1	0.3	tr	1.7	0.22	0.30
July	0.6	0.1	0.2	0.1	0.4	0.1	0.3	0.1	1.8	0.16	0.28
November	0.5	0.1	0.2	0.1	0.3	0.1	0.2	tr	1.4	0.21	0.21
LE											
March	0.4	0.1	0.1	0.1	0.3	0.1	0.3	tr	1.3	0.22	0.30
July	0.6	0.1	0.2	0.1	0.3	0.1	0.3	tr	1.7	0.18	0.28
November	0.5	0.1	0.2	0.1	0.3	0.1	0.3	0.1	1.5	0.18	0.28
UW											
March	0.5	0.1	0.1	0.1	0.7	0.2	0.3	0.2	2.1	0.17	0.29
July	0.6	0.1	0.1	0.1	0.8	0.3	0.3	0.2	2.4	0.16	0.34
November	0.4	0.1	0.1	0.1	0.6	0.2	0.2	0.2	1.8	0.17	0.29
UC											
March	0.4	0.1	0.1	0.1	0.7	0.2	0.2	0.2	2.0	0.18	0.30
July	0.6	0.1	0.1	0.1	0.9	0.3	0.3	0.2	2.7	0.14	0.34
November	0.5	0.1	0.1	0.1	0.7	0.2	0.2	0.2	2.2	0.15	0.30
UE											
March	0.4	0.1	0.1	0.1	0.6	0.2	0.2	0.2	1.7	0.14	0.31
July	0.6	0.1	0.1	0.1	0.8	0.3	0.2	0.2	2.4	0.11	0.31
November	0.7	0.1	0.1	0.1	0.9	0.3	0.2	0.3	2.7	0.12	0.32
Average Lower Stations	0.5	0.1	0.2	0.1	0.3	0.1	0.3	tr	1.7	0.19	0.28
Average Upper Stations	0.5	0.1	0.1	0.1	0.7	0.2	0.2	0.2	2.2	0.15	0.31

2.4 Discussion

2.4.1 Total organic carbon, C/N ratios and stable isotopes

The sediments from this study were rich in organic carbon (Table 2-1), which is relatively common in muddy near-shore environments. A study by Ferguson et al. (2003), on northern New South Wales estuaries recorded organic carbon levels up to 6%, a study in Boston Harbour, USA recorded organic carbon up to 6.1% (Giblin et al., 1997) while in Plum Island Sound, USA, organic carbon was measured as high as 10.3% (Hopkinson et al., 1999). It is not surprising that near-shore environments such as bays and estuaries are rich in organic carbon as inflows from rivers and streams carry with it high levels of particulate organic matter from catchment runoff, which settle on to the sediment as it is carried down the estuary. In contrast, deep ocean sediments generally have low levels of organic carbon with amounts recorded ranging between 0.1–3.2 % (Trimmer et al., 2003; Hulth et al., 1994; Rysgaard et al., 2001; Stahl et al, 2004). This occurs because most of the organic carbon is broken down before it reaches the sediment.

However, it is not only the quantity of organic matter reaching the sediment but also the quality (or lability) that is crucial in driving remineralisation of organic matter (Herbert, 1999). Organic carbon from marine sources, such as phytoplankton (low C/N ratio), is considered to be more reactive or labile than terrestrial sources (high C/N ratio), and is therefore important in driving diagenetic processes in sediments (Canuel and Martens, 1996). In the current study, sediments from the Upper location averaged molar C/N ratios of 17.2, indicating that organic matter is mostly of terrestrial origins while at the Lower location the average molar C/N ratio was 11.1, indicating mixed sources from both phytoplankton and terrestrial plants.

However, this approach is very approximate as degradation of organic matter may significantly alter these ratios (Thornton and McManus, 1994). C/N ratios of phytoplankton rich in nitrogen tend to increase during degradation because nitrogen is lost from organic matter at a greater rate than produced by decomposing bacteria. In contrast, C/N ratios of terrestrial organic matter tend to decrease during degradation as bacterial nitrogen production occurs at a faster rate than nitrogen loss from the decomposing organic matter (Thornton and McManus, 1994). Therefore, as new

material is constantly arriving at the seafloor and in different degradation states the surface sediment contains a mixture of 'fresh' and degraded material, and the longer organic matter has been deposited on the seafloor the more difficult it becomes to distinguish between the two sources using just C/N ratios.

Another approach is to use ratios of $^{12}C/^{13}C$ stable isotopes, which can provide a good estimate of the relative contribution of terrestrial and marine sources to sedimentary organic matter (Fry and Sherr, 1984). Terrestrial organic matter will generally have a $\delta^{13}C$ value of -26 to -30‰ and $\delta^{15}N$ values of about 1.5‰, whilst organic matter with a marine origin will generally have a $\delta^{13}C$ of -19‰ to -23‰ and $\delta^{15}N$ of about 7.5‰ depending on the particular organisms present (Heip et al., 1995).

The $\delta^{13}C$ results from this current study suggest that both sites are dominated by marine organic carbon, with the lower sites averaging -20.2‰ while the upper sites averaged –22.2‰. The $\delta^{13}C$ results for the lower estuary sites are consistent with the $\delta^{15}N$ values (average 7.7‰), which also indicate dominance from marine sources but differ with the C/N ratio, which indicated mixed sources of organic carbon. However, as discussed above C/N ratios are generally not good indicators of the source of organic matter because they are altered during remineralisation. Nitrogen rich sediments tend to increase during degradation, because nitrogen is lost from organic matter at a greater rate than produced by decomposing bacteria.

The $\delta^{13}C$ for the upper estuary were also reasonably consistent with the $\delta^{15}N$ values (average 4.9‰) which both suggested the organic matter had mixed sources of organic matter. However, this did not compare well with the C/N ratios for this location, which indicated sediments dominated with terrestrial organic matter. Perhaps, as discussed above, the C/N ratios have been altered significantly due to degradation processes and therefore do not give a very accurate indication of the likely sources of organic matter.

These results highlight the potential pitfalls of applying single techniques to describe the origins of the organic material. This is due in part to *in situ* biological processes (i.e. organic matter degradation) altering the composition of the organic matter and hence the C/N and stable isotope ratios. The following discussion compares the data

presented in this section with lipid biomarkers (e.g. fatty acids and sterols), which are powerful tools for determining the origins of organic matter to give a more complete picture of the sources of organic matter.

2.4.2 Fatty Acids

Fatty acids are generally the most abundant lipid type in surface sediments. Concentrations have been found as high as 3764 $\mu g\ g^{-1}$ dry weight in the highly productive Peru continental shelf (Smith et al., 1983). In contrast, fatty acid concentrations recorded during this study ranged between 5.7 and 16.3 $\mu g\ g^{-1}$ dry weight. Common sources of fatty acids found in sediments include bacteria, phytoplankton, higher plants, and marine fauna (Volkman, et al, 1998). Each of these sources has distinctive fatty acid profiles (Table 2-8); Phytoplankton are characterised by the presence of large amounts of PUFAs, some bacteria by *iso*- and *anteiso*-branched fatty acids (but others lack these fatty acids) and terrestrial plants by long-chain fatty acids > C_{22}.

Table 2-8. Distinctive fatty acids in major classes of organic matter found in marine sediments including phytoplankton, bacterial and terrestrial plants.

Source of organic matter	Distinctive Fatty Acids	Reference
Phytoplankton	20:5n-3, 22:6n-3, 18:2n-6, 18:3n-3, 18:5n-3; 18:1n-9; 16:1n-7;	Volkman et al. (1998); Viso and Marty (1993)
Bacteria	*iso*- and *anteiso*- branched FAs; 16:1n-7, 18:1n-7	Volkman et al. (1998); Perry et al. (1979);
Terrestrial Plants	Long chain fatty acids (>C_{22})	Carrie et al. (1998)

The predominance of C_{22}-C_{30} fatty acids, responsible for approximately a quarter of fatty acids at the Lower location and half of all fatty acids at the Upper location, is typical of material derived from terrestrial plants and probably derived from surface waxes of plants (Volkman et al., 1998). Linear regression (Figure 2-5) between Σ long chain fatty acids and other known terrestrial biomarkers (sitosterol, $C_{24:0}$ alcohol

and lupeol) shows that the long chain fatty acids are most likely from terrestrial sources and that the source of the terrestrial organic matter is from the same source at both locations. The likely source of the terrestrial organic matter is from the surrounding catchments which is still 94% naturally or semi-naturally vegetated (Butler, 2005). The Huon and Kermandie River are the major sources of particulate organic carbon (POC) from catchment runoff and generally carry loads of between 3–12mg kg^{-1} of suspended solids (Butler, 2005).

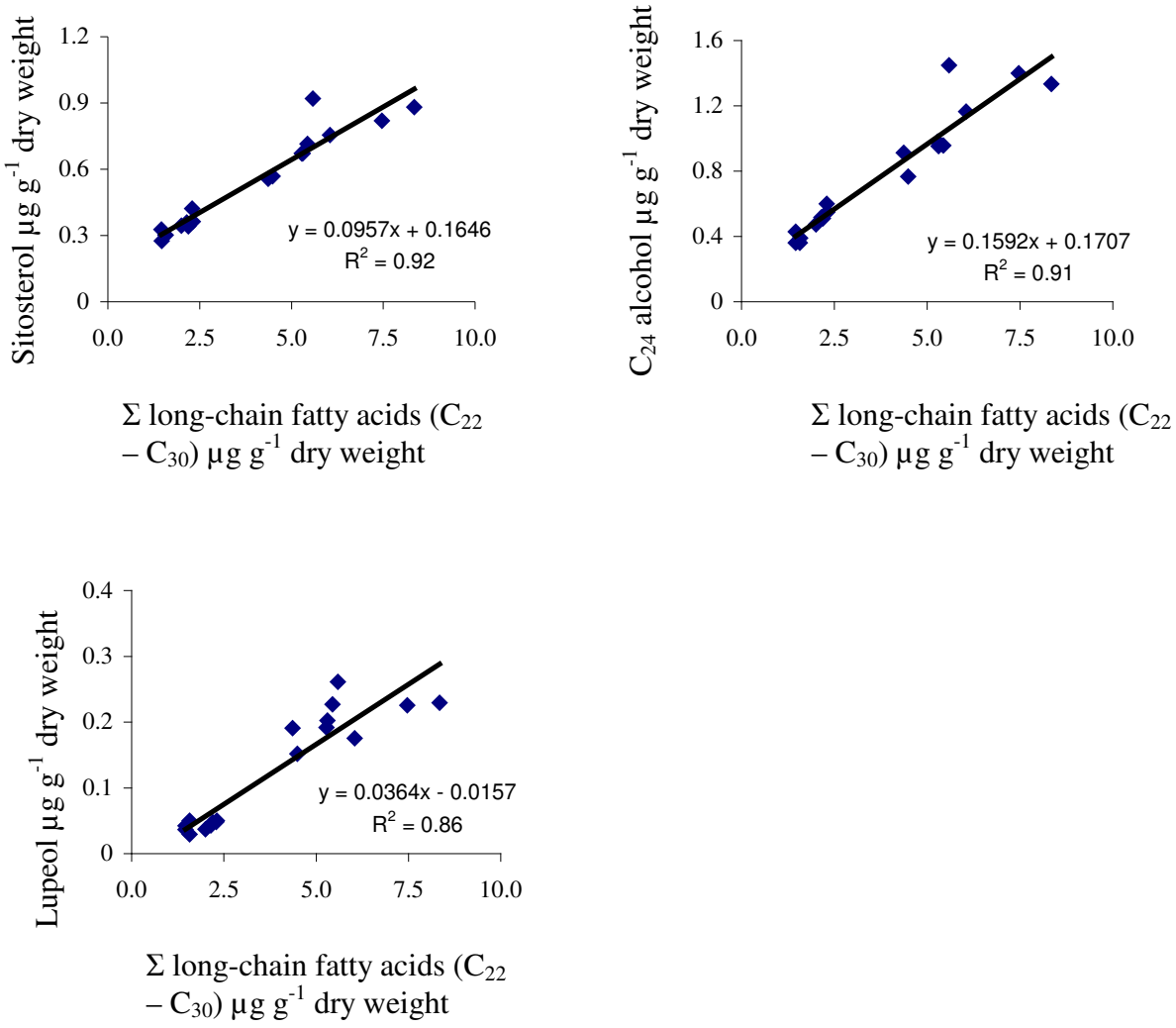


Figure 2-5. Linear regression of Σ Long-Chain Fatty Acids with other terrestrial lipid biomarkers including sitosterol, C_{24} alcohol and lupeol.

Other fatty acids that were present in relatively abundant amounts include 14:0, palmitic and stearic fatty acids (16:0 and 18:0 respectively), branched fatty acids *iso*-

15:0 and *anteiso*-15:0, palmitoleic acid (16:1n-7), vaccenic acid (18:1n-7) and oleic acid (18:1n-9). Both palmitic and stearic acid are ubiquitous in the environment and thus are difficult to assign to any particular source of organic material. Palmitoleic acid (16:1n-7) is often found in phytoplankton, with high concentrations found in *Bacillariophyceae* (diatoms), *Raphidophyceae*, *Xanthophyceae*, and *Cyanophyceae*. Diatoms are a common component in marine sediments with a high 16:1n-7 content (Smith et al., 1983; Wilson et al., 2001). The additional presence of PUFA 20:5n-3 along with 16:1n-7 fits the characteristic profile for diatoms (Table 1-1), and suggests their likely presence in the sediments of the present study.

However, 16:1n-7 is also found in bacteria. The presence of branched saturated fatty acids (accounting for 7.7–15.3% of total fatty acids) indicates that bacteria are a significant contributor of 16:1n-7. Linear regression of 16:1n-7 with characteristic phytoplankton and bacterial fatty acid biomarkers (Figure 2-6) shows that the relationship is better correlated with the bacterial fatty acids.

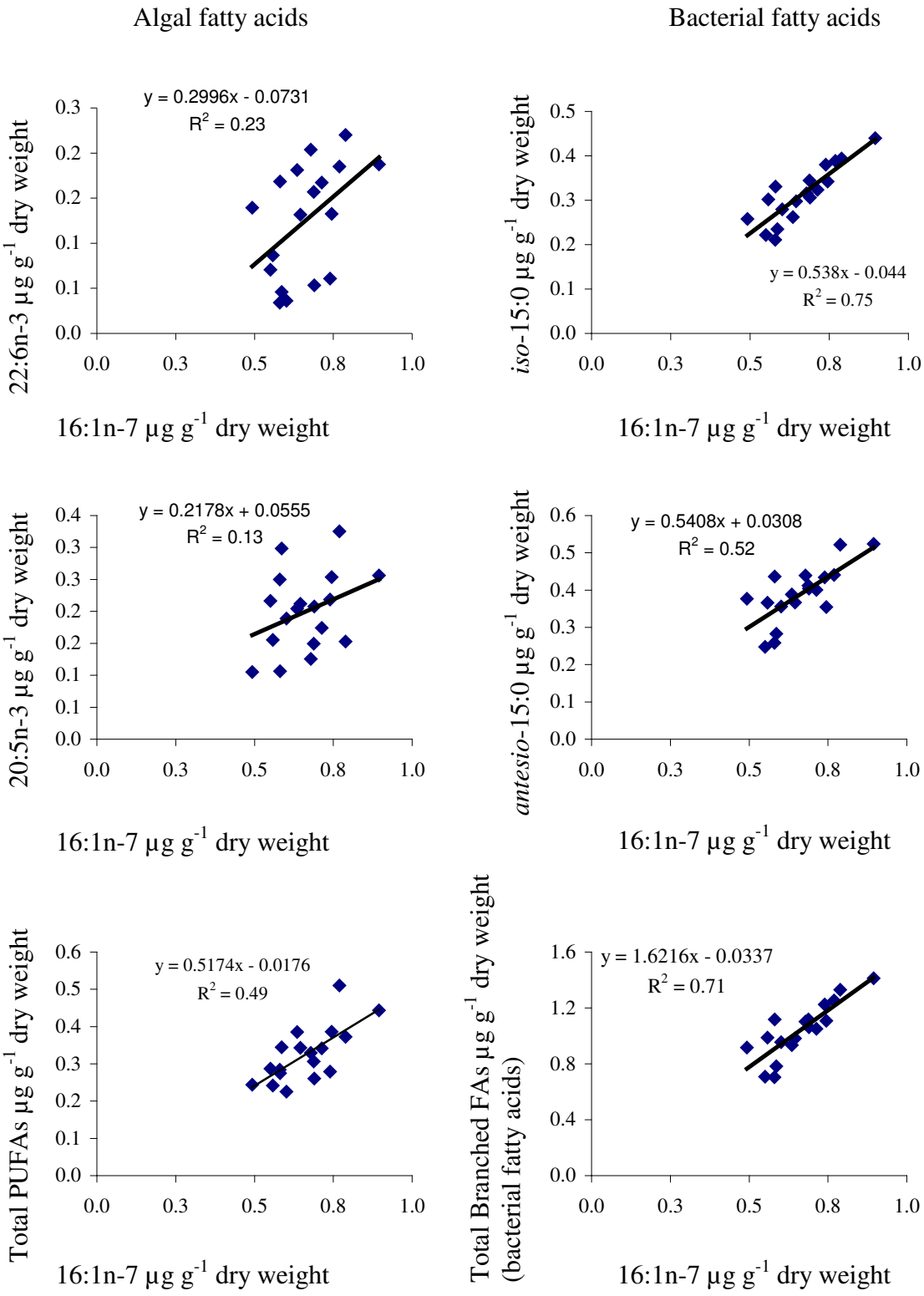


Figure 2-6. Linear regressions of 16:1n-7 plotted against characteristic microalgal and bacterial – fatty acid biomarkers including 22:6n-3, 20:5n-3, iso-15:0, anteiso-15:0, total PUFAs and total bacterial fatty acids.

Vaccenic acid has also been used in the past to indicate the presence of bacteria (Volkman et al., 1980; Perry, et al., 1979). Perry et al. (1979) found that vaccenic acid accounted for 10.5% of fatty acids in aerobic heterotrophs and 17.3% in anaerobic heterotrophs cultured from Low Isles sediment. However, as with 16:1n-7, vaccenic acid also has multiple sources and is commonly found in most phytoplankton species albeit in low abundance. Linear regression of 18:1n-7 with characteristic phytoplankton and bacterial fatty acid biomarkers shows that the relationship is better correlated with the bacterial fatty acids (Figure 2-7). As with 16:1n-7, the result indicates the sediments during this study have accumulated more 18:1n-7 from bacterial sources, however it does appear that some are from phytoplankton sources due to the presence of PUFAs. PUFAs rarely occur in bacteria (Wilson, et al., 2001and references therein) while they are commonly found in phytoplankton.

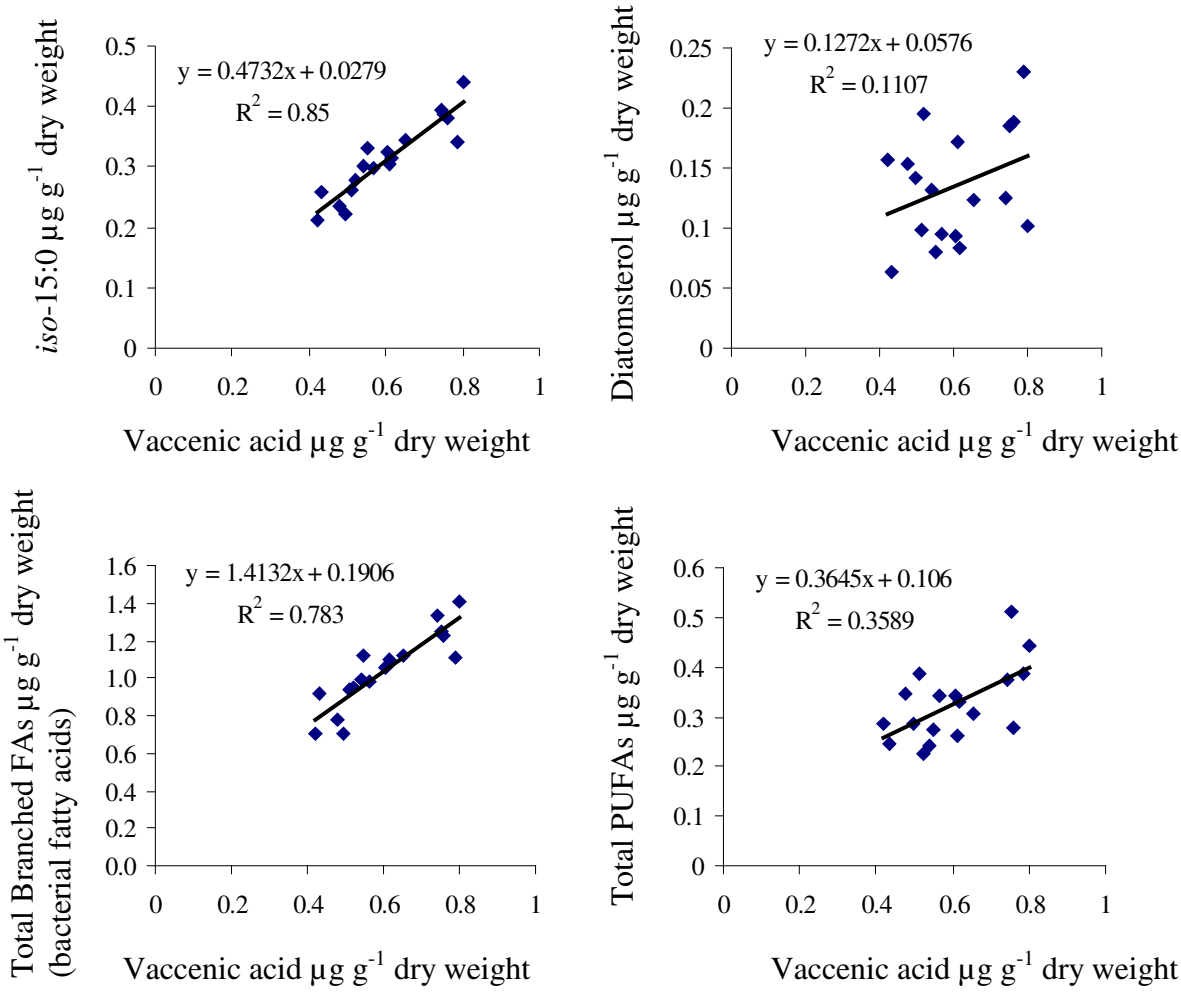


Figure 2-7. Linear regressions of Vaccenic acid plotted against characteristic microalgal and bacterial –fatty acid and sterol biomarkers including 22:6n-3, 20:5n-3, iso-15:0, diatomsterol, total PUFAs and total bacterial fatty acids.

Organic matter source markers for some of these inputs, in particular markers for terrestrial organic matter, showed distinct temporal trends. Terrestrial fatty acids were most abundant in winter, most likely due an increase in input from terrestrial sources (Figure 2-8). Rainfall patterns in Southern Tasmania are generally higher in winter, compared with other seasons (Butler, 2005). Increasing river flows, carry higher concentrations of particulate organic matter from catchment runoff, which inturn is likely to increase the terrestrial detritus load at the seafloor.

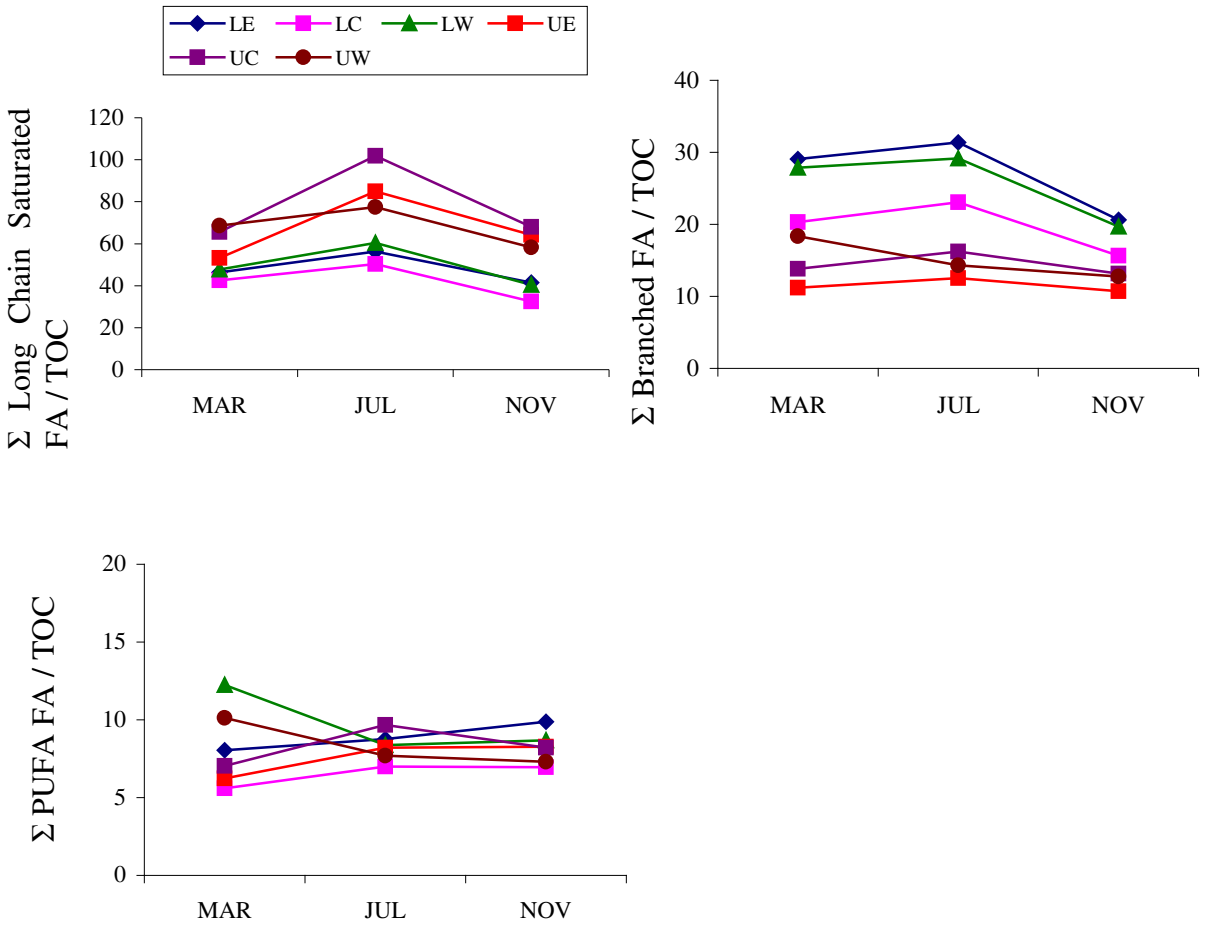


Figure 2-8 Temporal changes in fatty acid classes for the Upper and Lower locations

Overall, sediments at both locations have high inputs of organic material from terrestrial sources, dominating the fatty acid profiles at the upper locations. Bacteria and phytoplankton are also significant sources of organic matter and are more important at the Lower location than in the Upper location.

2.4.3 Alkanes and Alcohols

The high abundance of odd-chain alkanes between C_{25} and C_{31} at both locations (Lower 53.5 % and Upper 59% of total alkanes) and maximising at C_{27} is typical of alkanes from cuticular waxes of terrestrial plants (Eglinton et al., 1962; Eglinton and Hamilton, 1967; Birgel et al., 2004). The fact that all samples at both locations maximised at C_{27} indicates that the terrestrial source of organic matter is the same.

This is consistent with the findings for fatty acids. There was only a small contribution from alkanes $<C_{23}$, which are generally associated with phytoplankton.

The fatty alcohols detected in the surface sediments at both locations were dominated by even-numbered compounds ranging from C_{16} to C_{30}, maximising at C_{24}, which is commonly associated with higher plant waxes (Smith et al., 1983), complementing the findings for alkanes and fatty acids. A clear increase in long chain fatty alcohols in July, as discussed earlier with long chain fatty acids, indicates an increase in the flux of terrestrial organic matter during the winter months.

Phytol was also detected in the sediments. Phytol is the side-chain of chlorophyll *a* and thus its presence in sediments indicates a contribution from phytoplankton derived organic matter (Macleod et al., 2004). Phytol concentrations were only a minor component of lipids, and as we have seen with PUFAs, short chain fatty acids, alkanes and fatty alcohols, did not vary much over the study period indicating a steady but small flux of phytoplankton-derived organic matter to the seafloor.

Phytol normalised to TOC indicated that the sediments at the Lower location were richer in phytoplankton organic matter compared with the Upper location. Linear regression of phytol/TOC with the C/N ratio (used as a proxy for sources of organic matter – see earlier discussion), was reasonably strong ($r^2 = 0.77$). This result suggests that phytol normalised to TOC could be useful as another proxy measure for sediment organic matter quality.

2.4.4 Sterols

The major sterol found in the Upper location sediments was sitosterol, while sitosterol and cholesterol were the dominant sterols at the Lower location. Sitosterol is often used as a marker for organic matter derived from terrestrial plants (Hudson et al., 2001), however it has been shown that it can also be biosynthesised by some phytoplankton species (Volkman, 1986).

One potential method for determining if sitosterol is from terrestrial or marine sources is to look at the ratios of campesterol, stigmasterol and sitosterol (Volkman, 1986).

Where sitosterol is more abundant then the other two sterols, as was found in the Peru and Loch Clair sediments (Volkman, 1986), it can be assumed that the sterol is highly likely derived from terrestrial sources. Another approach is to compare inferences drawn from sterol data with information derived from other lipid classes. For instance, the presence of organic matter from terrestrial sources can be confirmed by the presence of long chain alkanes showing a high proportion of odd-chain lengths (Volkman, 1986).

Using both these approaches in this study confirmed that sitosterol is mostly from terrestrial sources. Sitosterol on average was 11 times more abundant then campesterol while 34 times more abundant then stigmasterol. We have also seen in earlier sections that biomarkers such as fatty alcohols and long chain alkanes indicate that terrestrial sources are highly abundant in the sediments from this present study. Strong correlations of sitosterol with $C_{24:0}$ alcohol ($r^2 = 0.97$) and long chain alkanes ($r^2 = 0.68$), and weak correlations with diatomsterol ($r^2 = 0.43$) and dinosterol ($r^2 = 0.10$) indicate that sitosterol is from terrestrial sources.

The presence of triterpenoid alcohols, taraxerol and lupeol, common in terrestrial plants (Currie and Johns, 1989 and references therein), provides additional evidence that sitosterol is from terrestrial sources. Linear regression between sitosterol with taraxerol ($r^2 = 0.72$) and lupeol ($r^2 = 0.92$) also indicates that the terrestrial organic matter is from the same source.

Cholesterol was the other major sterol present in the sediments and is commonly known as a biomarker for zooplankton or other marine fauna since it is the major sterol of most marine animals. It also occurs frequently in phytoplankton, such as diatoms (Volkman, 1986), but only rarely as the major sterol. Given the poor relationship between cholesterol with dinosterol, diatomsterol or phytol (Figure 2-9), all biomarkers commonly used to indicate the presence of phytoplankton, it is likely that most of the cholesterol in these samples can be attributed to animal matter. To support this assumption, benthic animals such as annelids and brittle stars were present in most cores during this study as discussed in Chapter 3.

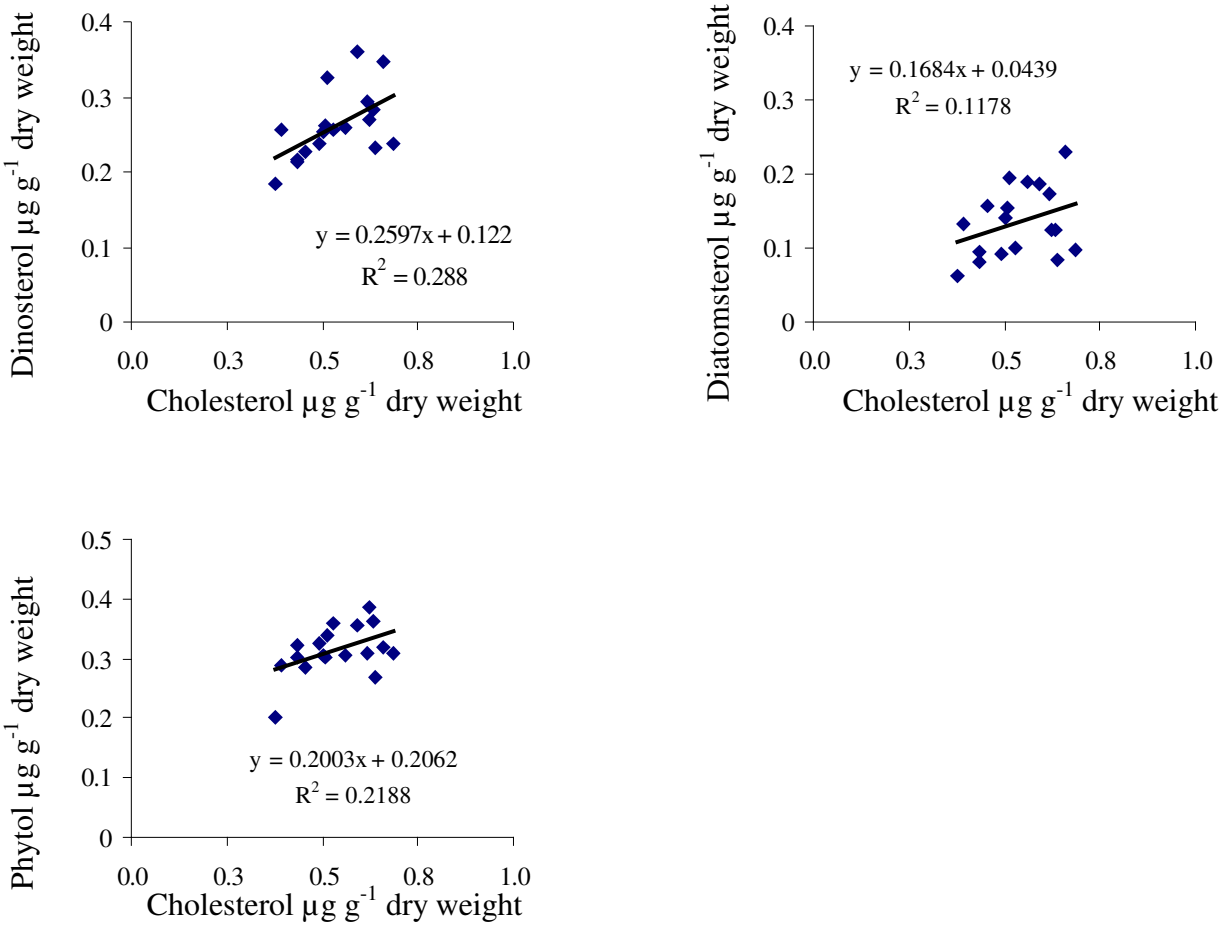


Figure 2-9. Linear regression of cholesterol with dinosterol, diatomsterol and phytol, indicators of phytoplankton.

The presence of dinosterol and diatomsterol, indicators of dinoflagellates and diatoms respectively, supports the earlier findings linking PUFAs and phytol to the presence of phytoplankton in the surface sediments. Diatoms and dinoflagellates commonly co-exist in the water column of the Huon estuary (Butler, 2005) so its not surprising to find a mix of the two types of phytoplankton in the surface sediments.

The ratio of dinosterol to diatomsterol ranged between 1.4 to 1.9 at the Lower location and 2.2 to 3.0 at the Upper locations. This indicates that dinoflagellates during this study were a greater source of organic matter to the sediments than diatoms. Normalisation of dinosterol and diatomsterol to TOC indicated that Lower station sediments were up to 3 times richer in dinosterol and up to 4 times richer in diatomsterol compared with the Upper station. This complements the findings for

phytol, clearly showing that phytoplankton were a greater source of organic matter at the Lower station compared with the Upper station.

Unlike higher plants, marine animals and phytoplankton, very few bacteria have been shown to synthesise sterols and caution must be used where reports of sterols in bacteria have been found, because the amounts are usually quite small and contamination from other organisms or from the laboratory is quite high (Volkman, 2003). However, the presence of stanols in sediments has been used to indicate the presence of microbial activity in previous studies (Rohjans et al., 1998). Bacteria can reduce stenols to stanols (Wilson et al., 2001 and references therein) and therefore, a high stanol content in relation to stenols can be associated with bacterial activity (Wilson et al., 2001). The presence of stanols in this study therefore supports the fatty acid data that indicates bacteria are a source of lipids in sediment samples. A slightly higher ratio of stanols to sterols in the summer sampling period for most sediments, indicates higher bacterial activity in summer supporting the respiration data in Chapter 3 where increased oxygen uptake rates and CO_2 effluxes out of the sediment during summer suggest increased remineralisation of organic matter by bacteria.

2.4.5 Source attribution of lipids in Huon Estuary

Based on the preceding discussion, lipids that I am confident of their origins have been assigned to potential source groups as outlined in Table 2-9, to determine the contributions of each source to the organic matter pool in the surface sediments. Lipids that have ambiguous sources, as has been discussed in the preceding sections (e.g. fatty acid C_{16}) have not been assigned to an organic matter source pool. Also, fatty acids such as palmitoleic acid and vaccenic acid have not been assigned since they have mixed bacterial and phytoplankton sources. Cholesterol also comes from more than one source, but was assigned to the marine animal pool because I was confident in this case that the majority of cholesterol was from marine animals (presence of various animals in the sediments and weak relationship between cholesterol and various phytoplankton biomarkers as discussed above). In total, up to 53% of identified lipids were assigned to a particular source at the lower estuary stations, while up to 60% of the identified lipids were assigned to a particular source at the upper estuary. A more basic approach was also taken to assign lipids to either a

marine or terrestrial source. In this case the assignment of cholesterol as "marine" is less contentious since it's other likely source, phytoplankton, is also grouped in marine.

Table 2-9. Lipid biomarker source groups

Source	Lipid Biomarkers
Phytoplankton	**Sterols:** 24-methylcholesta-5,22E-dien-3β-ol, 24-methyl-5α-cholest-22E-en-3β-ol, 24-methylcholesta-5,24(28)dien-3β-ol, 4 α,23,24-trimethyl-5α-cholest-22E-en-3β-ol; Phytol **Fatty acids:** 20:4n-6, 20:5n-3, 22:6n-3
Bacteria	**Fatty acids:** *iso* 15:0, *anteiso* 15:0, *iso* 17:0, *anteiso* 17:0
Terrestrial Plants	**Sterols:** 24- ethylcholesta-5,22E-dien-3β-ol, friedoolean-14-en-3β-ol, 24-ethylcholest-5-en-3β-ol, lup-20(29)-en-3β-ol; **Alkanes:** C_{25}, C_{27}, C_{29}, C_{31} **Alcohols:** C_{22}, C_{24}, C_{26} **Fatty acids:** C_{22}, C_{24}, C_{26}, C_{28}, C_{30}
Marine Animals	**Sterols:** Cholest-5-en-3β-ol
Marine	Phytoplankton, Bacteria, Marine animals
Terrestrial	Higher plants

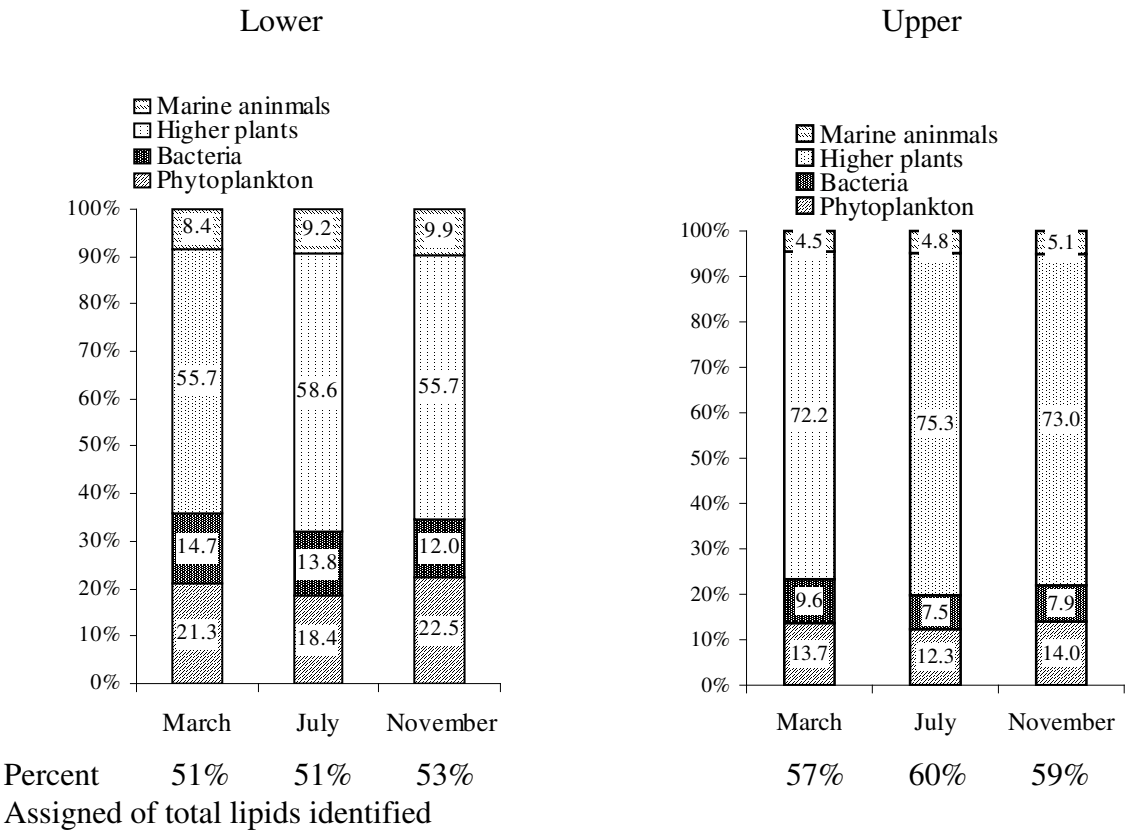


Figure 2-10. Lipid biomarkers assigned to potential sources including marine animals, higher plants, bacteria and phytoplankton (calculated as a % of the total of the particular biomarkers assigned to source categories).

The assigned lipids in Figure 2-10 shows that the major source of lipids at both study locations was dominated by terrestrial organic matter. At the Lower location, terrestrial organic matter accounted for 55.7–58.6% of the total lipid biomarker set followed by phytoplankton sources (18.4–22.5%), bacteria (12.0–14.7%) and marine animals (8.4–9.9%). In contrast, lipid biomarkers assigned to terrestrial sources accounted for about three quarters (72.2– 75.3%) of organic matter, with minor contributions from phytoplankton (12.3 – 14%) bacteria (7.5 - 9.6%) and marine animals (4.5 – 5.1%). The relative proportions of lipid sources did not change much over the sampling period, however as we saw earlier with temporal changes in fatty acids, terrestrial organic matter peaks in winter, while the contribution from phytoplankton is lowest in winter. This indicates that the increased rainfall during winter in southern Tasmania provides increased organic matter from terrestrial land

runoff, while the decreased input of phytoplankton can be attributed to lower primary productivity during winter.

Table 2-10. Lipid biomarkers normalised to Total Organic Carbon (as % of assigned biomarker set) from marine and terrestrial sources

	Lower			Upper		
	March	July	November	March	July	November
Marine	40.7	40.3	45.3	27.8	24.7	27.0
Terrestrial	59.3	59.7	54.7	72.2	75.3	73.0

Table 2-10 shows the assignment of organic matter into either marine or terrestrial sources. This reinforces the dominance of terrestrial organic matter in the lipid profile at the Upper location while the Lower location presents a more 'mixed' profile of marine and terrestrial inputs. This agrees well with the C/N ratios discussed earlier where the upper location had a molar C/N ratio of 17.2 indicating that organic carbon comes predominantly from terrestrial sources while the Lower location had an average molar C/N ration of 11.1 indicating a more mixed input of marine and terrestrial sources.

2.5 Conclusions

This study, using a variety of organic geochemical approaches, has shown that the organic matter accumulated in sediments studied within the Huon estuary, are dominated by allochthonous, river-supplied terrestrial organic carbon. Spatial differences were observed between the Upper and Lower locations with the Upper estuary receiving most of its organic carbon from terrestrial sources. The Lower location displayed a mixed organic carbon profile, with just over half of its organic carbon from terrestrial sources, with lesser amounts from phytoplankton sources and bacteria. This difference in proportion of labile organic matter is likely to impact on respiration rates and nutrient recycling at both locations as discussed in the following chapters.

Marine organic matter, made up largely of phytoplankton detritus, was only found in small amounts during this study. This is due either to the lack of a phytoplankton bloom during this study, or that it undergoes rapid mineralisation when it reaches the seafloor and thus is not detected in the integrated top cm of the sediments. Given this, future studies should include taking measurements of primary production, sedimentation rates and benthic mineralization at the same time as an integrated study. This approach would therefore help to identify the organic matter that is actually undergoing mineralisation and help resolve the problems associated with integrated sediments.

Chapter Three

Benthic Respiration in the Huon Estuary

Chapter 3 Benthic Respiration

3.1 Introduction

The sediment surface of coastal environments including estuaries and bays are dynamic zones of organic matter degradation. Organic matter produced in situ (i.e. phytoplankton) or from allochthonous sources such as land runoff will settle on the sea floor and the labile portion will be rapidly degraded within days to weeks (Grenz et al., 2000; Ferguson et al., 2003). On the other hand, the refractory component will be degraded over months to years or buried within the sediment (Thornton and McManus, 1994; Loh et al., 2002; Ferguson et al., 2003). Organic matter degraded by micro-organisms proceeds by the oxidation of compounds in the order of most energy-yielding terminal electron acceptor, starting at the top of the sediment down (O_2, NO_3, Mn^{3+}, Fe^{3+}, SO_4^{2-}, CO_2) (Caffrey et al., 2002; Papadimitriou et al., 2002).

Aerobic respiration, the most energy yielding, is confined to a relatively thin layer near the sediment surface. In coastal environments with high organic matter, oxygen may only penetrate a few millimetres into the sediment. In contrast, areas with low organic matter contents such as deep oceans, oxygen penetration may increase up to centimetres in depth (Glud et al., 1994; Mackin and Swider, 1989 and references within).

The depth of the oxygen layer, and hence rates of oxygen consumption at the surface are regulated by a number of factors including temperature, quality and quantity of organic matter (Ferguson et al., 2003), bioturbation (Kristensen and Mikkelsen, 2003; Vopel et al., 2003; Nielsen et al., 2004) and the oxygen concentration of the bottom water (Rasmussen and Jorgensen, 1992). It is very important when undertaking incubations that both temperature and oxygen concentrations of the overlying water match in situ conditions. Rasmussen and Jorgensen (1992) found that reduced bottom water oxygen concentrations affected oxygen uptake by sediments by:

- Reducing the O_2 gradient across the DBL thus reducing the diffusive flux
- Reducing the thickness of the oxic zone, thereby reducing the population of aerobic microorganisms which could respire with O_2

Their results show the importance of measuring O_2 microgradients under in situ O_2 concentrations.

Over the past two decades many new studies have shed light on benthic metabolic processes in sediments. However most of these studies have been devoted to eutrophic estuaries and coastal environments of Europe and North America. Very little information exists on mesotrophic estuarine systems. This current study was conducted in the Huon estuary, situated in the south-east corner of Tasmania, Australia. It is a relatively pristine environment (Butler et al., 2000) and thus will provide a good contrast to the more commonly studied eutrophic estuaries.
The Huon estuary is the site of an expanding salmon farming industry and thus a need has arisen to understand chemical and biological processes inherent in the estuary to calculate the carrying capacity of the estuary. To date, most of the studies have been confined to the water column with very little information on sediment processes. Hence, the purpose of this chapter was to gain a better understanding of benthic respiration.

The main aims were to:

- Elucidate temporal and spatial variability in respiration
- Compare oxygen fluxes from core incubations and microelectrodes
- Understand the impact of benthic fauna
- Compare benthic processes in the Huon Estuary with other studies from around the world

3.2 Materials and Methods

3.2.1 Study Site

The study was conducted in the Huon Estuary, located in the southeast corner of Tasmania. The Huon estuary is a drowned river valley, and stretches 39km southeast to Huon Island. The estuary has a mean flushing time of 7 days, with riverine input averaging approximately 90 cumecs. It is a microtidal, salt wedge system, with a tendency to become partially mixed at the mouth (Butler et al., 2000). The system is classified mesotrophic, and has low nutrient input from the catchment, which is

mostly wilderness. The Huon Estuary Study (Butler et al., 2000) described the estuary as a 'substantially natural' water way and that the estuary is likely to function in much the same way as it did in a pristine state. The waters of the Huon estuary are highly coloured due to high concentrations of colour dissolved organic matter (CDOM) which is derived from subalpine and "buttongrass" (*Gymnoschoenus sphaerocephalus*) moorlands as well as ti-tree stands (*Leptospermum* sp.) in the upper catchment. (Cook, 2002). For more detail on the estuary please see section 1.4 of the introduction.

Two locations were chosen to study benthic respiration: the 'Upper estuary' zone, located between Brabazon point and Pillings bay, and a 'Lower estuary' zone, located between Huon Island and Huon point at the mouth of the estuary (Figure 1-3). Within each of these two locations, a transect covering 3 sites starting from the west side of the estuary, and finishing on the east side was sampled. In the upper estuary zone the sites were called Upper estuary east (UE), Upper estuary centre (UC) and Upper estuary west (UW). The lower estuary sites were called Lower estuary east (LE), Lower estuary centre (LC) and Lower estuary west (LW).

The rationale behind selecting these two locations was to elucidate the impact of different sources of organic carbon on benthic respiration and nutrient cycling (see chapter 4) and to examine the spatial variability within a sampling location (e.g. variation between cores at site UW), between sites within the same location (i.e. UW vs. UE) and between sampling locations (i.e. Upper estuary vs. Lower estuary). Faunal data was also collected in order to help explain any inter-core variability. The faunal data was not intended to describe the actual fauna populations at a site.

3.2.2 Sediment Collection

The upper and lower locations were visited three times in 2004, including March, July and November, using a small research vessel. The two locations were sampled on separate occasions, generally 3 days to a week apart, due to resource and labour limitations. Data from an additional sampling trip in April 2005 from Hideaway bay and Garden Island (Figure 1-3, Chapter 1) have also been used in this chapter and will

serve as a comparative study of the benthic processes with the upper and lower locations of the main study.

At each station a box corer was used to take intact sediment samples. From each box-core sample, an undisturbed sub-core was taken by 25-cm-long polyethylene tubes with an inner diameter of 9.7 cm to measure benthic respiration and nutrient exchange (see chapter 3) at the sediment – water interface. Three replicates were obtained at each site. The remaining surface sediment (top 1cm) that was not collected in the sub-cores was collected from the box core and placed into glass jars and frozen until analysed for stable isotopes $\delta^{13}C$ and $\delta^{15}N$, carbon and nitrogen content, lipid biomarkers and sediment grain size. Duplicate Cores (4.8cm inner diameter, i.d) for oxygen porewater analysis were taken from separate grab samples.

The cores were then held in an ice-cooler at in situ temperatures. Near bottom water (~160 litres) was collected using an 8l Niskin bottle and stored in 20 litre plastic carboys. Temperature and salinity were determined in surface and bottom water on each occasion using a digi-thermo thermometer and refractometer respectively. Secchi depth was also measured in July and November. The cores and water were then transported back to the lab within six hours of sampling. On arrival, the bottom water was placed into an incubation trough and glass aquarium (60 litres) and brought to in-situ temperatures using a recirculating water bath and temperature controlled thermostat probe respectively.

3.2.3 Molecular Diffusive Fluxes and Oxygen Profiling

Before submerging the cores (4.8 cm I.D) into the glass aquarium the sediment was pushed up so that only 1 – 2 cm gap existed between the sediment surface and the top of the core. This ensured that there was good mixing of water above the sediment surface. Mixing was achieved by continuously bubbling air through an air-stone, which created turbulence at the water surface. This also served to maintain 100% saturation of dissolved oxygen in the aquarium water. The cores were then left in the dark overnight to re-equilibrate.

The day after sampling, oxygen microelectrodes (Clark type with a guard cathode from Unisense A/S Aarhus, Denmark) were positioned vertically above the sediment surface on a hydraulic computer-controlled micromanipulator. The microelectrode was then inserted stepwise into the sediment in 100µm intervals to measure oxygen concentration across the sediment-water interface to the bottom of the oxic zone. The sediment surface and tip of the microelectrode were observed continuously using a stereomicroscope to record when the microelectrode tip penetrated the sediment surface. This ensured the correct positioning of the measured oxygen profiles relative to the sediment-water interface and consequently the determination of the diffusive boundary layer (DBL).

This method was then compared to the method described by Glud et al. (2003) where the upper DBL boundary was determined as the intersection between the extrapolated linear O_2 gradient in the DBL and the constant O_2 value in the overlying water (Figure 3-1). The thickness of the DBL was estimated from the intersection point and the position of the sediment, which was identified from a distinct break in the concentration profile (Figure 3-1). Where the two methods diverged, and average of the two methods was taken to determine the position and thickness of the DBL. Three to six profiles were measured in duplicate cores from each site at random positions.

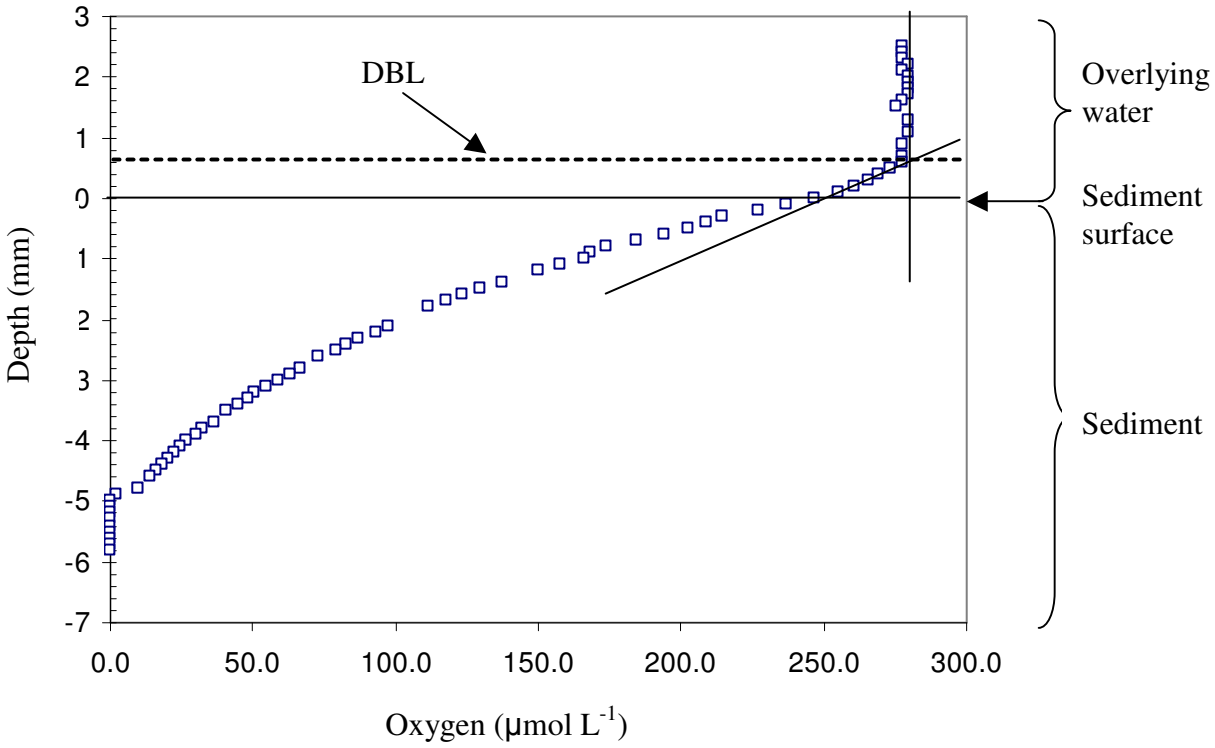


Figure 3-1. A typical O_2 microprofile measured during November 2004 at the mid estuary location. The solid horizontal line indicates the estimated position of the sediment surface, and the broken line indicates the estimated position of the upper DBL boundary. The slope of the concentration gradient within the DBL is shown.

The output (mV) of the microelectrode was automatically recorded onto a computer. The output was then converted to micromolar concentrations of dissolved oxygen by comparing to Winkler titrations of the aquarium water for which the temperature, salinity and electrode output was also recorded. The daily calibration was a 3-point linearity test with air saturated, oxygen saturated and nitrogen-saturated water as the 3 points along the straight line. The standard calibration was then calculated by using the following equation: $O_2 – N_2$ / air – N_2 to give the signal output ratio.

At standard atmospheric conditions, the proportion of O_2 at saturation [100%] / air saturation [20.9] is 4.78 and one would expect the microsensor to respond similarly. On each occasion the microelectrodes gave acceptable output ratio of 4.64 – 4.75, that this is, ± 2.5% of expected value of 4.78 (Revsbech and Jørgensen, 1986).

The diffusive flux of oxygen across the sediment-water interface and downwards into the sediment (J_{DBL}) was calculated according to Rasmussen and Jorgensen (1992). Briefly, molecular diffusive fluxes of oxygen were calculated through the DBL using Fick's first law of diffusion as:

$$J_{DBL} = \frac{D(C_w - C_s)}{Z\delta}$$

Where:

J_{DBL} = the flux across the DBL in μmol O_2 m^2 d^{-1}

D = the apparent diffusion coefficient in cm^2 s^{-1}

C_w = the concentration of oxygen in the overlying water (μmol)

C_s = the measured concentration of oxygen at the sediment surface (μmol) and,

Z_δ = the effective depth of the DBL (cm)

These rates were then compared to molecular diffusive fluxes mathematically modelled from oxygen sediment gradients (J_{SED}). In order to compute this, knowledge of the porosity and of the coefficient of diffusion in the sediments was required. Porosity in the top 1cm sediment of each core was determined according to the method in the Nitrogen Cycling in Estuaries (Dalsgaard et al., 2000) handbook while the coefficient of diffusion was calculated according to Rasmussen and Jorgensen (1992).

Oxygen Consumption Profiles

Oxygen consumption profiles were modelled using PROFILE (version 1) developed by Berg et al. (1998), which uses a diffusion model to calculate sediment production and consumption rates as a function of depth.

Briefly, 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.

An estimate of the bioturbation coefficient (D_B) was obtained by comparing the total oxygen fluxes as measured in the core incubations (see method in section 3.2.4) to those calculated from O_2 micro-profiles (method described above). D_B was calculated according to Berg et al. (2001):

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

Where Ds 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 porewater profiles.

3.2.4 Total O_2, TCO_2 and Alkalinity Fluxes

Prior to submerging the cores (9.7 cm I.D) into the incubation trough the cores were standardised so that there was a sediment depth between 8 and 10 cm and that there was 11 to 13cm of overlying water. The uncapped cores were then left to re-equilibrate in darkness over night prior to measurements. To ensure a good exchange between the water phase of the core and the exterior seawater during the re-equilibration period, a Teflon-coated stirrer bar (5 cm length) was suspended ~5 cm above the sediment surface. This was driven by an external rotating magnet at 50 – 60 rpm.

The following morning rates of oxygen (O_2), Carbon dioxide (TCO_2), alkalinity and pH were measured in the dark only. Light incubations were not performed as in a preliminary field trip light was not detected at the surface of the sediments using a Licor Quantum light meter. Also no difference was detected in oxygen penetration depths following light experiments (results not shown) suggesting that there was no photosynthetic activity occurring. It was therefore assumed that surface sediments were predominately exposed to darkness and that surface algae or microphytobenthos had a minor role if any in the ecology of the sediments in this current study.

The incubations were started by capping the cores and waiting 1 hour prior to making the first measurement. For the core incubations, it was assumed that oxygen concentrations were saturated or close to saturation, which is based on previous measurements of oxygen concentration in the Huon estuary. To maintain oxygen saturation in the tank water prior to beginning the core incubations, air was bubbled into the tank water. During the incubations in situ temperature and salinity (two variables that will effect oxygen saturation) was maintained throughout the incubations in order to mimic in situ conditions as close as possible.

Oxygen and pH were measured in the core, through a sampling port, during incubations using electrodes. Water samples for alkalinity and nutrient (see chapter 4) analysis determinations were taken from the core by withdrawing 40 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. The water samples were then filtered through Acrodisc 0.45µm cellulose acetate filters into 10 ml screw-cap polypropylene containers; alkalinity samples were stored at 4^{o}C in the dark and analysed within 2 weeks of sampling. An incubation time was used that ensured dissolved oxygen (DO) did not drop below approximately 20% of the saturated concentration.
The flux across the sediment-water interface was calculated as:

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

Where:

α = linear regression of analyte concentration (corrected for addition of replacement water) versus time in sediment core (µmol l^{-1} h^{-1})

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

V = water column volume (l) and,

A = sediment surface area (m^2).

Fluxes for each replicate core were calculated from the linear regression of the change of an analyte, in this case, alkalinity, over time. This method is commonly used in the

literature and is referenced in the Protocol Handbook for Nitrogen Cycling in Estuaries (Dalsgaard et al, 2000). This method was preferred over the alternative method of calculating fluxes, which is to only measure analyte concentrations at the start and finish of the incubation period. It is generally regarded that obtaining more measurements of analyte concentration over time and establishing a regression is more accurate than only measuring concentrations at the start and finish of an incubation experiment.

However, to ensure the statistical rigour of the flux estimate calculated from the linear regression, the flux was only taken to be significant if the standard error of the slope of the regression line was less than the magnitude of the flux. This approach was taken from Cook et al., (2004II).

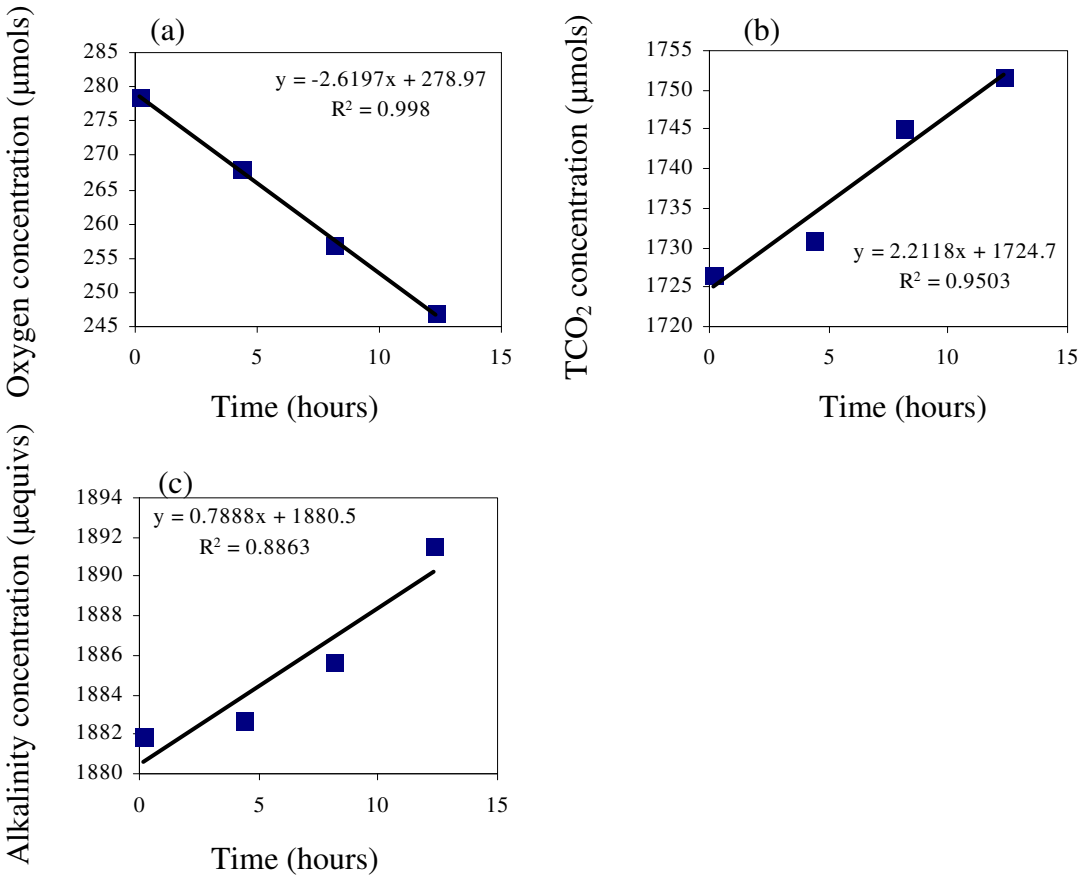


Figure 3-2. Examples of sediment core flux data plots (a) Oxygen, (b) TCO_2 and (c) Alkalinity taken from station UC during the November 2004 field trip.

3.2.5 Benthic Fauna

After completion of the incubations, the entire sediment content of each core was sieved through 500 micron mesh to collect the benthic infauna, which were stored in 10% seawater buffered formalin until analysis. The animals were grouped into major faunal groups (i.e. echinoderms, gastropods) and any dominating species was identified. Wet weights and relative abundance of each group was recorded for each core.

3.2.6 Sample Analysis

Alkalinity was measured using a Gran titration, which was performed using an Orion 960 Autochemistry system (precision = ± 1.0%). A total of 10ml of sample at 20^{o}C was titrated with 0.01 M HCL acid. pH was measured using an Orion (91-55) pH probe connected to a PHM85 (Radiometer) pH meter (precision = ± 0.002%). TCO_2 was calculated using alkalinity, pH, salinity and temperature according to equations given by Almgren et al. (1983).

3.2.7 Statistical Analysis

Statistical analysis was carried out using SPSS vers.11.0 (Microsoft). Two-way analysis of variance (ANOVA) was performed with time and location as independent factors and station as a random factor. Homogeneity of variance was checked by examination of residual plots and log transformed if necessary. This approach allowed for the detection of variability within each of the two study locations while also allowing for the detection of differences between the two locations. Correlation and regression analysis were used to explore relationships between variables. The level of significance to reject the null hypothesis was set at $p<0.05$.

3.3 Results

3.3.1 Temporal and Spatial Patterns

3.3.1.1 Total Oxygen fluxes

Oxygen fluxes peaked at 639.2 μmol m^{-2} h^{-1} at the UE station in March and the lowest flux was recorded in July at the LW station recording 126.1 μmol m^{-2} h^{-1}. Oxygen fluxes were significantly different between each of the 3 sampling periods (Table 3-1 & Table 3-2) and total oxygen fluxes measured in the sediment reactors followed clear temporal trends at all stations at both locations (Figures 3-3 & 3-4). Spatially, there was no significant difference between sites within either the upper or lower estuary locations. However, there was a significant statistical difference between the upper (398.2 μmol m^{-2} h^{-1}) and lower estuary locations (292.5 μmol m^{-2} h^{-1}) (Table 3-1).

Table 3-1 ANOVA table for total oxygen fluxes for the sampling period March 2004 – November 2004 (data was log transformed)

Source	df	Mean Square	F	Sig. (p)
Location	1	15.61×10^{-4}	25.342	**0.000**
Station	2	46.04×10^{-2}	0.747	0.482
Time	2	26.54×10^{-4}	43.056	**0.000**
Location x Station	2	67.33×10^{-2}	1.092	0.347
Location x Time	2	29.12×10^{-2}	0.472	0.628
Station x Time	4	23.23×10^{-2}	0.377	0.823
Location x Station x Time	4	34.08×10^{-2}	0.553	0.698
Error	33	61.63×10^{-2}		
Total	51			

Table 3-2 Tukey HSD homogenous subsets for total oxygen fluxes with time as fixed factor

		Subset		
Time	*N*	1	2	3
	16	207.8250		
November	18		358.1667	
March	17			458.0588
Sig.		1.000	1.000	1.000

* The mean difference is significant at the 0.05 level

3.3.1.2 TCO_2 fluxes

TCO_2 fluxes at the lower estuary site ranged between 228 µmol m^{-2} h^{-1} TCO_2 at LE and LC stations during July and 644 µmol m^{-2} h^{-1} TCO_2 at LE during March (Figure 3-3). The TCO_2 fluxes at the lower estuary sites also showed a clear temporal trend with highest in March and lowest in July. The TCO_2 fluxes at the upper estuary location had a higher range and varied between 267 µmol m^{-2} h^{-1} TCO_2 at UE station in November and 839 µmol m^{-2} h^{-1} TCO_2 at station UC in March (Figure 3-4). No obvious temporal trend could be discerned at the upper estuary sites. A significant difference was found between March and July and between March and November, but not between July and November (Table 3-3 & Table 3-4)

TCO_2 fluxes between sites at the two locations were not statistically different from one another while no significant difference was found between the two locations (Table 3-3). Community respiration quotients (CRQ = TCO_2/O_2 flux ratio) across the lower estuary stations averaged 1.4 and ranged between 1.1 and 1.8. In comparison, the CRQ across the upper stations averaged 1.2 and ranged between 0.6 and 2.7. The highest CRQ was measured at UC at the upper estuary site during July when oxygen consumption rates were lowest.

Table 3-3 ANOVA table for total CO_2 fluxes for the sampling period March 2004 – November 2004(data was log transformed)

Source	df	Mean Square	F	Sig. (p)
Location	1	46.42×10^{-3}	3.054	0.093
Station	2	18.10×10^{-3}	1.190	0.321
Time	2	30.20×10^{-4}	19.867	**0.000**
Location x Station	2	12.07×10^{-2}	0.079	0.924
Location x Time	2	39.61×10^{-3}	2.606	0.094
Station x Time	4	16.95×10^{-3}	1.115	0.371
Location x Station x Time	1	84.32×10^{-2}	0.555	0.463
Error	25	15.20×10^{-3}		
Total	40			

Table 3-4 Tukey HSD table for total CO_2 fluxes with time as fixed factor

		Subset	
Time	*N*	1	2
November	17	343.5941	
July	12	411.9583	
March	11		661.7545
Sig.		1.000	1.000

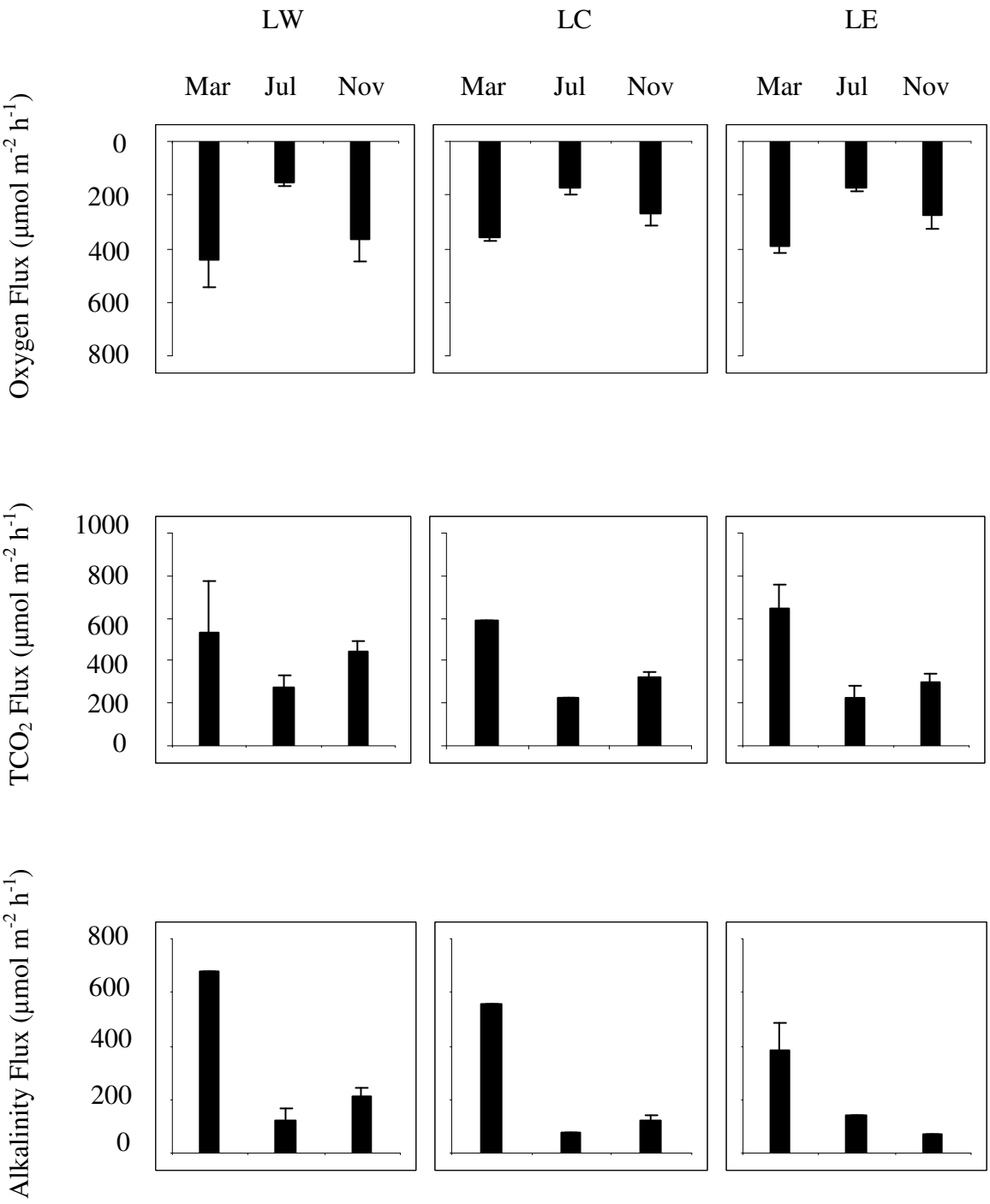


Figure 3-3. Oxygen, ∑CO2 and alkalinity fluxes at the lower estuary transect during March, July and November. Error bars represent the standard error of the mean (n = 2 or 3). N.B. where no error bars are present, the data only represents one flux result. This was due to some estimates of fluxes not being significant because of erratic changes over the incubation period.

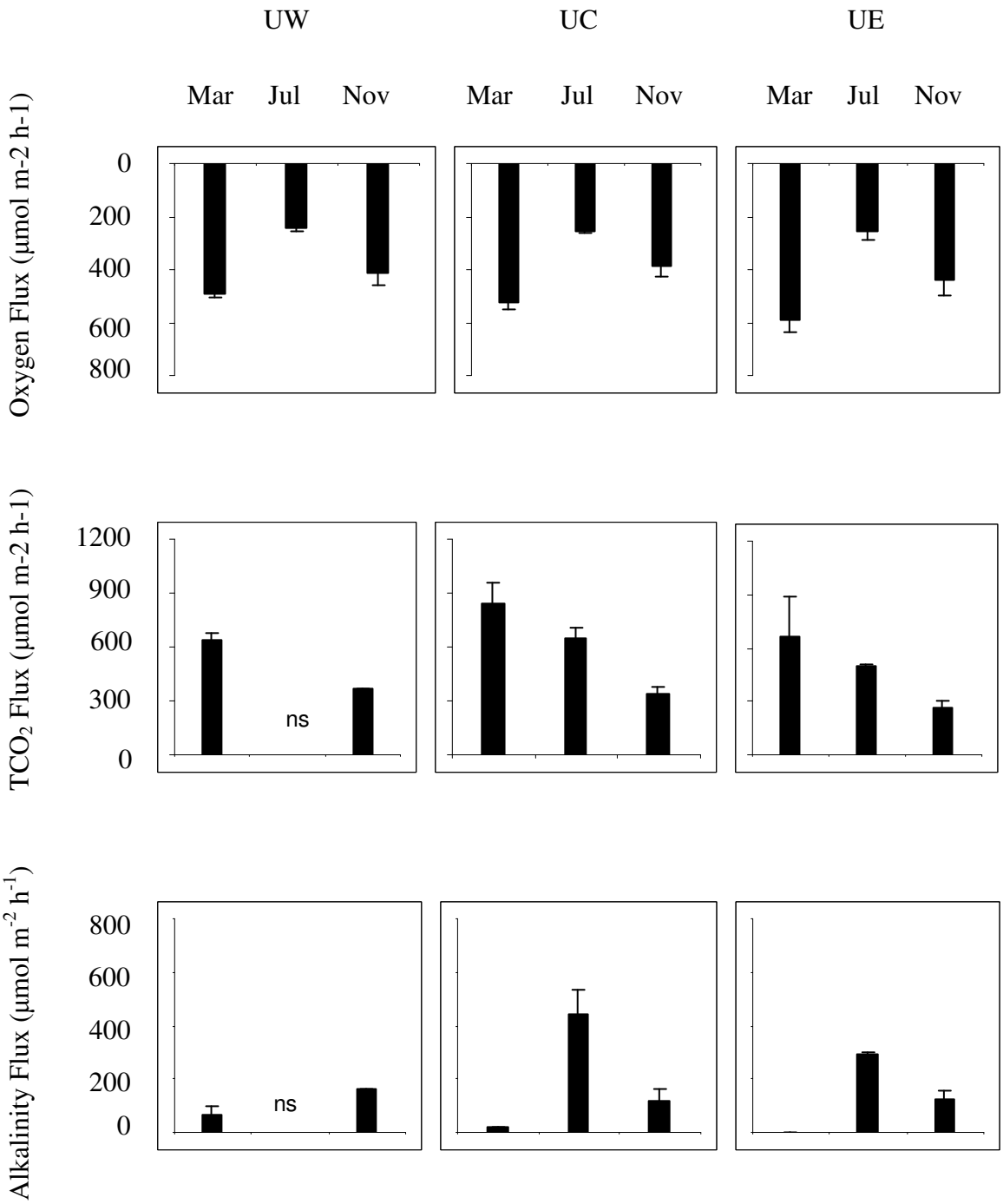


Figure 3-4 Oxygen, ∑CO2 and alkalinity fluxes at the upper estuary transect during March, July and November. Error bars represent the standard error of the mean (n=2-3). N.B. where no error bars are present, the data only represents one flux result. This is due to some fluxes been not significant because of erratic changes over the incubation period.

3.3.1.3 Depth of oxygen penetration and molecular diffusive flux rates

The O_2 penetration depth at both locations and all stations displayed a clear temporal trend. The shallowest depth of the oxic zone was measured in March and the deepest in July. The range of oxic zone depths was greater at the lower estuary transect (3.7 - 9.0 mm), than at the upper estuary transect (3.1 – 7.4 mm) (Table 3-5).

Table 3-5 Mean measured oxygen penetration depths and molecular diffusive fluxes. The fluxes were calculated from the diffusive boundary layer (J_{DBL}) and compared with fluxes modelled from the oxygen gradient within the sediment (J_{SED}). Numbers in brackets represent ± standard error. (n = 2).

Site		Oxygen Penetration Depth (mm)	Molecular Diffusive Flux Rates (μmol m^{-2} h^{-1})	
			J_{DBL}	J_{SED}
LW	March	5.2 (±0.5)	264 (±16.5)	259 (±15.9)
	July	8.3 (±0.3)	157 (±18.5)	155 (±17.9)
	November	7.2 (±1.1)	181 (±33.1)	176 (±31.3)
LC	March	3.7 (±0.9)	435 (±162.2)	422 (±150.4)
	July	8.2 (±2.2)	144 (±29.3)	142 (±142)
	November	4.7 (±1.1)	264 (±48.0)	258 (±46.4)
LE	March	4.6 (±0.7)	233 (±36.2)	227 (±36.3)
	July	9.0 (±0.4)	134 (±7.2)	132 (±7.2)
	November	6.9 (±1.1)	178 (±34.6)	173 (±32.5)
UW	March	3.7 (±0.4)	347 (±60.6)	334 (±57.1)
	July	7.4 (±0.6)	175 (±35.4)	171 (±34.3)
	November	5.1 (±0.4)	297 (±50.4)	286 (±47.9)
UC	March	3.1 (±0.5)	489 (±77.7)	468 (±78.5)
	July	5.5 (±1.1)	244 (±78.8)	237 (±75.9)
	November	4.9 (±0.6)	314 (±34.9)	301 (±33.7)
UE	March	nm	nm	nm
	July	6.2 (±0.4)	186 (±41.5)	182 (±40.2)
	November	4.0 (±0.5)	320 (±53.2)	310 (±50.8)

The molecular diffusive fluxes of oxygen (J_{DBL}) at the lower estuary transect ranged between 134 (±7.2) μmol m^{-2} h^{-1} at LE in July and 435(±162.2) μmol m^{-2} h^{-1}at LC in March (Table 3-5). In comparison, the upper estuary transect ranged between 175 (±35.4) μmol m^{-2} h^{-1} at UW in July and 489 (±77.7) at UC in March (Table 3-5). There was an obvious inverse relationship between molecular diffusive fluxes and oxygen penetration depths; as oxygen penetrations depths decreased, molecular

diffusive fluxes increased and vice versa. There was also good agreement between J_{DBL} and J_{SED} with less than 3% difference in flux rates between them.

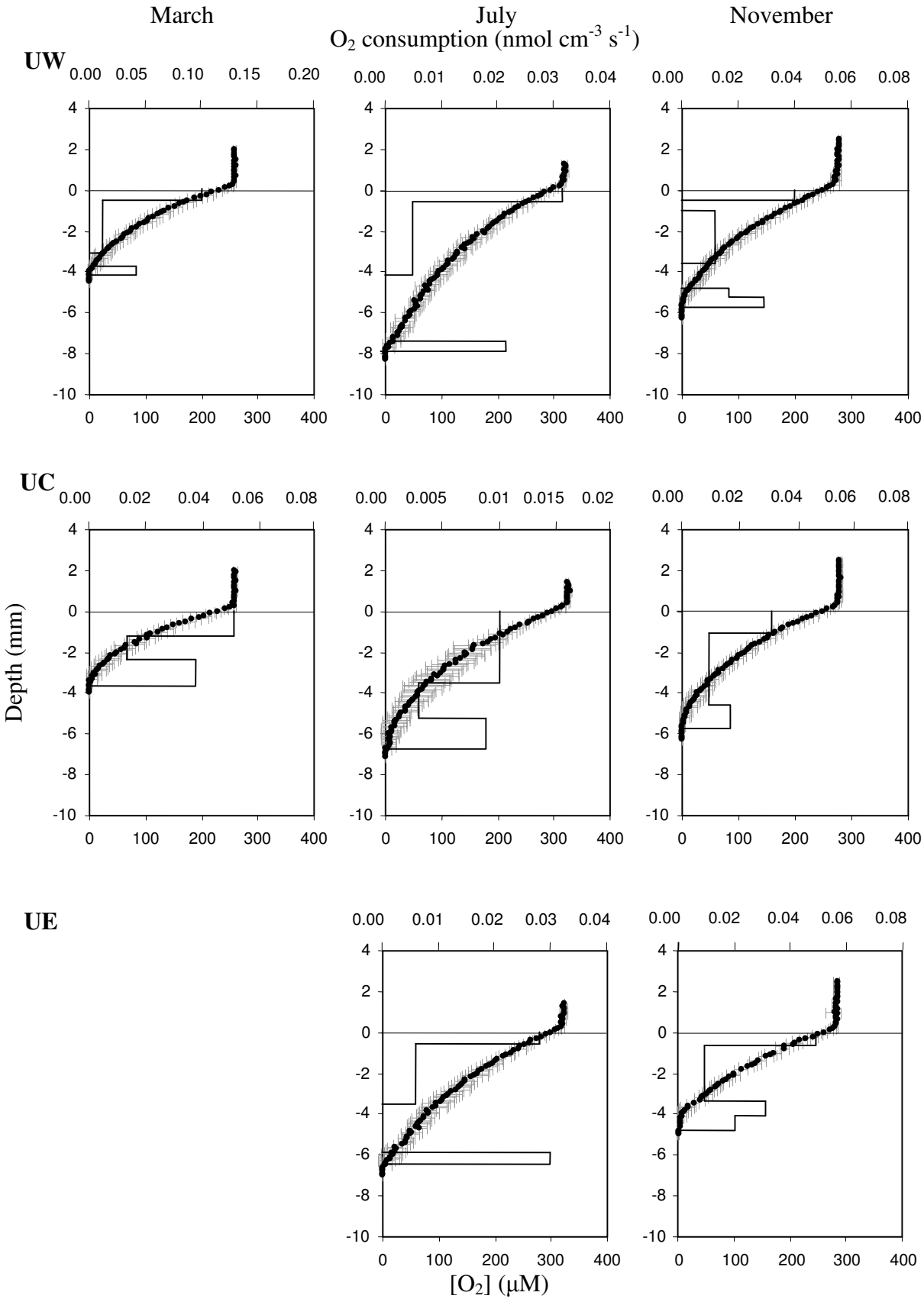


Figure 3-5 Measured oxygen microgradients and consumption profiles during March, July and November at the upper estuary station sites. The microgradients and consumption profiles represent the mean profiles from two cores at each site. The error bars on the microgradients represent the standard deviation of the mean.

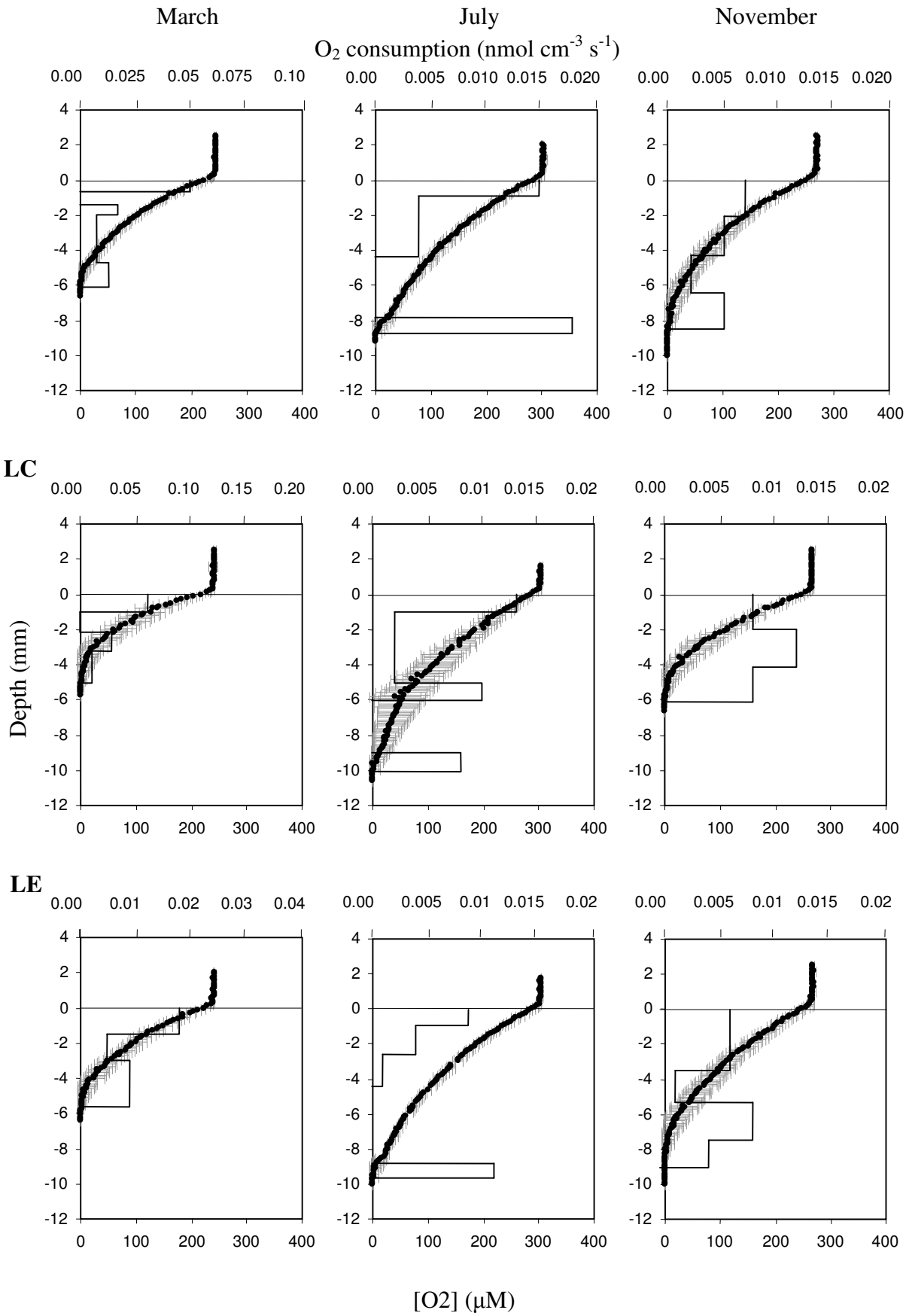


Figure 3-6 Measured oxygen microgradients and consumption profiles during March, July and November at the lower estuary station sites. The microgradients and consumption profiles represent the mean profiles from two cores at each site. The error bars on the microgradients represent the standard deviation of the mean.

3.3.1.4 Oxygen Microgradient Profiles

The oxygen microgradient profiles for the most part (Figures 3-5 and 3-6) reflected smoothly declining O_2 concentrations with inward curvature within the sediment. On no occasions were the profiles broken. The microgradient profiles were also characterised by small error bars suggesting a relatively homogenous sediment microstructure. No large benthic fauna were ever found to be present in the cores used for the oxygen microprofiles although small disturbances just beneath the sediment were witnessed on a few occasions, which may have been caused by worms. Thus, the results should reasonably reflect the diffusive oxygen uptake minus fauna within the sediments and will serve as a useful comparison with the total oxygen uptake measurements (see later) measured in sediment reactors that had large fauna such as the brittle star present on a number of occasions.

The oxygen consumption profiles were distinctly different between the three sampling times at the lower estuary (Figure 3-6). In March, most of the oxygen was consumed near the sediment-water interface with a small peak in oxygen at the oxic-anoxic interface also observed. However, the oxygen consumption profiles in July, were characterised by large peaks at the oxic-anoxic interface, while in November, the profiles were relatively uniform through the oxic layer. In contrast, the oxygen consumption rates modelled at the upper estuary (Figure 3-5) transect exhibited intense activity at the sediment surface and at the oxic-anoxic interface during all three sampling periods.

The intense activity at the sediment surface is due presumably to aerobic degradation of labile organic carbon deposited onto the sediment surface. The increased O_2 consumption rates at the oxic-anoxic interface is most likely related to gradual oxidation of reduced solids (FeS) by the downward migration of oxygen in the colder winter months due to increased oxygen concentrations in the bottom water (> 300µm O2 in winter versus >250µm O_2 in summer). The source of the reduced solids (e.g. sulphide) is most likely related to anaerobic mineralization such as sulphate reduction occurring during the summer months.

3.3.2 Benthic fauna

The incubated sediment cores were dominated by three major groups, echinoderms, annelids and gastropods, while bivalves and crustaceans were present on occasion (Table 3-6). At the Upper estuary sites, echinoderms dominated the faunal populations, and consisted entirely of the Brittle star *Amphiura elandiformis*. These were found to inhabit between 63.8 and 100% of the populations at these sites except for UW in November when no brittle stars were present in the incubated cores. At the lower estuary, a more mixed population was present with gastropods including whelks and screw shells always present in the incubated cores. Once again brittle stars were present and were found in approximately 40% of the cores incubated at the lower estuary sites. A much higher biomass was observed at the lower estuary (range 43.0 to 249.5 ww (g) m^{-2}) compared with the upper estuary (range 2.9 to 77.8 ww (g) m^{-2}).

Table 3-6. Summary of benthic fauna at the Upper and Lower locations during all three sampling times. Biomass was recorded as wet weight. Bivalves and gastropods were weighed with shells intact. However, before weighing, bivalves and gastropods were examined for fresh tissue mass to ensure they were viable at the time of sampling.

	LW						LC						LE					
	Mar ww(g) m^{-2}	%	Jul ww(g) m^{-2}	%	Nov ww(g) m^{-2}	%	Mar ww(g) m^{-2}	%	Jul ww(g) m^{-2}	%	Nov ww(g) m^{-2}	%	Mar ww(g) m^{-2}	%	Jul ww(g) m^{-2}	%	Nov ww(g) m^{-2}	%
Enchinoderms	34.0	13.6	16.9	8.8	16.3	5.8	150.3	71.9	0	0	0	0	50.9	24.7	16.6	10.6	47.2	57.6
Annelida	10.9	4.4	3.2	1.7	48.9	17.5	18.6	8.9	38.9	90.5	27.2	51.1	4.5	2.2	24.3	15.4	0	0
Bivalves	42.5	17.0	0	0	0	0	5.1	2.4	0	0	0	0	20.2	9.8	7.3	4.6	2.2	2.7
Gastropods	162.1	65.0	171.2	89.5	214.5	76.7	35.1	16.8	4.1	9.5	26.0	48.9	130.1	63.3	109.4	69.4	32.5	39.7
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	249.5	100	191.3	100	279.7	100	209.1	100	43.0	100	53.2	100	205.7	100	157.6	100	81.9	100

	UW						UC						UW					
	Mar ww(g) m^{-2}	%	Jul ww(g) m^{-2}	%	Nov ww(g) m^{-2}	%	Mar ww(g) m^{-2}	%	Jul ww(g) m^{-2}	%	Nov ww(g) m^{-2}	%	Mar ww(g) m^{-2}	%	Jul ww(g) m^{-2}	%	Nov ww(g) m^{-2}	%
Enchinoderms	55.2	86.2	9.0	63.8	0	0	58.0	74.6	27.3	73.6	27.7	100	18.9	88.3	27.5	78.1	16.6	86.5
Annelida	5.3	8.3	0	0	0	0	6.2	8.0	1.0	2.7	0	0	0	0	0	0	0	0
Bivalves	3.0	4.7	0	0	0	0	0	0	0	0	0	0	2.5	11.7	4.5	12.8	0	0
Gastropods	0.5	0.8	5.1	36.2	0	0	13.6	17.4	8.8	23.7	0	0	0	0	3.2	9.1	2.6	13.5
Other	0	0	0	0	2.9	100	0	0	0	0	0	0	0	0	0	0	0	0
Total	64.0	100	14.1	100	2.9	100	77.8	100	37.1	100	27.7	100	21.4	100	35.2	100	19.2	100

3.4 Discussion

3.4.1 Spatial and temporal variability

Sources of variability that will alter sediment respiration rates include temperature (Thamdrup et al., 1998), organic supply (Kristensen, 2000), bioturbation (Aller, 1994; Kristensen, 2000) advection and sediment grain size (Janssen et al., 2005). It was assumed in this study that advection was not significant due to the silty nature of the sediments. The high silt contents of the sediments indicates the study locations were indicative of high depositional areas, due to low hydrodynamic activity, and are therefore relatively impermeable to advective water flows from the overlying water. It is therefore proposed that temperature, organic matter supply and bioturbation (which will be discussed in another section of this chapter) are potentially the main drivers of respiration in the current study.

In this study, temperature appeared to strongly influence sediment respiration rates in the Huon estuary. Sediment oxygen fluxes and diffusive oxygen fluxes significantly correlated with temperature at all stations and locations as did TCO_2 fluxes at the lower estuary although not at the upper estuary sites. Regression analysis between total oxygen fluxes and temperature revealed that between 81 and 86% of the variability at the upper stations could be attributed to temperature while between 53 and 76% at the lower stations. Likewise, regression analysis between temperature and diffusive fluxes showed that 72 – 97% of the variability could be attributed to temperature at the upper sites, while 71 – 78% of the variability could be attributed to temperature at the lower sites.

Temperature affects both oxygen penetration depth and flux rates because: 1) increasing temperature increases reaction rates, therefore increasing metabolic rates and the demand for O_2; 2) increasing temperature decreases O_2 solubility, therefore in summer the bottom water has less dissolved oxygen, so diffusion into the sediment is slower because it is coming from a lower concentration.

The regression analysis should be interpreted cautiously however as they rely on three different sampling periods within one year only and therefore lack robustness. Many

studies in the literature (Forja et al., 1994; Cowan and Boynton, 1996; Vidal et al., 1997; Hopkinson et al., 2001 and Eyre and Ferguson, 2005) however have shown that sediment respiration is strongly influenced by temperature and therefore it is proposed that temperature is one of the main drivers of respiration in this study given the observed trends in the data.

Another possible driver of respiration is the supply of organic matter to the sediments. Many studies have shown that when 'fresh' labile material such as phytoplankton or fish farm waste deposit onto the sediment surface, a rapid response occurs with respiration rates increasing by up to six times the initial rate (Enoksson, 1993; Sloth, 1995; Christensen et al., 2000; Grenz et al., 2000 and Witte et al., 2003). However in an estuarine system that receives organic material from land runoff and fluvial systems the sources of organic carbon generally are mixtures of terrestrial and marine (algal) inputs. It is generally accepted that organic material from terrestrial sources is refractory (Loh et al., 2002; Ferguson et al., 2003) or in other words already heavily degraded. It is typically composed of compounds such as lignin (Loh et al., 2002) and longer chain (>C22) saturated alcohols (Jeng et al., 2003), which are not easily broken down by bacteria. In contrast marine material is considered to be highly labile (Loh et al., 2002; Ferguson et al., 2003), rich in PUFA's and amino acids, both of which are easily broken down by bacteria.

However, no significant relationship between various indicators of organic carbon content (including total fatty acids, PUFAs or phytols) with respiration for all stations was found, suggesting that either organic carbon had little control on respiration, which is contrary to most studies, or more likely that the sediment organic content does not reflect the supply of organic material actually respired in the sediments (Hopkinson et al., 2001). This could be resolved by measuring sedimentation rates in the Huon estuary.

While no statistical relationship was observed between respiration and various organic matter parameters, benthic respiration was higher at the upper estuary sediments, which on average had twice as much organic carbon compared to the lower estuary sediments. Therefore, it is likely that the main reason for the difference in respiration rates between the two study locations is due to the amount of organic

carbon present in the surface sediments. However, while the upper estuary had higher organic carbon in surface sediments it was generally dominated by refractory organic carbon. As a comparative indicator of the proportion of labile organic carbon in the sediments between the two sampling locations, I normalised total oxygen consumption to organic carbon content. This shows that the lower estuary had much higher rates of oxygen consumption normalised to carbon compared to the upper estuary for all sites and times (Table 3-7), which suggests the lower estuary sediments had higher proportions of labile organic matter.

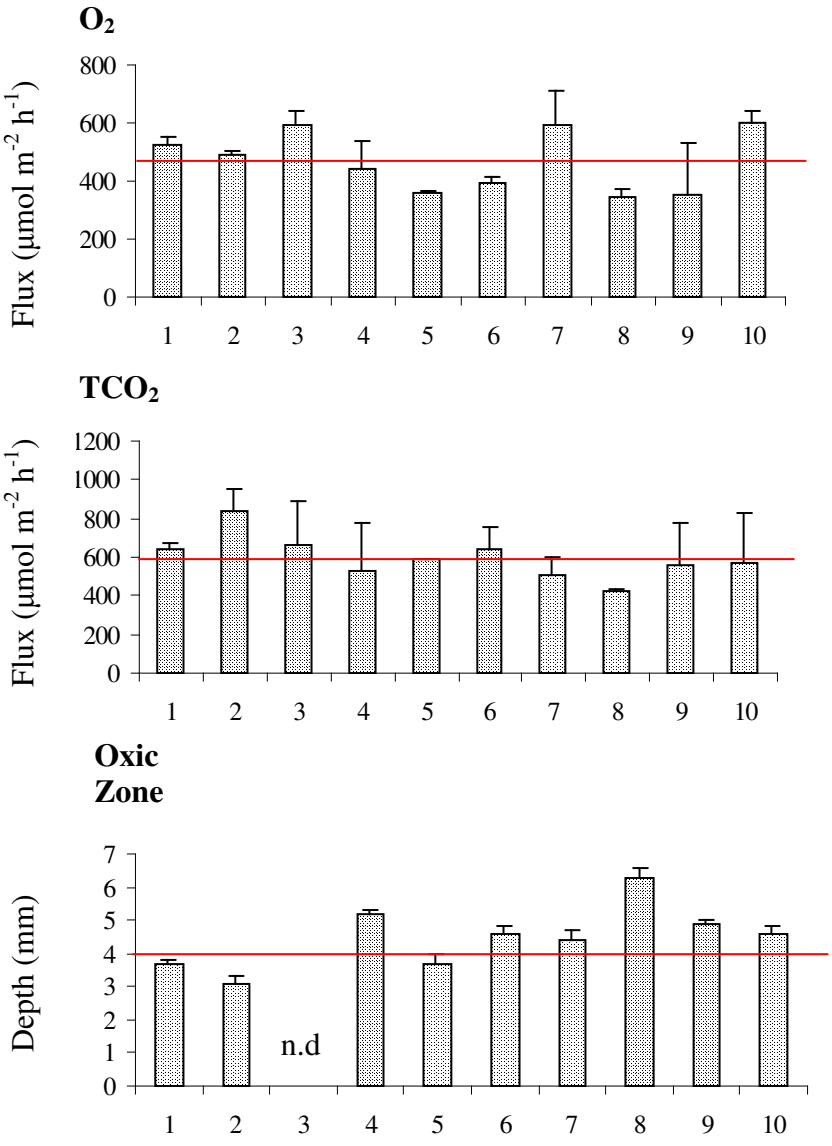
Table 3-7. Normalisation of sediment oxygen consumption to total organic carbon in surface sediments

Location	Time	Total Oxygen Consumption (TOC) µmols m^{-2} d^{-1}	Organic Carbon %C DW	TOC normalised to organic carbon
LW	March	442	4.5	98
	July	152	3.8	40
	November	363	3.6	101
LC	March	356	4.7	76
	July	176	4.6	38
	November	269	4.5	60
LE	March	391	3.4	115
	July	172	3.9	44
	November	273	3.8	72
UW	March	523	7.7	68
	July	256	7.8	33
	November	387	7.7	50
UC	March	489	8.1	60
	July	244	8.2	30
	November	415	8.0	52
UE	March	592	8.2	72
	July	253	8.8	29
	November	441	8.7	51

Broader spatial variability of respiration within the Huon estuary was examined, which included data from a separate study conducted in 2005 (Hideaway bay and Garden island) along with data obtained from Stringers cove during a 2001 – 2 study (see Figure 1.3 for locations). Oxygen uptake rates ranged between 350.5 and 600.5 µmol m^{-2} h^{-1} across all stations (Figure 3-7). Both the highest and lowest rates were recorded at Stringers cove. The average for all stations was 468.5 (±33.4) µmol m^{-2} h^{-1}. The reproducibility of the oxygen flux measurements (SD/mean) was within 22.6%

indicating good precision between replicate measurements. TCO_2 fluxes measured in late summer ranged between 428.8 at P4 near Garden Island and 839.8 at UC at the mid estuary study location during 2004. The average TCO_2 flux was 597.2 and the reproducibility of the fluxes was within 18.6%. The high reproducibility in respiration fluxes observed across the broader Huon estuary / Port Esperance region probably reflects the similar nature of sediments at each of the sampling sites. All sites were dominated by the silt/clay fraction and had relatively high inputs of terrestrial organic matter.

In contrast, the reproducibility of the oxic zone was 41.5%, indicating lower precision in comparison to oxygen and TCO_2 fluxes. At very small scales respiration is likely to be very heterogenous and dynamic in comparison to the larger integrated fluxes. These findings are consistent with Rabouille et al. (2003) who observed much greater variation at very small scales (decimetres) than at the station levels. This suggests that oxygen microgradients and modelled diffusive fluxes will vary greatly over a large area. The dynamic nature of microgradients can be influenced by a number of factors including the microtopography (surface roughness) of the surface sediment (Glud et al., 2003); the variable distribution of organic material on the sediment surface; or faunal irrigational activity or enhanced supply of reduced species from the deeper anoxic zones (Rabouille et al., 2003).



Number	Location	Station	Sampling date	Temperature
1	Brabazon Point	UE	06/03/2003	15.6
2	Brabazon Point	UC	06/03/2003	15.6
3	Brabazon Point	UW	06/03/2003	15.6
4	Huon Island	LE	01/03/2003	15.7
5	Huon Island	LC	01/03/2003	15.7
6	Huon Island	LW	01/03/2003	15.7
7	Hideaway Bay	P3	12/04/2005	15.7
8	Garden Island	P4	12/04/2005	15.7
9	Stringers Cove	R2 (O_2 & CO_2)	22/02/2001	~16.0
	(Macleod et al., 2004)	R2 (oxic zone)	24/04/2001	
10	Stringers Cove	R2 (O_2 & CO_2)	18/02/2002	~16.0
	(Macleod et al., 2004)	R2 (oxic zone)	06/02/2002	

Figure 3-7. Spatial patterns of benthic respiration (O_2 and TCO_2 fluxes and oxic zones: means ± SE) during late summer/ early Autumn. Temperatures ranged between 15 – 16°C for all sampling periods. (n.d.= no data). Red line indicates the average value.

3.4.2 Carbon diagenesis pathways

Numerous techniques have been used to measure sediment respiration rates to determine organic matter decomposition in sediments (Hopkinson et al., 2001). Most commonly, oxygen fluxes measured in benthic chambers or core incubations have been used. However due to the complexity of biochemical reactions within sediments, measuring oxygen consumption can underestimate organic matter decomposition when oxygen is not the only terminal electron acceptor for respiration. That is, the oxygen demand from reduced ions is ultimately derived from organic carbon respiration that has occurred at an earlier time. So the oxygen consumption measurements are actually integrating several carbon mineralisation events, rather than giving the mineralisation rate at the time of measurement. It is however, not feasible to separate out which component of sulphide (and all other reduced ions that may be present) has resulted from current carbon mineralisation without direct measurements of anaerobic respiration, particularly of sulphate reduction.

A more direct approach of determining organic matter remineralisation is to measure CO_2 production as it is an end product in respiration as seen in the stoichiometric equation below (Hopkinson et al., 2001).

$$106(CH_2O)16(NH_3)(H_3PO_4) + 106O_2 \rightarrow 106\ CO_2 + 16\ NH_3 + PO_4$$

A useful comparison can be made between CO_2 production and oxygen consumption, known as the community respiratory quotient (CRQ), which can be used to infer the relative importance of aerobic and anaerobic decomposition. If the molar ratio of CO_2 produced to oxygen consumed is 1 to 1 then it can be concluded that the majority of organic matter is decomposed via aerobic respiration. However when the molar ratio is >1 then organic matter is also being decomposed anaerobically producing reduced end products such as sulphide (Hopkinson et al., 2001). Figure 3-8a shows the observed TCO_2 versus O_2 plot with the majority of the plotted ratios falling close to the ratio expected for aerobic respiration alone (1:1). The low Alkalinity:O_2 flux ratios (Figure 3-8b) also suggest that organic matter degradation occurs mainly by aerobic respiration (Forja et al., 1994).

Measuring alkalinity fluxes, in tandem with CO_2 and O_2 fluxes allows some basic stoichiometric relationships to be inferred. Alkalinity may be produced by (Berelson et al., 1998):

1. respiration
2. denitrification
3. sulphate reduction
4. net dissolution of $CaCO_3$

However it can also be consumed by:

1. nitrification
2. pyrite oxidation
3. sulphide oxidation

In this study, aerobic respiration is significant, but does not account for all mineralisation, because CO_2:O_2 variability (fig 3.8) indicates that anaerobic respiration is also occurring. The relatively shallow oxic zones found in this study support the conclusion that aerobic respiration is constrained due to the limited availability of oxygen. It is therefore likely that both of aerobic and anaerobic processes are responsible for the alkalinity effluxes measured in this study. However, alkalinity is also likely to be consumed via nitrification (see chapter 4 for more information on nitrification). Thus a complex reaction of metabolic processes can occur at the same time, some producing alkalinity and some consuming it. To unravel this, more specific experiments need to be done to target the processes responsible for producing or consuming alkalinity.

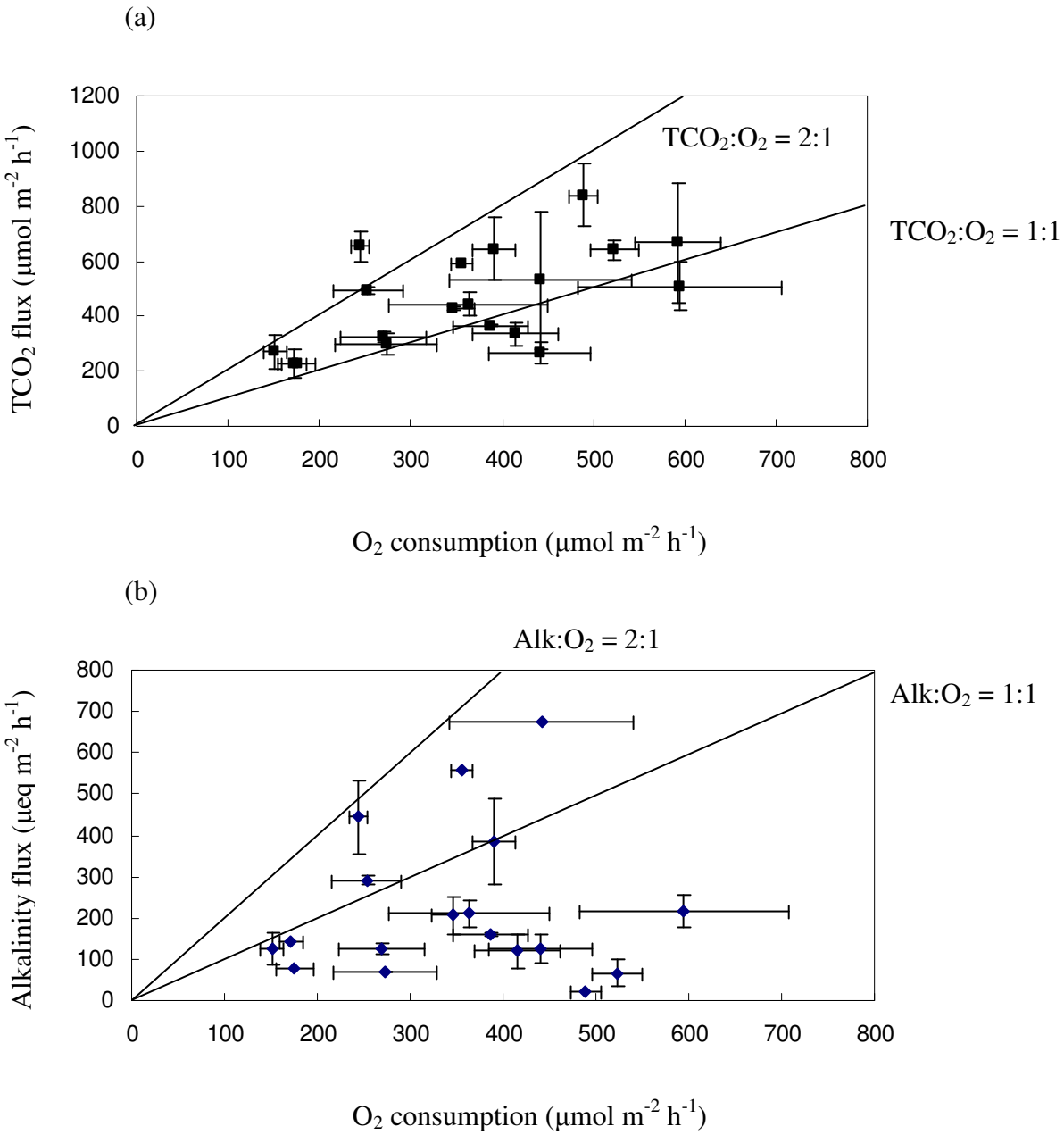


Figure 3-8. (a) TCO_2 flux versus O_2 flux. (b) Alkalinity flux versus O_2 flux. Data shown are from all core incubations at all sites during the 3 sampling periods.

3.4.3 Comparison of diffusive (DOU) and total oxygen uptake (TOU) rates and the role of benthic infauna

The processes investigated are very different when measuring sediment oxygen demand by either core incubation or with microelectrodes. Core incubations (often referred to as total flux) integrate all processes involved in oxygen transfer: diffusion, advection and faunally-mediated transport. Benthic fauna may enhance oxygen transfer and thus consumption by either bioirrigation whereby fauna such as polychaete worms actively pump oxygenated water into their burrows or by biodiffusion caused by the erratic movements of the fauna, such as the movement of brittlestar appendages. In comparison, microelectrode profiles measure the diffusive flux and assume that the transfer of oxygen is by molecular diffusion only (Rabouille et al., 2003).

Therefore comparing the two measurements should allow some insight into faunally mediated processes assuming physical advective processes such as that caused by wave energy or groundwater pumping can be ignored. Janssen et al. (2005) found that total oxygen uptake rates were significantly enhanced by advection in medium to coarse sandy sediments, but they could not measure advection in fine grain sandy sediments. In the current study locations, the sediments were very silty indicating high depositional areas and thus low wave energy; I therefore assumed that physical advection is likely to be of minor importance in these locations. Therefore the difference between TOU and DOU can be attributed mainly to faunally mediated processes in this study.

However, it is likely that the core incubations will underestimate total oxygen uptake due to the exclusion of large fauna and the disturbance to animal burrows and therefore to animal behaviour in general (Glud et al., 2003; Rabouille et al., 2003). During the current study, brittlestars were seen to be active at the surface during incubations and thus contributing to TOU, however we cannot estimate the damage to worm burrows and therefore it is possible that TOU values were underestimated in this study.

Molecular diffusive fluxes can be underestimated when using microelectrode profiles with a one-dimensional diffusion model, which assumes a smooth sediment surface and therefore consistent thickness of the DBL layer over the surface. The DBL, however, is affected by the microtopography of the sediments, with the DBL layer thinner over protruding surfaces and thicker over interjacent areas (Glud, 2008). The one-dimensional approach assumes a flat plane, therefore underestimating the surface area of the potential diffusive exchange (Jørgensen and Des Marais, 1990); vertically measured profiles may protrude the DBL on an angle, which artefactually deepens the DBL; and horizontal diffusion is not accounted for. Quantitatively, this maybe important as Glud et al., (2003) found that the difference between 3D and 1D calculations was a factor of 1.12 ± 0.01, highlighting the importance of the effect of surface roughness on the diffusive flux.

Another under or overestimation of the diffusive flux is that the DBL in the incubation cores may not reflect in situ conditions due to different flow velocity of the bottom water and therefore can lead to errors in flux calculations. Glud et al., (2003) found that in areas that have very high respiration rates along with shallow oxic zones a change in the DBL due to change in water flow velocity could have significant effects on the calculated diffusive fluxes. On the other hand they also found that in sites with relatively deep oxic zones, changes in DBL thickness would only affect the diffusive fluxes marginally. Rasmussen & Jorgensen (1992) calculated that the impedance of the diffusive flux by changes in the DBL thickness was between 3 – 5% in winter when the oxic zone was 4 to 5mm thick and 12 – 16% in summer when the oxic zone was 1.5 to 5mm thick. In the current study, the DBL thickness was altered by up to 10% when modelling the diffusive flux from oxygen profiles and it was found that the effect on the modelled diffusive fluxes were only minor ranging between 1.6 and 3.4%. Thus, it was concluded that changes in the DBL thickness would only cause minor changes to the diffusive oxygen fluxes in cores from this study. Overall, it has been found that DBL impedance can have important consequences on the DOU over short time scales, in particular where sediments are highly reactive due to the presence of labile organic carbon and the oxic zones are relatively shallow (e.g. <2.0mm). Over the long term however DBL impedance is likely to be marginal in coastal sediments.

Selecting the interface location between the sediment and water can also lead to an under or overestimation when calculating the diffusive flux (Glud et al., 2003). Comparison between diffusive fluxes calculated from the DBL and modelled from the oxygen profile in the sediments in this study found little difference between the two independent measures and therefore it is likely that selection of the interface location between the sediment and water was reasonably accurate in this study.

In the current study the ratio between TOU and DOU ranged between 0.8 and 2.0, with an average of 1.3 (Figure 3-9). On one occasion the diffusive flux was greater than the total flux. The reason this occurred was because there were some very large values calculated from some of the microprofiles in one core, inflating the overall average. Removal of the two highest fluxes would bring the flux in line with the total flux. This highlights the small-scale spatial heterogeneity in sediments. The TOU/ DOU ratios are similar to other reported data. Burke (1999) reported an average TOU/DOU of 2.3 in Port Phillip Bay, in South-Eastern Australia, and attributed the difference to advection via benthic invertebrate activity through bioturbation or bioirrigation. Rabouille et al. (2003) found that on a number of occasions, the diffusive flux was close to the TOU, while Rasmussen and Jorgensen (1992) found a TOU/DOU of 1.4 in coastal Danish sediments and attributed the difference to large burrowing fauna such as bivalves and polychaetes. Hulth et al. (1994) recorded TOU/DOU between 1.1 and 4.8, however, they could find no relationship between the values >2.5 and observed bioirrigating infuana. Thus Hulth et al. (1994) attributed the differences to sediment topography and possible core decompression at the very deep sites.

In this study a weak regression ($r^2 = 0.15$) was found between TOU/DOU and total faunal weight (g m^{-2}). As gastropods were weighed with shells, they were removed from the fauna population to remove the possible effect of shell weight and the regression was run again. This slightly improved r^2 to 0.24. The regression analysis suggests that benthic fauna were only responsible for up to 24% of the variability between TOU and DOU. However, to get a better understanding of the role benthic fauna play in mediating sediment respiration, a more detailed study of the benthic fauna present in the sediments is required. Experiments of how individual faunal species, such as the brittle star, mediate sediment respiration should be conducted so

that the importance of different benthic faunal species on benthic respiration can be ascertained. Concurrent experiments to distinguish between effects of benthic fauna versus artefacts of sediment sampling and of the experimental protocol should also be done.

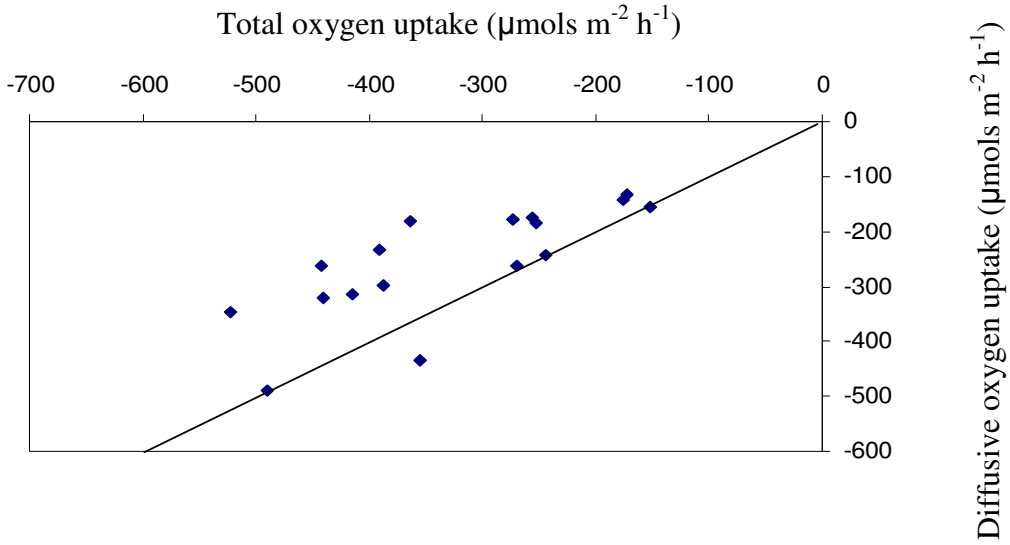


Figure 3-9. Total oxygen uptake versus diffusive oxygen uptake. Results presented are averages from all stations at both locations during the 3 sampling times. The line indicates a 1:1 relationship. Any points above the line suggest that processes such as faunal mediated oxygen consumption are also occurring along with diffusive processes.

3.4.4 Ecological Significance of carbon mineralisation in Huon estuary sediments

An important aspect of studying carbon mineralisation in sediments is to place it in the context of the whole estuary system. Comparing benthic decomposition rates of organic carbon with primary production rates in the water column allows an estimate of the amount of primary productivity carbon mineralised by the sediments. This can then tell us if the carbon cycle within the estuary or bay is tightly coupled between the seafloor and water column. As primary production rates in the Huon Estuary were not made as part of this study, data from Cook (2002) have been used for the analysis. Cook (2002) found that primary productivity in the water column ranged between 55 and 280 g C m^{-2} yr^{-1} and averaged 86 g C m^{-2} yr^{-1} in the upper estuary. At Port

Cygnet, which is a marine site, primary production ranged between 63 to 2200 g C m^{-2} yr^{-1} and averaged 210 g C m^{-2} yr^{-1}.

The average annual rates of primary production at the upper estuary and at Port Cygnet were then compared with average annual rates of carbon mineralisation (CO_2 fluxes were used as estimates of Carbon mineralisation rates) at the upper estuary and lower estuary (including Garden island and Hideaway bay sites) respectively. At the upper estuary, average annual carbon mineralisation rates were calculated to be 55 g C m^{-2} yr^{-1} compared to average primary productivity rates estimated as 86 g C m^{-2} yr^{-1}. Likewise, the lower estuary sites average annual carbon mineralisation rates were calculated to be 42 g C m^{-2} yr^{-1} compared to average primary productivity rates estimated as 210 g C m^{-2} yr^{-1}. The comparison of loading to sediment respiration indicates that about 64% of total organic carbon entering the upper estuary as planktonic primary production is mineralised on the bottom while only 20% of primary productivity is mineralised on the bottom at the lower estuary sites.

The amount mineralised in the lower estuary sites is lower then reported by Giblin et al. (1997) (46%) and Glud et al. (2003) (46%) while the upper estuary sites were higher. As phytoplankton depositional rates are unknown it is difficult to speculate why these differences occur. Clearly then, to resolve this, algal sedimentation rates need to be measured and compared with measured primary productivity rates and sediment carbon mineralisation rates

3.5 Conclusions

Benthic respiration rates were generally low and oxygen penetration depths relatively deep for coastal sediments rich in organic carbon. The low respiration rates are likely due to the highly refractory nature of the organic carbon. An inverse relationship was observed between oxygen consumption and oxygen penetration depths, for example as oxygen penetration depths increased, oxygen consumption decreased. Aerobic respiration is considered the main pathway for carbon cycling in Huon estuary sediments. The low CO_2:O_2 and Alkalinity:O_2 flux ratios present evidence for this along with peaks of oxygen consumption at the sediment-water interface. Peaks in

oxygen consumption at the oxic-anoxic interface also occurred and were most likely due to the oxidation of sulphides diffusing up from the anoxic zone.

Temperature and carbon availability were considered the main drivers for the temporal and spatial differences in respiration respectively during this study. The role of benthic fauna was less clear as it only accounted for a small proportion of the variability between total versus diffusive oxygen uptake rates.

This study of benthic respiration has provided the foundations for further work on carbon cycling in Huon estuary sediments. It has also identified the knowledge gaps that remain. Some of the more important gaps include that lack of knowledge on primary productivity and sedimentation rates in the Huon estuary. This study provided a first estimate of the importance they play in carbon cycling, however to gain a better understanding of carbon cycling between the water column and the sediments, measurements of primary productivity and sedimentation rates are crucial to understanding how much carbon produced in the water column is mineralised in the sediments and then either released back into the water column as CO_2 or permanently stored in the sediments via burial. In addition to this, in situ measurements should be made using benthic chambers so that the role of benthic fauna can be better understood. Microcosm experiments could also be set up to study the roles of individual species in relation to bioturbation and bioirrigation.

Chapter Four

Sediment Nutrient Fluxes in the Huon Estuary

Chapter 4. Sediment Nutrient Fluxes

4.1 Introduction

The Huon Estuary is recognised for its relatively pristine environment and high value Atlantic salmon farming industry. It is also known to be strongly stratified, with two-layer circulation driven by relatively high annual freshwater discharge from the Huon River (Butler et al., 2000). It also has high annual NO_x and phosphate concentrations in deep water at the mouth of the estuary (in the D'Entrecasteaux Channel). This, combined with the 2-layer circulation, results in a large influx of NO_x and phosphate into the estuary bottom waters (Butler et al., 2000).

There is however, some doubt about the source of NO_x in bottom waters at the mouth of the estuary during summer due to high nitrite:nitrate ratios. These observations suggest that a substantial fraction of the nitrate may be due to nitrogen recycling and nitrification in the water column and sediments, in both the lower Huon Estuary and the D'Entrecasteaux Channel. If this is the case, it may have significant implications for the impact of finfish-farm loads on the estuary (Butler et al., 2000).

While the precise nature of these events is not well understood for this system, they are most likely related to sediments breaking down organic matter and releasing nutrients back to the water column, where they are readily available for phytoplankton uptake. At present, however, these events cannot be predicted due to the lack of knowledge about how the sediments function within the Huon estuary.

Thus the research described in this chapter was undertaken in order to understand nutrient cycling at the sediment – water interface, to assess the contribution from sediments to the nutrient mass balance in the Huon estuary and to compare the observations in this study to other benthic nutrient cycling studies, most of which have been undertaken in eutrophied systems in North America and Europe, which makes it difficult to relate those findings back to a mesotrophic system such as the Huon estuary (Butler et al., 2000). Thus, in order to understand the functioning of sediment systems in a mesotrophic system and to compare with eutrophic systems such studies as the current one are important.

The aim of this research was to measure rates of nutrient fluxes at the sediment – water interface concentrating on: ammonia, nitrite, nitrate, phosphate and silicate across both temporal and spatial scales. Flux stoichiometry was then used to define the processes that are of importance in the recycling and burial of nutrients in Huon estuary sediments. Additionally, ammonia, nitrate and nitrite in pore waters were measured during the last sampling campaign in spring to compare measured nutrient fluxes with diffusive fluxes calculated from sediment pore water profiles.

4.2 Methods

4.2.1 Sediment Collection

The upper and lower estuary locations were visited three times in 2004, in March, July and November, using a small research vessel. The two locations were sampled on separate occasions, generally 3 days to a week apart, due to resource and labour limitations. Results from Hideaway bay and Garden Island (Figure 1-3) sampled in April 2005 have also been included and will serve as a comparative study of the benthic processes with the upper and lower estuary locations. At each site a box corer was used to take intact sediment samples. From each box-core sample, an undisturbed sub-core was taken by 25-cm-long polyethylene tubes with an inner diameter of 9.7 cm to measure benthic respiration and nutrient exchange. Three replicates were obtained at each site.

The remaining surface sediment (top 1cm) that was not collected in the sub-cores was collected from the box core and placed into glass jars and frozen until analysed for stable isotopes $\delta^{13}C$ and $\delta^{15}N$, carbon and nitrogen content, lipid biomarkers and sediment grain size. Cores (4.8cm inner diameter, i.d) for nutrient porewater analysis were taken separately, in duplicate in November 2004 at the Upper and Lower locations and again in April 2005 at Hideaway bay and Garden island.

The cores were then held in an ice-cooler at in situ temperatures. Near bottom water (~160 litres) was collected using an 8l Niskin bottle and stored in 20 litre plastic carboys. Temperature and salinity were determined in surface and bottom water on each occasion using a digi-thermo thermometer and refractometer respectively.

Secchi depth was also measured in July and November. The cores and water were then transported back to the laboratory within six hours of sampling. The bottom water was placed into an incubation trough and glass aquarium (60 litres) and brought to in-situ temperatures using a thermostatically controlled recirculating water bath and temperature thermostat probe respectively.

Prior to submerging the cores for benthic respiration and nutrient exchange into the incubation trough, the sediment volume was standardised so that there was a sediment depth between 8 and 10 cm and that there was 11 to 13cm of overlying water. The uncapped cores were then left to re-equilibrate in darkness over night prior to measurements. To ensure a good exchange between the water phase of the core and the exterior seawater during the re-equilibration period, a Teflon-coated stirrer bar (5 cm length) was suspended ~5 cm above the sediment surface in each core. This was driven by an external magnet rotating at 50 – 60 rpm.

4.2.2 Sediment - water Nutrient fluxes

Rates of nutrient exchange (ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), phosphate (PO_4^{3+}) and silicate (Si^-)) in the dark as well as O_2 and TCO_2 (data presented in chapter 3) were measured on the day after sampling following re-equilibration. The incubations were started by capping the cores and waiting 1 hour prior to making the first measurement. Oxygen and pH were measured by inserting electrodes into the core water sampling port. Water samples for nutrient and alkalinity analysis determinations were taken from the core water by withdrawing 40 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. The water samples were then filtered through Acrodisc 0.45µm cellulose acetate filters into 10 ml screw-cap polypropylene containers; alkalinity samples were stored at 4^{o}C in the dark and analysed within 2 weeks of sampling while nutrient samples were stored at -20^{o}C and were analysed within 3 months of sampling unless otherwise stated in the text. Measurements were taken every 2 – 4 hours depending on the rate of decrease in oxygen saturation. Core incubations were terminated prior to dissolved oxygen (DO) decreasing to 80% saturation of the starting concentration and total incubation times ranged between 8 to 12 hours.

4.2.3 Nutrient Porewater analysis

For porewater extractions, cores were rapidly sectioned under atmospheric conditions at 0.5cm intervals from 0 to 2cm, at 1cm intervals from 2 to 4 cm, and at 2 cm intervals from 4 to 8 cm. Slices were placed in a centrifuge tube and then centrifuged at 2000 rpm for 10 minutes. The water was then extracted and filtered through Acrodisc 0.4 micron filters into 10ml polystyrene tubes and frozen at -20^{o}C for analysis of NH_4^+, NO_2^- and NO_3^-.

4.2.4 Analytical Methods

4.2.4.1 Ammonium

NH_4^+ was analysed using OPA derivatisation and fluorescence detection (Watson et al., 2004). The precision of the method was between 5–8% with a detection limit of 0.07–0.2 µM.

4.2.4.2 Nitrate + Nitrite

Nitrate was measured quantitatively by reducing nitrate to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) was then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion was coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. Nitrate concentrations were obtained by subtracting nitrite values, which have been previously analysed, from the nitrite + nitrate values. The precision of the method was between 2 - 4% with a detection limit of 0.03 – 0.07 µM for nitrate and a precision of between 1 -12% with a detection limit of 0.005 – 0.01 µM for nitrite (Diamond, 1999).

4.2.4.3 Phosphate

Phosphate was analysed by ammonium molybdate and antimony potassium tartrate reacting in an acid medium with phosphate to form an antimony-phospho-molybdate complex. This complex was reduced to an intensely blue-colored complex by ascorbic acid. The color produced is proportional to the phosphate concentration in the sample. The precision of the method was between 1 - 3% with a detection limit of 0.02 – 0.03 µM (Diamond, 1998).

4.2.4.4 Silicate

Soluble silica species react with molybdate at 45 ℃ and a pH of 1.2 to form a yellow Silica molybdate complex. This complex was subsequently reduced with stannous chloride to form a heteropoly blue complex, which has an absorbance maximum at 820 nm. The absorbance is proportional to the concentration of "molybdate reactive" silica. The precision of the method was between 2 - 3 % with a detection limit of 0.05 – 0.07 µM (Wolters, 2003).

4.2.4.5 Total Nitrogen

Inorganic and organically bound nitrogen was converted to soluble nitrate species by digestion with an alkaline potassium persulphate solution in an autoclave. At the start of the digest the solution is alkaline and the breakdown of nitrogen species occurs. The total nitrogen is measured as soluble nitrate. 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 1 - 2% and a detection limit of 0.01µM (Latham, 2000).

4.2.5 Sediment – water flux calculations

The flux across the sediment-water interface was calculated as:

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

Where:

α = linear regression of analyte concentration (corrected for addition of replacement water) versus time in sediment core (µmol l^{-1} h^{-1})
α_w = linear regression of analyte concentration (corrected for addition of replacement water) versus time in "blank" core
V = water column volume (l) and,
A = sediment surface area (m^2).

Fluxes for each replicate core were calculated from the linear regression of the change of an analyte, in this case, alkalinity, over time. This method is commonly used in the literature and is referenced in the Protocol Handbook for Nitrogen Cycling in Estuaries (Dalsgaard et al, 2000). This method was preferred over the alternative

method of calculating fluxes, which is to only measure analyte concentrations at the start and finish of the incubation period. It is generally regarded that obtaining more measurements of analyte concentration over time and establishing a regression is more accurate than only measuring concentrations at the start and finish of an incubation experiment.

However, to ensure the statistical rigour of the flux estimate calculated from the linear regression, the flux was only taken to be significant if the standard error of the slope of the regression line was less than the magnitude of the flux. This approach was taken from Cook et al., (2004II).

4.2.6 Modelled sediment NH_4^+ production rates

Estimates of the upward flux of NH_4^+ within the sediments were calculated according to the method described in Cook et al (2004II). Briefly NH_4^+ fluxes were calculated using Fick's first law:

$$F = -\varphi(D_s + D_e)\frac{\Delta C}{\Delta x}$$

Where φ is porosity, $\Delta C/\Delta x$ is the concentration gradient obtained using linear regression of the linear portion of the concentration profiles (4 to 5 data points), D_e is the enhanced sediment diffusion of solutes and D_s is the sediment diffusivity. D_s was calculated according to Iversen and Jorgensen (1993). An estimate of enhanced diffusion (D_e) was obtained by comparing the measured fluxes of oxygen to those calculated from O_2 micro-profiles (See chapter 3 for more details). D_e was calculated according to Berg et al. (2001):

$$D_e = D_s\left(\frac{F_{meas}}{F_{calc}} - 1\right)$$

Where F_{meas} is the measured flux in intact cores and F_{calc} is the calculated diffusive flux profile assuming $D_e = 0$. 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 Rasmussen and Jorgensen (1992).

4.2.7 Statistical Analysis

The statistical analysis used SPSS version 11. Two-way analysis of variance (ANOVA) was used to test for spatial and temporal differences within and between sampling locations**.** Homogeneity of variances was checked visually by examining residual plots. Data that did not meet this assumption of ANOVA were log transformed. Significant factors were then compared using Tukey's HSD. All statistical tests were tested at $\alpha = 0.05$. Where log-transformed data failed to correct heteroscadisticity, a non-parametric test was used. Correlation and regression analysis were used to explore relationships between variables.

4.3 Results

4.3.1 Physio-chemical water column conditions

Physio-chemical water column conditions are reported in Table 4-1. Salinity was stable at the Lower location, but dropped slightly in winter at the Upper location, reflecting its position in the mixing zone of the estuary and exposure to greater freshwater inflows during winter months. Water temperature followed a temporal pattern, with highest recorded in March (late summer/ early autumn) and lowest in July (winter). Nitrate concentrations were low, reflecting the low nutrient runoff from the catchment, and peaked in winter, most likely derived from the marine end of the estuary (Butler et al., 2000). Ammonium and phosphate concentrations were generally low and variable, reflecting the low inputs to the estuary. Silicate varied over the sampling period and was highest in July at both study locations, when it was most likely derived from the terrestrial end of the estuary (Butler et al., 2000).

Table 4-1 Physico-chemical conditions of bottom water at the Upper and Lower locations during 2004. (nm – not measured)

		Lower	Upper
Water Depth(m)		25 - 37	10 - 19
Salinity (‰)	- Mar	35	35
	- Jul	35	28 - 34
	- Nov	35	34
Temp (^{o}C)	- Mar	15.5	15.7
	- July	10.3	8.0 – 8.2
	- Nov	13.0	12.8
Secchi Depth	- Mar	nm	nm
(m)	- Jul	1.5	<1
	- Nov	2.0	<1
Ammonium	- Mar	0.8	1.3
(µM)	- Jul	0.1	0.3
	- Nov	0.3	3.1
Nitrate	- Mar	0.7	0.6
(µM)	- Jul	5.1	5.0
	- Nov	1.6	1.5
Phosphate	- Mar	0.5	0.3
(µM)	- Jul	0.6	2.0
	- Nov	0.4	0.5
Silicate	- Mar	3.8	1.5
(µM)	- Jul	9.0	18.1
	- Nov	5.3	5.8

4.3.2 Sediment nutrient fluxes

Fluxes of dissolved inorganic nitrogen (DIN) [$NH_4^+ + NO_2^- + NO_3^-$] were always directed out of the sediment, with the highest mean flux rate recorded in March at UW (18.1 ± 9.0 µmol m^{-2} h^{-1}) and the lowest mean flux rate recorded in July at UM (0.9 ± 4.7 µmol m^{-2} h^{-1}). DIN fluxes were found to be significantly different between the 3 sampling periods (Kruskal-Wallis, $P < 0.05$). Mann-Whitney pair-wise comparisons (Table 4-2) found that March had significantly higher flux rates compared to July ($P = 0.00001$) and to November ($P = 0.010$). July had significantly lower flux rates compared to November ($P = 0.00001$).

Table 4-2 Mann-Whitney pair-wise comparisons between the three sampling times, March, July and November and DIN fluxes

(a)Time	**(b) Time**	**Sig.**
March	July	0.000
	November	0.010
July	March	0.000
	November	0.000
November	March	0.010
	July	0.000

Kruskal-Wallis analysis was also used to determine differences across spatial scales. No significant difference was found between stations at either the lower (P = 0.2202) or the upper (P = 0.4158) locations. All the data from the three stations was then pooled for each of the locations and the two locations were compared on an annual basis. The annual mean flux at the upper and lower locations was not significantly different (P = 0.5913).

Mean fluxes of ammonium were generally directed out of the sediment during March with the exception of site LC (Figure's 4-1 & 4-2). During July all sites except LE had mean ammonium fluxes directed into the sediment. In November, all sites at the lower estuary had small fluxes directed out of the sediment, but all sites at the upper estuary location ammonium were directed from the water column to the sediments. During the course of the study, none of the ammonium fluxes differed significantly between stations at the upper location (Kruskal-Wallis, p = 0.8394) while at the lower location, LC and LE were significantly different (Mann-Whitney, p = 0.0170). The highest ammonium efflux rate across all sites during the sampling period occurred at site LE during March with a mean flux of 6.4 ± 3.7μmol m^{-2} h^{-1}out of the sediment.

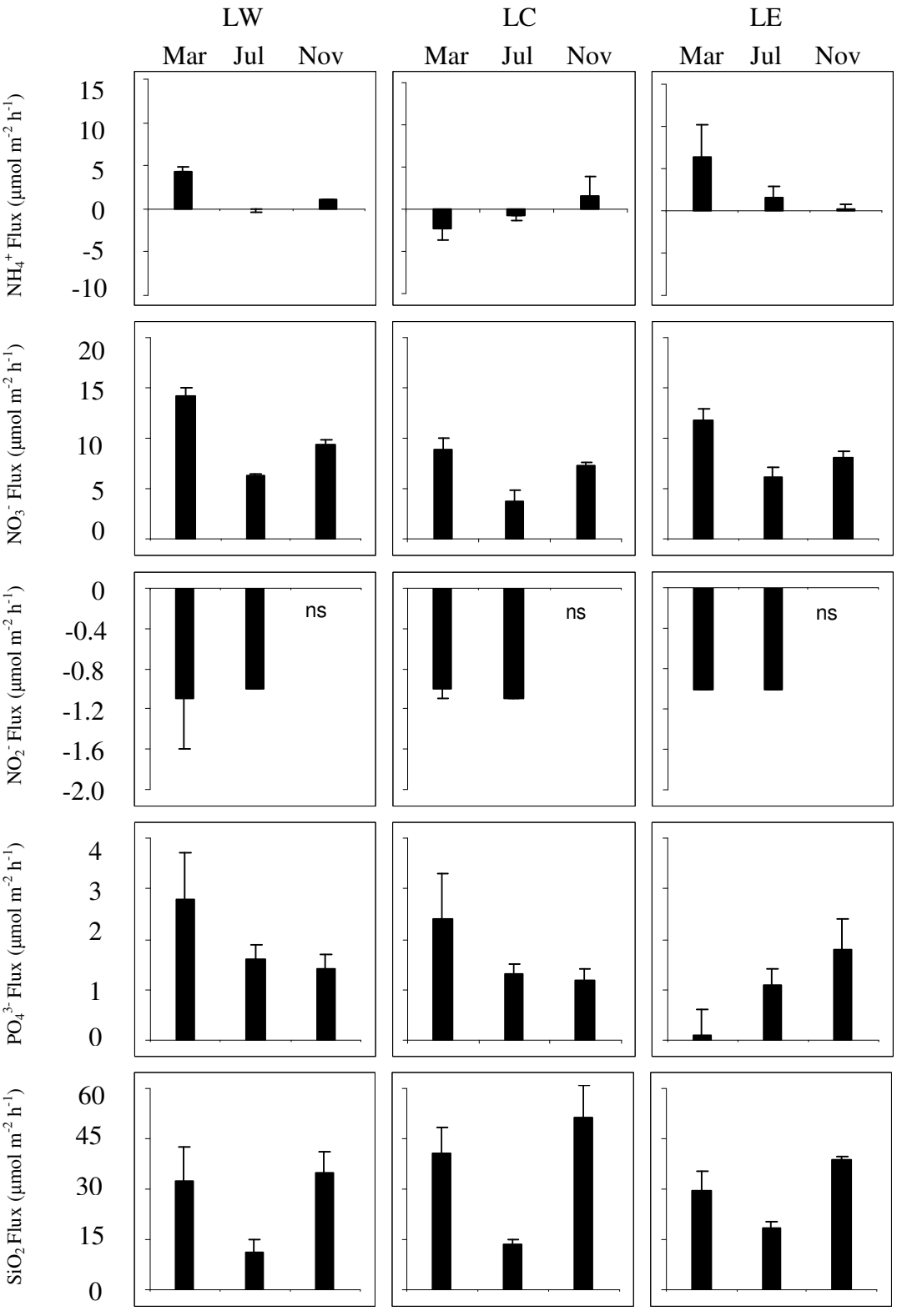


Figure 4-1 Fluxes of NH_4^+, NO_2^-, NO_3^-, $PO_4^{3-}$and Si^- at the sediment – water interface at the three sample stations during March, July and November at the Lower estuary sites of the Huon estuary. Error bars represent the standard error of the mean (n=2-3). N.B. where no error bars are present, the data only represents one flux result. ns = fluxes not significant because of erratic changes over the incubation period.

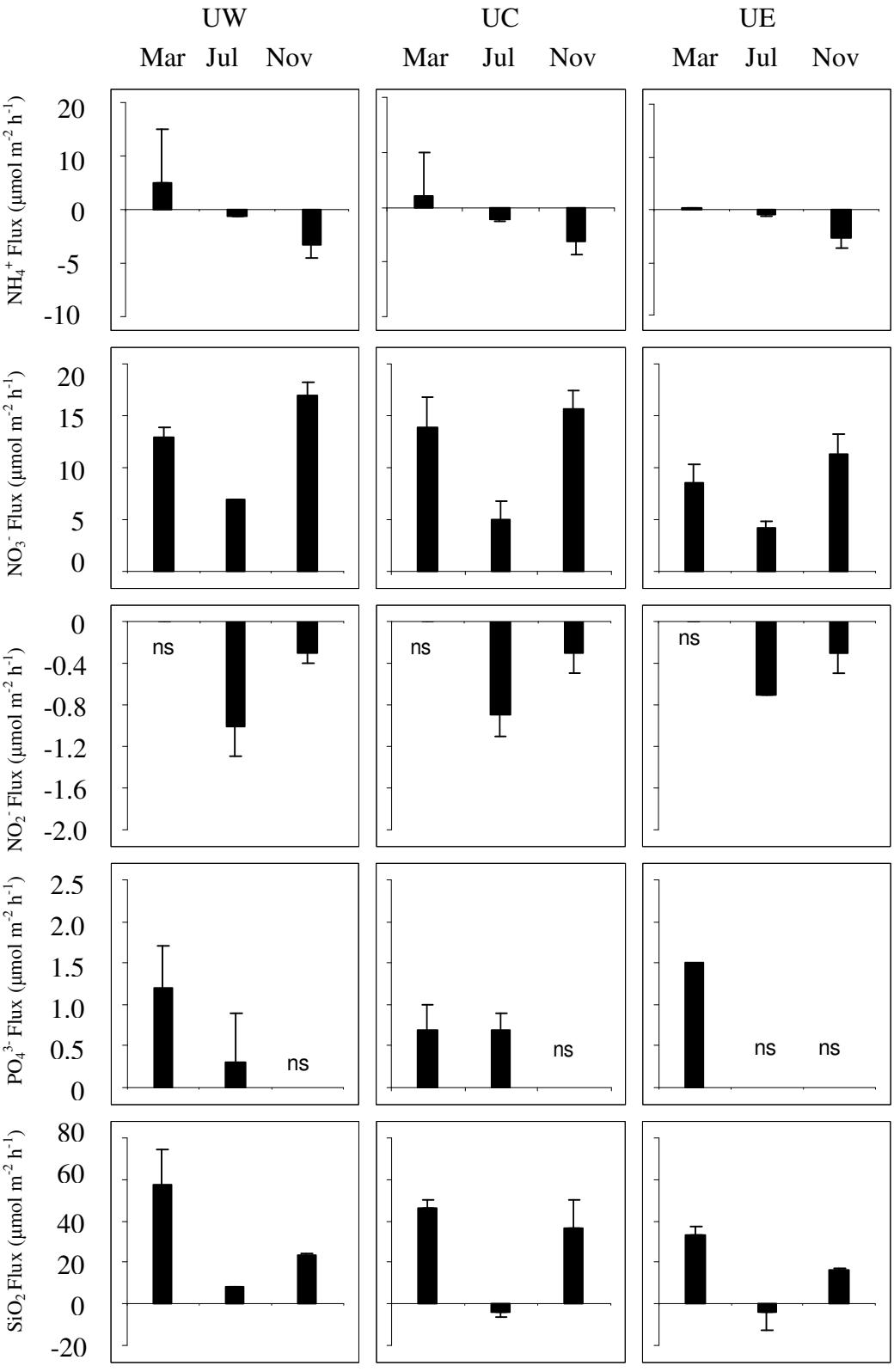


Figure 4-2 Fluxes of NH_4^+, NO_2^-, NO_3^-, $PO_4^{3-}$and Si^- at the sediment – water interface at the three sample stations during March, July and November at the Upper estuary sites of the Huon estuary. Error bars represent the standard error of the mean (n=2-3). N.B. where no error bars are present, the data only represents one flux result. N.S = fluxes not significant because of erratic changes over the incubation period.

Nitrate was always directed out of the sediment (Figure's 4-1 & 4-2) and was the dominant form of DIN flux (mean range 3.7 to 17 μmol m^{-2} h^{-1}). Nitrite was a small component of DIN with a flux of ≤ 1 μmol m^{-2} h^{-1} and always directed into the sediment. The highest nitrate efflux rate across all sites during the sampling period occurred at site UW during November with a mean flux of 17.0 ± 1.2μmol m^{-2} h^{-1}out of the sediment. Two-way analysis of variance (ANOVA) was used to compare flux rates with time and location as fixed factors and significant interactions between Location x Station ($F = 5.982$, df = 2,30; $P = 0.007$) and between location x time ($F = 10.576$, df = 2,30, $P = 0.000$) were found. Table 4-3 displays the occurrence of significantly different results.

Table 4-3 ANOVA table for nitrate fluxes for the sampling period March 2004 – November 2004 (data was log transformed)

Source	df	Mean Square	F	Sig. (p)
Location	1	51.81	9.833	**0.004**
Station	2	25.08	4.760	**0.016**
Time	2	140.69	26.700	**0.000**
Location x Station	2	31.52	5.982	**0.007**
Location x Time	2	55.73	10.576	**0.000**
Station x Time	4	3.32	0.631	0.644
Location x Station x Time	3	5.70	1.082	0.372
Error	30	5.27		
Total	47			

New variables were defined for the significant interactions found in the two-way ANOVA. Tables 4-4 and 4-5 show post hoc tests of station/location and time/location combinations respectively. Table 4-4 shows that station UW and UC were the most different from all others. Table 4-5 shows that nitrate fluxes measured in July at both locations were significantly smaller compared with the other sampling times, but not significantly different too each other. Also, nitrate fluxes measured in March were not significantly different when comparing fluxes at the two locations. Table 4-5 also

shows the nitrate fluxes measured in November at the Upper location was not significantly different to measurements taken in March.

Table 4-4 Tukey HSD Homogenous subsets for station/location combinations for nitrate fluxes. The station/location variables include 11 (west/lower), 21 (central/lower), 31 (east/lower), 12 (west/upper), 22 (central/upper) and 32 (east/upper).

		Subset		
Station/Location	**N**	**1**	**2**	**3**
21	8	6.9375		
32	8	7.9375		
31	9	8.6444		
11	8	10.3875	10.3875	
22	8		12.3500	12.3500
12	6			14.9833
Sig.		.061	.555	.245

* The mean difference is significant at the 0.05 level

Table 4-5 Tukey HSD Homogenous subsets for time/location combinations for nitrate fluxes. The time/location variables include 11 (march/lower), 21 (july/lower), 31 (november/lower), 12 (march /upper), 22 (july /upper) and 32 (november /upper).

		Subset			
Time/Location	**N**	**1**	**2**	**3**	**4**
22	5	4.5400			
21	7	5.5143	5.5143		
31	9		8.2000	8.2000	
11	9			11.5556	11.5556
12	8				12.1625
32	9				14.6889
Sig.		.961	.240	.080	.118

* The mean difference is significant at the 0.05 level

Fluxes of silicate were directed out of the sediment at all sites with the exception of sites UM and UE during winter (Figure 4-1 & 4-2). Two-way ANOVA was used to compare flux rates with time and location as fixed factors. A significant interaction was found between location and time ($F = 4.976$, $df = 2,28$, $P = 0.014$). Table 4-6 displays the occurrence of significantly different results.

Table 4-6 ANOVA table for silicate fluxes for the sampling period March 2004 – November 2004 (data was log transformed)

Source	df	Mean Square	F	Sig. (p)
Location	1	2.64	1.950	0.174
Station	2	1.98	1.462	0.249
Time	2	19.79	14.619	**0.000**
Location x Station	2	1.68	1.239	0.305
Location x Time	2	6.74	4.976	**0.014**
Station x Time	4	0.95	0.698	0.600
Location x Station x Time	3	0.20	0.146	0.932
Error	30	1.35		
Total	47			

New variables were defined for the significant interaction found in the two-way ANOVA. Table 4-7 silicate fluxes measured in March at the Upper location were significantly smaller compared to the other sampling times except for silicate fluxes measured at the Lower location, also in July. Table 4-7 also shows that silicate fluxes measured in March and November were not significantly different.

Table 4-7 Tukey HSD Homogenous subsets for time/location combinations for silicate fluxes. The time/location variables include 11 (march/lower), 21 (july/lower), 31 (november/lower), 12 (march /upper), 22 (july /upper) and 32 (november /upper).

		Subset		
Time/Location	**N**	**1**	**2**	**3**
22	2	.8271		
21	8	1.1210	1.1210	
32	9		1.3521	1.3521
11	9			1.4917
31	9			1.6002
12	8			1.6416
Sig.		.140	.366	.151

* The mean difference is significant at the 0.05 level

Fluxes of phosphate were directed out of the sediment with mean rates always < 3µmol m^{-2} h^{-1}. No phosphate fluxes at the lower estuary sites during summer were reported due to erratic changes in concentration over the incubation period leading to non-significant flux results. DON concentrations were small with changes over time generally non-linear, leading to insignificant flux results, so they were not reported.

4.3.3 Porewater profiles

Ammonium concentrations at all sites increased down the profiles to a maximum between 20 and 40 µM at a depth of 4cm before decreasing again (Figure 4-3). Calculated rates of ammonium upward fluxes were generally low, with the highest rate recorded at UW (5.7±1.2 µmol m^{-2} h^{-1}) and the lowest rate recorded at Garden Island in April 2005 (1.9 ±1.2 µmol m^{-2} h^{-1}).

Nitrate concentrations (Figure 4-4) generally peaked in the top centimetre of the sediments, within the oxygenated zone for all sites Nitrate concentrations were highest in the Lower estuary sites with LW recording 12.1 (±1.5) µM in the top centimetre. The highest concentration in the Upper estuary sites was 3.5 (±0.6) µM at UM. Nitrate concentrations in the surface sediments were greater than nitrate concentrations in the water column (see Table 4-1). No nitrite was detected in the porewaters.

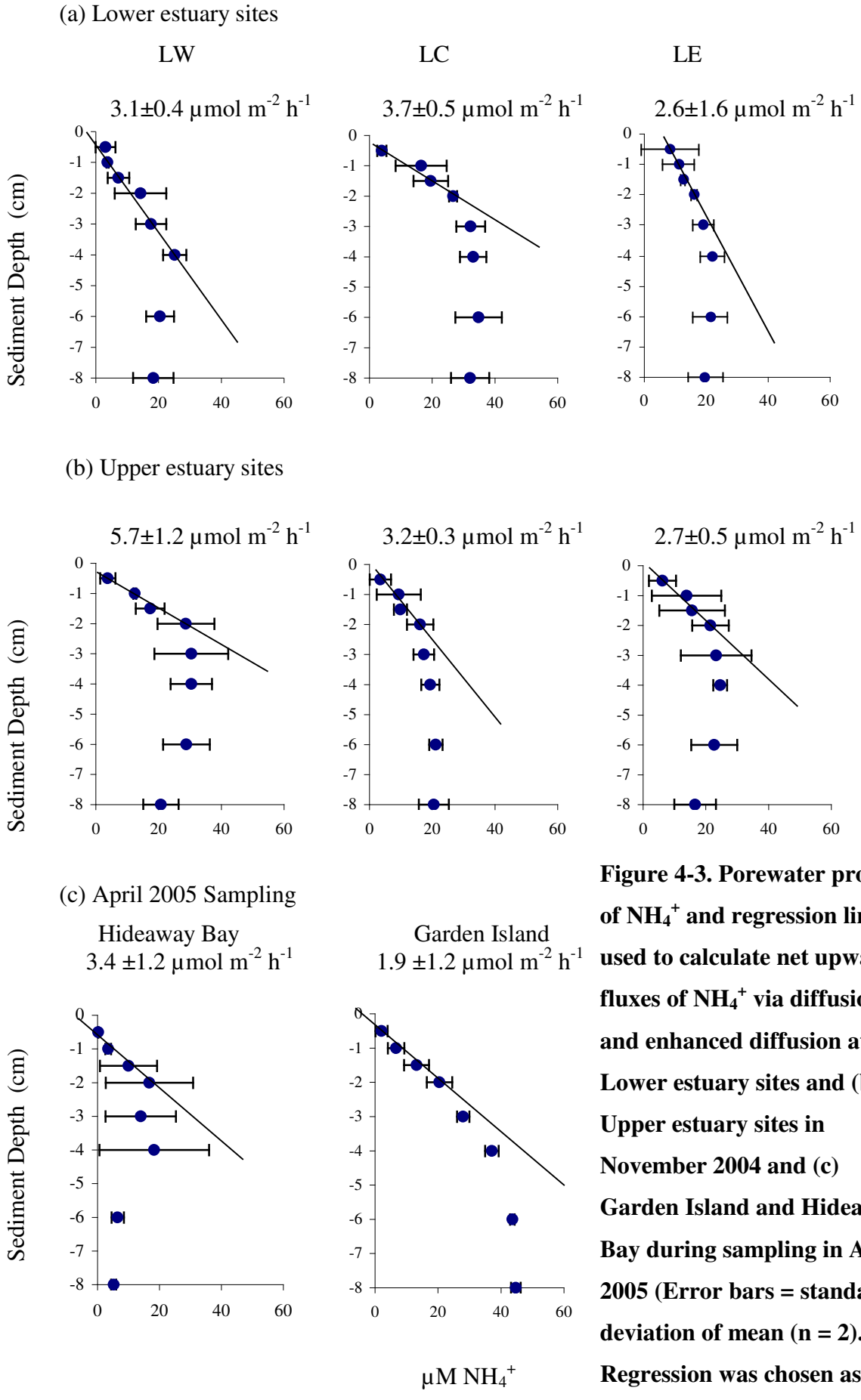
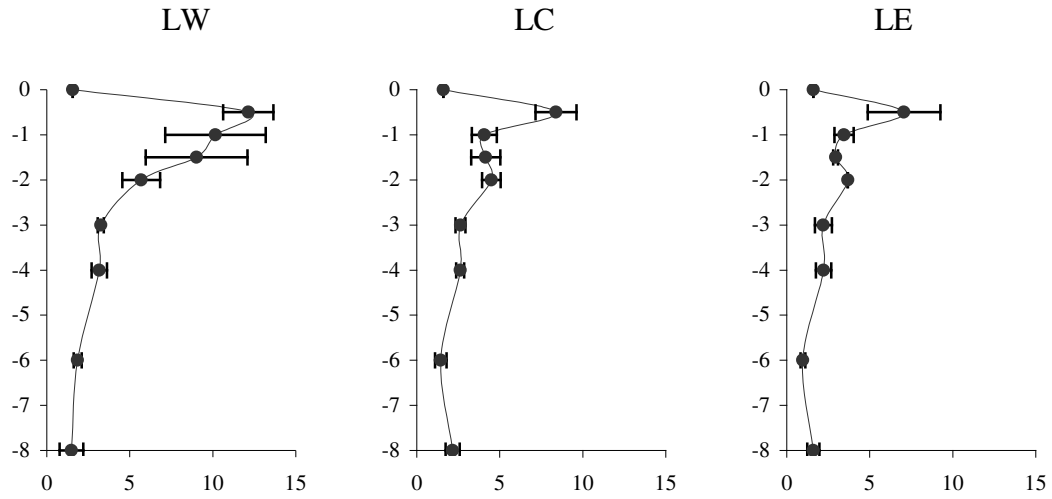
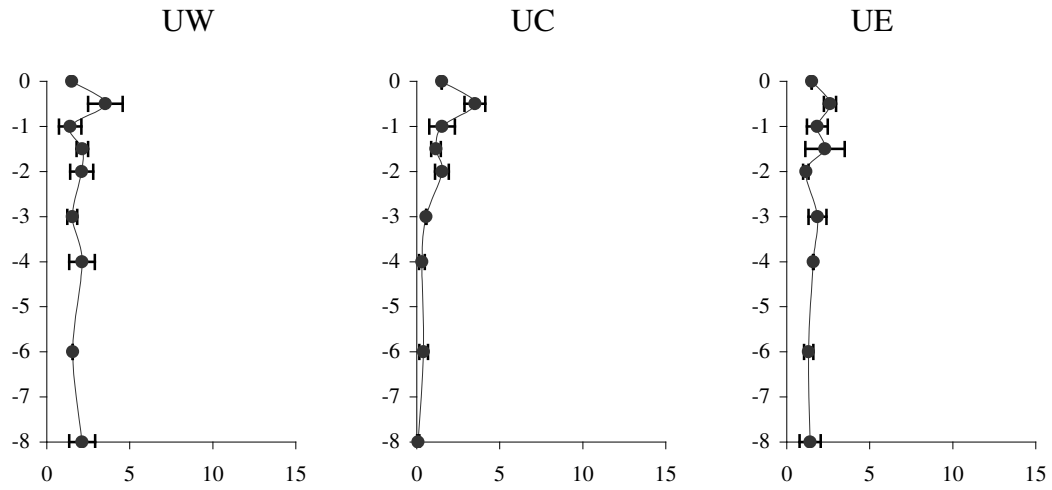


Figure 4-3. Porewater profiles of NH_4^+ and regression lines used to calculate net upward fluxes of NH_4^+ via diffusion and enhanced diffusion at (a) Lower estuary sites and (b) Upper estuary sites in November 2004 and (c) Garden Island and Hideaway Bay during sampling in April 2005 (Error bars = standard deviation of mean (n = 2). Regression was chosen as the best fitting line according to R^2.

a) Lower estuary stations



b) Upper estuary stations



(c) April 2005 Sampling

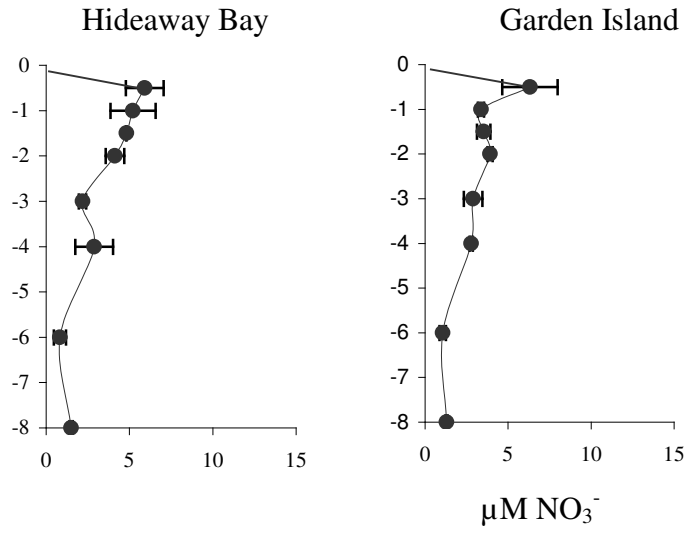


Figure 4-4 Porewater profiles of NO_3^- at (a) Lower estuary sites and (b) upper estuary sites in November and (c) Garden Island and Hideaway Bay during sampling in April 2005 (Error bars = standard deviation of mean (n = 2)

4.4 Discussion

4.4.1 Spatial variability of nutrient fluxes

Benthic nutrient fluxes often have large spatial variability, which may occur due to the presence or absence of benthic fauna which irrigate or bioturbate the sediments (Glud et al., 2003; Kristensen, 2000; Caffrey, 1995), the quantity and source of organic matter in the sediments (Hall et al., 1996; Forja et al., 1994), the sediment texture (Caffrey, 1995) and the presence of benthic algae (Sundback et al., 1991; Caffrey, 1995; Magalhaes et al., 2002; Cook et al., 2004II). All these factors can have a significant effect on nutrient fluxes across the sediment-water interface. In this study, spatial variability was examined across a number of scales including sediment cores <10m apart (cores within a station), cores 0.5 - 2km apart (between stations in the same location) and cores >20 km apart (between the two locations).

Small-scale nutrient flux variability was examined by calculating the coefficient of variation (SD/mean) as a measure of precision between replicate cores at the same sampling station. Table 4-8 shows the coefficients of variation for each type of nutrient flux across all stations and during all sampling times. The results show that ammonium fluxes demonstrated considerable spatial heterogeneity in values, but that all the other fluxes were, with one or two exceptions, less variable in time and space.

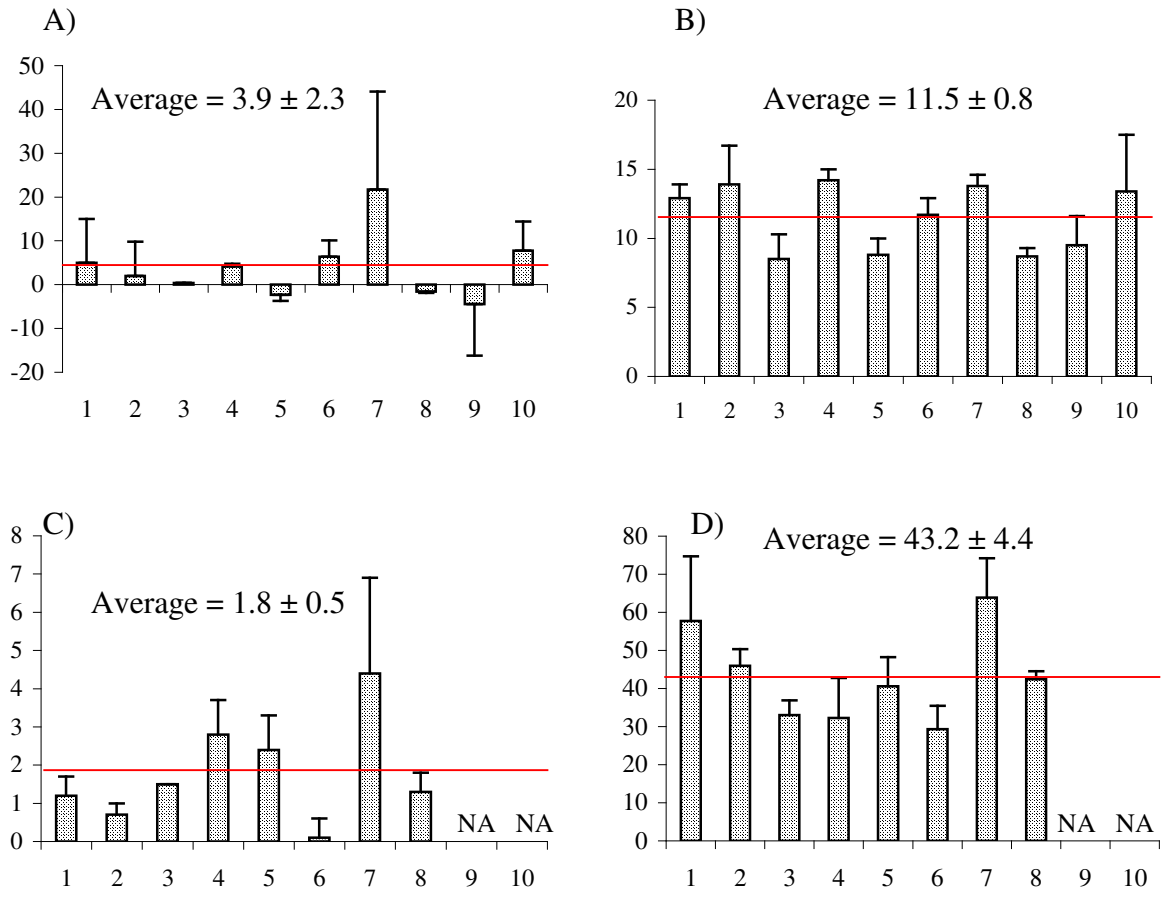
Ammonium fluxes were highly variable both between cores within a station and between cores within a location (Table 4-8). This high variability was caused by some cores releasing ammonium to the overlying water column while other cores at the same station or location consumed ammonium from the water column as indicated by negative fluxes. While the exact causes of this variability have not been identified, likely causes are: the presence or absence of fauna in the cores during incubation, the variable nature of ammonium retention in the sediments due to adsorption onto sediment particles, ammonium uptake by bacteria, nitrification and the presence of ‘hot spots’ of organic carbon on the sediment surface.

Table 4-8 Coefficients of variation of nutrient fluxes within each station. Values are given for replicate cores at each site during each sampling time and are reported as percentages. The closer the values are to zero, the greater the precision of the flux measurements. Cores from each site within each location were also pooled and precision values given for each sampling time.

Flux	LW	LC	LE	UW	UC	UE	Lower Sites Combined	Upper Sites Combined
DIN								
March	17.5	82.1	34.2	86.3	29.6	54.1	52.7	61.2
July	125.8	133.8	48.6	363.7	521.0	39.6	86.0	193.3
November	12.6	47.4	28.5	38.4	9.4	31.0	28.1	35.5
Ammonium								
March	22.7	105.5	81.3	344.4	535.5	122.9	198.7	325.1
July	424.3	88.4	122.6	12.9	9.0	12.9	693.5	43.4
November	739.9	242.6	-	63.4	68.5	59.5	244.6	56.6
Nitrate								
March	9.9	24.5	17.5	12.9	34.5	29.3	24.9	30.4
July	4.5	45.9	25.0	-	48.1	24.8	30.5	33.1
November	9.6	6.8	24.4	12.0	20.1	31.3	17.3	25.0
Nitrite								
March	76.4	14.8	-	-	-	-	45.9	-
July	5.6	6.7	5.6	37.2	35.3	7.9	5.0	27.8
November	-	-	-	66.7	115.5	84.9	-	79.8
Phosphate								
March	612.8	51.2	23.1	52.0	60.6	-	86.6	48.7
July	33.1	21.8	50.6	311.1	32.6	-	36.2	115.5
November	26.2	22.0	47.1	-	-	-	34.5	-
Silicate								
March	56.3	32.5	37.0	50.9	16.2	16.7	39.5	40.9
July	60.9	15.7	17.6	-	69.0	346.1	36.8	569.0
November	31.5	32.1	25.4	11.4	62.4	17.1	32.2	58.5

Broader spatial variability within the Huon estuary and surrounding bays was also examined by including data from a separate study conducted in 2005 from Hideaway bay and Garden island and from Stringers cove during a 2001 – 2 study (see map in Figure 1.3 in chapter 1). Nitrate and silicate fluxes had relatively high precision, with coefficients of variation of 20.8% and 28.7% respectively, in comparison, ammonium and phosphate fluxes were highly variable with coefficients of variation equal to 188.8% and 75.5% respectively (Figure 4-5).

The relatively high precision of nitrate and silicate fluxes suggest that sediments are functioning in a similar way across the estuary, while the highly variable ammonium fluxes suggest sediments are functioning quite differently. The presence of benthic fauna does not explain this variability as no significant correlation was found between macro-fauna abundance and ammonium fluxes. It could also be expected that nitrate and silicate fluxes should have greater variability then observed if there was significant bioturbation. Benthic algae can also be ruled out as a source of variability as experiments from chapter 3 indicated that no photosynthetic activity occurred at the surface of the sediments. Also the Huon estuary has extremely limited light penetration due to highly turbid waters and therefore severely inhibits benthic algal activity.



Number	Location	Station	Sampling date	Temperature
1	Upper Huon Estuary	UE	06/03/2003	15.6
2	Upper Huon Estuary	UC	06/03/2003	15.6
3	Upper Huon Estuary	UW	06/03/2003	15.6
4	Lower Huon Estuary	LE	01/03/2003	15.7
5	Lower Huon Estuary	LC	01/03/2003	15.7
6	Lower Huon Estuary	LW	01/03/2003	15.7
7	Hideaway Bay	P3	12/04/2005	15.7
8	Garden Island	P4	12/04/2005	15.7
9	Stringers Cove (Macleod et al 2004)	R2	22/02/2001	~16.0
10	Stringers Cove (Macleod et al 2004)	R2	18/02/2002	~16.0

Figure 4-5. Spatial patterns of benthic nutrient fluxes A) Ammonium, B) Nitrate, C) Phosphate, D) Silicate during late summer / early autumn. Temperatures ranged between 15 – 16^{o}C for all sampling periods. (n.d.= no data). Redline indicates average flux. Fluxes = µmol m^{-2} h^{-1}. Error bars reflect the standard error of the mean.

The high variability in phosphate and ammonium fluxes could be explained by the removal of phosphate (Hall et al., 1996) and ammonium (Mackin and Aller, 1984; Blackburn, 1986) by adsorption in oxidised sediments, in particular by clay (Hall et al., 1996). Ammonium may also be removed via nitrification (Nedwell et al., 1993; Blackburn and Henriksen, 1983) or incorporated into bacterial biomass through assimilation during the decomposition of organic matter (Blackburn, 1986; van Duyl et al., 1993; Lomstein et al., 1998). Oxygen concentration can also play a major regulatory role in benthic phosphate release (Sundby et al., 1986) and in nitrification. However, the Huon estuary bottom water is generally well oxygenated throughout the year (Butler et al., 2000) as are the surficial sediments. During this study, oxygen penetration depths of 3 – 9mm were observed, comparable to depths obtained by Macleod et al. (2004) who reported a range of 4.7 – 7.1 mm for the oxic zone in unimpacted sites near the Huon Estuary. All this would suggest that dissolved oxygen was not sufficiently variable to account for the observed changes in ammonium or phosphate fluxes.

4.4.2 Temporal variability of nutrient fluxes

A change in water temperature can alter flux rates by causing a change in the rate of microbially mediated organic matter remineralisation (Klump and Martens, 1989). Several studies have found that temperature has a significant effect on nutrient fluxes including studies undertaken in Port Phillip Bay (Nicholson and Longmore, 1999), in the Bay of Cadiz (Forja et al., 1994) and in Alfacs Bay (Vidal et al., 1997). In addition to temperature effects, temporal variability of nutrient fluxes may also be attributed to the annual supply and source of organic matter (Caffrey et al., 1995). Previous findings by a number of researchers has shown that spring phytoplankton blooms can often be associated with increased nutrient flux rates (Berelson et al., 2003; Caffrey, 1995; Hammond et al., 1985)

Temporal trends of nutrient fluxes during this study were different at both study locations and for each of the nutrients measured. At the lower estuary stations, ammonium fluxes were generally directed out of the sediment in both March and November, with highest effluxes in March, whilst ammonium effluxes were generally directed into the sediments in July. Silicate fluxes however were highest in November

and lowest in July. In contrast, fluxes of ammonium at the upper stations were directed out of the sediments only in March, and were directed into the sediments during both July and November, with the highest uptake rates observed in November. In November at the upper location stations, the relatively high uptake rates of ammonium coincided with the largest nitrate fluxes observed during the study. The relatively large nitrate effluxes and uptake of ammonium by the sediments suggests that nitrification may have been an important process at this time (November). Potentially nitrifiers may have being limited by ammonium production in the sediments and therefore obtained the rest of their ammonium requirements from the water column. Interestingly, the high ammonium uptake rate relative to the rest of the ammonium fluxes observed during this study, occurred when ammonium concentrations in the water column were highest (3 μM). This may suggest that sediments have the potential to act as net sinks of ammonium when concentrations in the water column are elevated and therefore may provide an important regulatory mechanism for ammonium levels in the estuary.

The temporal trends in the data were further explored by running correlations between temperature and nutrient fluxes (Table 4-9) on both the lower and upper stations. Significant correlations were found between temperature and nitrate and DIN at the lower stations and for nitrate, DIN and silicate at the upper stations and therefore may explain most of the difference between fluxes across the different study times. Temperature however could not explain differences in ammonium and phosphate fluxes with no significant correlation between these two variables and temperature found (Table 4-9).

In addition to temperature, correlations were also run between organic carbon contents in the surface sediments and nutrient fluxes. No significant correlations were found between sediment organic carbon contents and nutrient fluxes and therefore temporal variability of the nutrient fluxes observed during this study could not be attributed to the organic content of the sediments.

Table 4-9. Correlations between temperature and nutrient fluxes at both the Lower and Upper locations

Location	Variable	R^2	P value
Lower	Nitrate	0.85	Significant at 0.01 level (2-tailed)
	Ammonium	0.42	Not Significant
	DIN	0.75	Significant at 0.05 level (2-tailed)
	Silicate	0.65	Not Significant
	Phosphate	0.29	Not Significant
Upper	Nitrate	0.68	Significant at 0.05 level (2-tailed)
	Ammonium	0.36	Not Significant
	DIN	0.93	Significant at 0.01 level
	Silicate	0.91	Significant at 0.01 level (2-tailed)
	Phosphate	0.48	Not Significant

4.4.3 Nutrient Cycling

4.4.3.1 Nitrogen

The ratio of measured fluxes TCO_2:DIN can be used as an indicator of DIN reactions within sediments assuming that organic material undergoing degradation on the sea floor conforms to Redfield proportions (Berelson et al., 2003). If it does, then it is expected that resultant fluxes of carbon and nitrogen would have a ratio approximating 6.6C:1N (Berelson et al., 2003; Giblin et al., 1997). During this study, the ratio of TCO_2 (used as a proxy for oxidised organic carbon) to DIN flux strongly deviated from the expected. The measured ratios were always above 6.6 and had a range between 14.4 and 724.7 (average 45.4) at all stations, including the samples taken at Hideaway bay and Garden Island (Figure 4-6a).

The higher than expected ratios suggest that a large portion of DIN released during organic matter decomposition is retained in the sediments. Assuming TCO_2 flux reflects remineralisation of Redfield material, it was calculated that 54 – 99% (average 85%) of the nitrogen remineralised in sediments at all stations was not released to the overlying water as DIN.

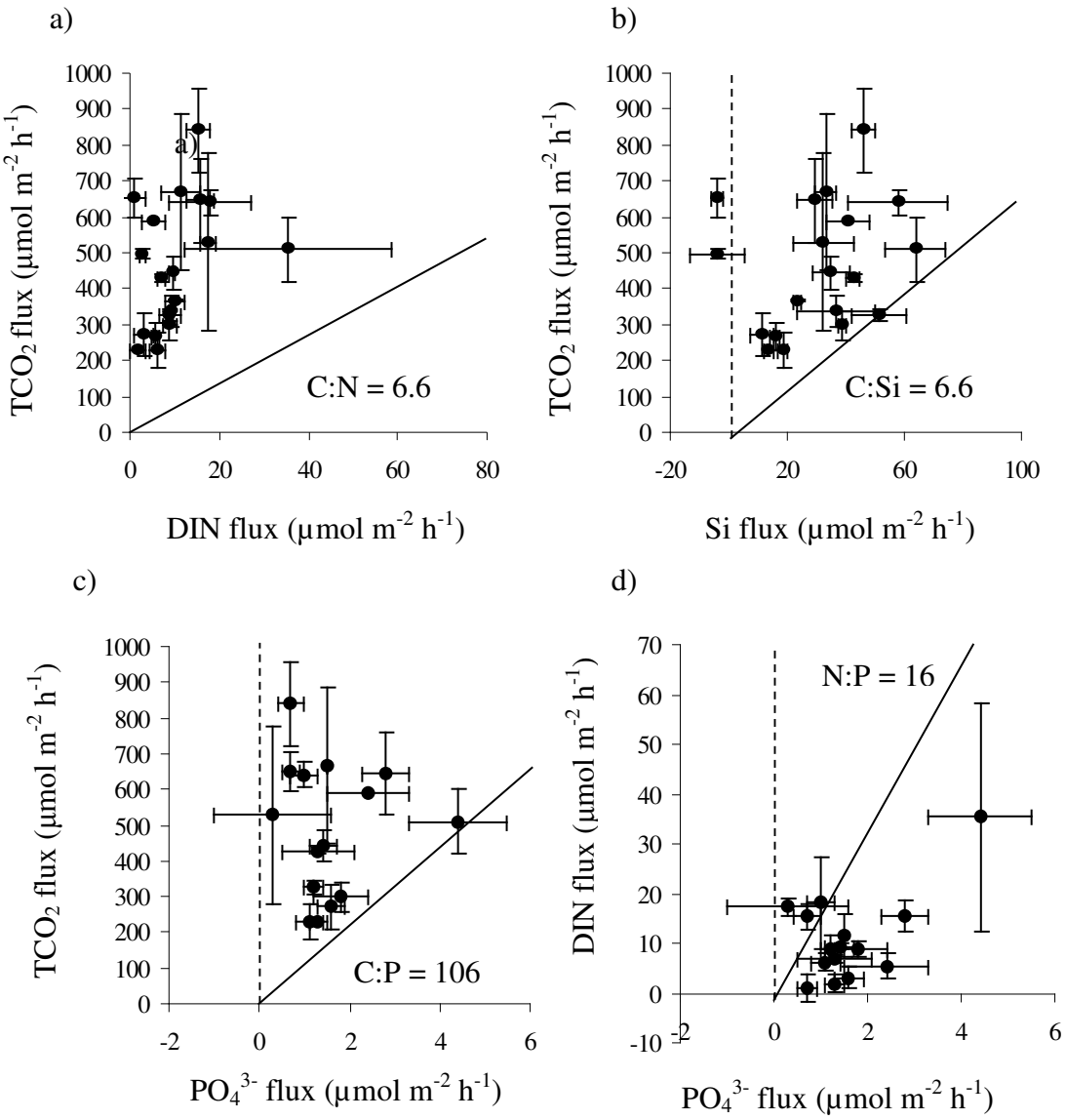


Figure 4-6. Nutrient flux stoichiometry. Line represents the Redfield ratio. Error bars = SE of mean (n = 2 – 3)

One possible mechanism that may account for this 'missing' nitrogen is the conversion of ammonium to nitrate via nitrification and then nitrate converted to di-nitrogen gas (N_2) via denitrification (Hulth et al., 2005). On all occasions during this study, the DIN flux was dominated by nitrate, which was always released from the sediment to the overlying water. Nitrite fluxes were always taken up by the sediment from the overlying water, while ammonium fluxes were erratic, but almost always smaller then nitrate effluxes. Aerobic conditions were prevalent in the upper 3 – 9mm of the sediment during this study (refer chapter 3) providing an environment

conducive for aerobic processes like nitrification. Peaks of nitrate within the aerobic zone observed from nitrate pore water profiles (Figure 4-4) also provided evidence that nitrate is produced in the oxic zone. All these observations suggest that the effluxes of nitrate and influxes of nitrite and ammonium in Huon estuary sediments are associated with intensive nitrification (Hall et al., 1996) stimulated by the presence of relatively deep oxygenated zones.

Numerous studies have found that a large portion of nitrate produced in sediments is denitrified resulting in the release of N_2 into the overlying water column. Seitzinger (1987) found that 80% of the nitrate produced in Ochlockonee Bay was denitrified. Jenkins and Kemp (1984) observed tight coupling between nitrification and denitrification in the Patuxent River where over 99% of nitrate produced in sediments was reduced to N_2 while Henriksen et al. (1981) found approximately 50% of the nitrate produced was reduced to N_2. Macleod et al. (2004) observed that denitrification accounted for 39 to 60% of total nitrogen fluxes in Stringers Cove, which is adjacent to the Huon estuary and has similar sediment geochemistry.

Direct denitrification measurements were not made during this study however denitrification rates (DR) estimates can be made using the following equation (Geoscience Australia, 2007):

$$DR = TDIN_p - DIN_m$$

Where $TDIN_m$ is the measured dissolved inorganic nitrogen flux and $TDIN_p$ is the total dissolved inorganic nitrogen flux predicted on the basis of stoichiometric relationship with benthic carbon dioxide flux. This is calculated by the following equation (Geoscience Australia, 2007):

$$TDIN_p = C_{ox} \times (N:C)$$

Where C_{ox} is benthic carbon dioxide flux and N:C is the stoichiometric relationship of the decomposing organic matter (C:N ratios used to model denitrification were 11.1 and 17.2 at the lower and upper estuary locations respectively).

The denitrification efficiency (DE) of the sediments can also be calculated as (Geoscience Australia, 2007):

$DE\% = (TDIN_p - DIN_m) \times 100 / TDIN_p$

Figure 4-7 shows calculated denitrification rates and efficiencies during the current study. Denitrification rates were estimated to be highest in July at the upper location and highest in March at the lower location. Denitrification efficiencies were also estimated to be high, between 55 and 94%, and were most efficient during July when nitrate concentrations were elevated in the water column. These estimates of denitrification imply that most of the DIN is ultimately converted to N_2.

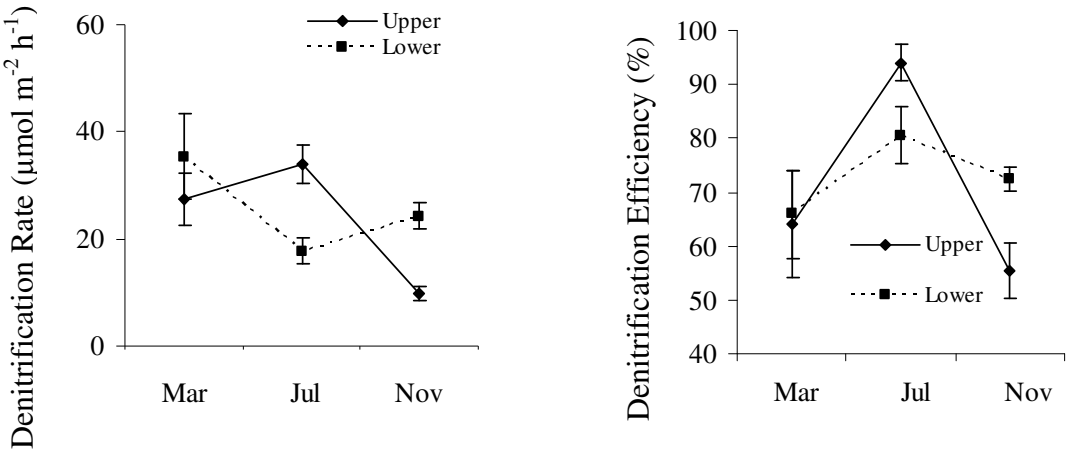


Figure 4-7. Calculated denitrification rates and denitrification efficiencies during the current study at the upper and lower locations

Support for the case for nitrification-denitrification occurring in Huon Estuary sediments is given by the porewater profiles of oxygen (Figure 3-5 & 3-6) and NO_3^- (Figure 4-4). Nitrate was produced in the oxic zone of the sediments indicating the presence of nitrification, which is generally considered an aerobic process. Furthermore, nitrate penetrates into the suboxic zone of the sediments where it is consumed, presumably by the denitrifiers. This supports the classical view of coupled nitrification-denitrification where nitrification takes place in the upper oxic zone of

the sediments and denitrification in the suboxic zone just below the oxic/suboxic interface (Hulth et al., 2005).

However an alternative pathway to this classical view is that of anaerobic ammonium oxidation (ANAMMOX) which bypasses the aerobic nitrification phase. ANAMMOX is the oxidation of ammonium coupled to the reduction of NO_2^-, which results in the production of N_2 (Trimmer et al., 2003). Anaerobic ammonium oxidation has recently been reported to account for as much as 24 and 60% of N_2 formation in continental shelf sediments while as low as 2% of N_2 formation in eutrophic shallow coastal bay sediments (Thamdrup and Dalsgaard, 2002). It is not possible to determine the relative proportions of ANAMMOX and denitrification occurring in these sediments, however given that ANAMMOX has now being detected in marine sediments, future work on nitrogen cycling in the Huon estuary should involve determining the relative contributions of ANAMMOX and denitrification.

The stoichiometric approach used in the study however has some limitations as it assumes that the missing nitrogen from the DIN has been denitrified, when it maybe assimilated by bacteria (Lomstein et al., 1998) or benthic algae in the sediment. However, the latter can probably be ruled out as no benthic algal activity was detected in the sediments (see chapter 3). Another assumption made using the stoichiometric approach is that the organic matter being remineralised conforms to the Redfield ratio. However, if the C:N ratio is significantly higher, then this assumption will overestimate the predicted DIN release and therefore the denitrification rate.

The calculated denitrification rates in this study are high compared with results from a study by Macleod et al. (2004) in which observed mean denitrification rates ranged between 8 – 19 $\mu mol\ m^{-2}\ h^{-1}$ in unimpacted sediments at Stringers Cove adjacent to the Huon estuary. The surface sediments in Stringers Cove have similar organic matter content, oxygen penetration depth and DIN, nitrate and ammonium fluxes to those measured in the Huon estuary here. Therefore the calculated denitrification rates in this study are questionable and most likely overestimate actual rates of denitrification and therefore future work should incorporate direct measurements of denitrification.

DON fluxes may also account for some of the missing nitrogen, however they are unlikely to be significant. In recent studies, it has been shown that DON fluxes are only significant when there is a high deposition of fresh phytoplankton (Cook et al., 2004II and references therein; Sloth et al., 1995). During this study, no algal blooms were apparent during sampling periods and results from lipid biomarkers in chapter 2 suggested that phytoplankton accounted for only a small proportion of organic matter in the sediments. DON fluxes in this study were considered to be insignificant because changes in DON concentrations overtime were small and non-linear. This leads to the conclusion that DON fluxes are not likely to be a significant source of the missing nitrogen flux in this case.

To summarise then, the missing nitrogen flux is most likely due to coupled nitrification and denitrification or bacterial nitrogen assimilation, although ANAMMOX may also play an important role. Future work should therefore be undertaken to determine the occurrence and rates of these processes in order to get a more comprehensive view of nitrogen cycling in Huon estuary sediments.

4.4.3.2 Phosphate

TCO_2 to PO_4^{3-} flux ratios (Figure 4-6c) indicate strong phosphate retention is occurring in sediments at all sites throughout this study. The flux ratios ranged between 116– 1762, which is higher than predicted by the 106:1 Redfield ratio. As suggested earlier, one possible reason for this to occur is that the efflux of remineralised phosphate is controlled in the surface oxic layer by sorption to iron oxyhydroxides (Hopkinson et al., 2001) and is only released when iron (III) is reduced to iron (II) (Butler et al., 2000). The oxic nature of the sediments in this study would suggest that most of the iron is in the form of $Fe3^{+}$ and therefore binding phosphate to sediment particles. More research is required to explore this hypothesis further.

PO_4^{3-} was also compared to DIN with the flux ratio (16:1) expected from material with Redfield composition undergoing degradation. The majority of DIN:PO_4^{3-} flux ratios (Figure 4-6d) fell below 16:1, suggesting the majority of nitrogen was either retained in the sediments or exported as N_2 to the overlying water.

4.4.3.3 Silicate

The TCO_2:Si flux ratio (Figure 4-6b) ranged between 6.3 and 24.3 for all sites over the sampling period, with an average ratio of 14.3. Some cores had a ratio close to the expected ratio of 6.6 for Redfield material (Berelson et al., 2003) indicating that the organic matter undergoing decomposition was largely diatomaceous, in particular cores LC and LE measured in November. However, only a weak positive correlation ($r^2 = 0.39$) existed between TCO_2 production and silicate fluxes, suggesting that the sources of organic carbon are variable in their silicate content. Evidence from chapter 2 demonstrates that the sediment organic matter is mainly terrestrial and thus likely to have much lower silicate content. Therefore the silicate fluxes result from decomposition of different sources of organic matter having variable silicate:carbon content.

4.4.4 Upward fluxes of ammonium in the sediments

An alternative approach to measuring total sediment-water fluxes to assess the nitrogen cycle in sediments is to calculate upward fluxes of NH_4^+, which can than be compared to the measured total sediment-water NH_4^+ fluxes. Large differences between the 2 techniques can occur due to the enhanced transport of solutes across the sediment–water interface.

The enhanced transport of solutes across the sediment–water interface in cohesive sediments in these and previous studies is attributable to either the pumping activity of fauna (bio-irrigation) or the random movements of fauna within the sediment (enhanced diffusion). Enhanced diffusion can be simply modelled using Fickian diffusive models, whereby an enhanced diffusion coefficient (De) is calculated, as done by Berg et al. (2001). Bio-irrigation is more complicated to model, and should theoretically be modelled using a non-local exchange model.

In the present study I used the enhanced diffusion model to estimate the upward fluxes of NH_4^+. This is justified because the sediments were checked after core incubations for fauna and very few polychaetes and pumping bivalves were observed. In addition, the NH_4^+ profiles obtained presented little evidence of bio-irrigation, with the profiles showing an outward curvature. Under the influence of bioirrigation one

would expect to see a more sigmoid profile shape for NH_4^+ (Mortimer et al. 1999, Tuominen et al. 1999).

Furthermore, the average De of 3.92 ± 2.77 x 10^{-6} cm^2 s^{-1} found in this study is comparable to the De found by Berg et al 2001 (4.6 ± 1.0 ×10–6 cm^2 s^{-1}) and Cook et al 2004 (10.2 × 10–6 cm^2 s^{-1}) for enhanced diffusion, and agrees well with the enhanced pore water transport, caused by the movement of meiofauna found by Aller & Aller (1992). Therefore based on the evidence presented, the De values used in this study are considered appropriate as an estimate for calculating upward ammonium fluxes.

In this study, the calculated upward fluxes of NH_4^+ were greater than those actually measured in the dark (Figure 4-8), except for at Hideaway Bay, where one core from the replicates recorded a total flux rate of > 80 µmol m^{-2} h^{-1}. These results suggest that the sediments were consuming the ammonium before it could diffuse out into the water column.

In many instances, particularly at the upper estuary location, there was in fact an uptake of NH_4^+ from the water column, which indicates that NH_4^+ was being consumed at the sediment surface. Nitrification and subsequent denitrification is potentially the NH_4^+ consuming process for reasons discussed in the above section.

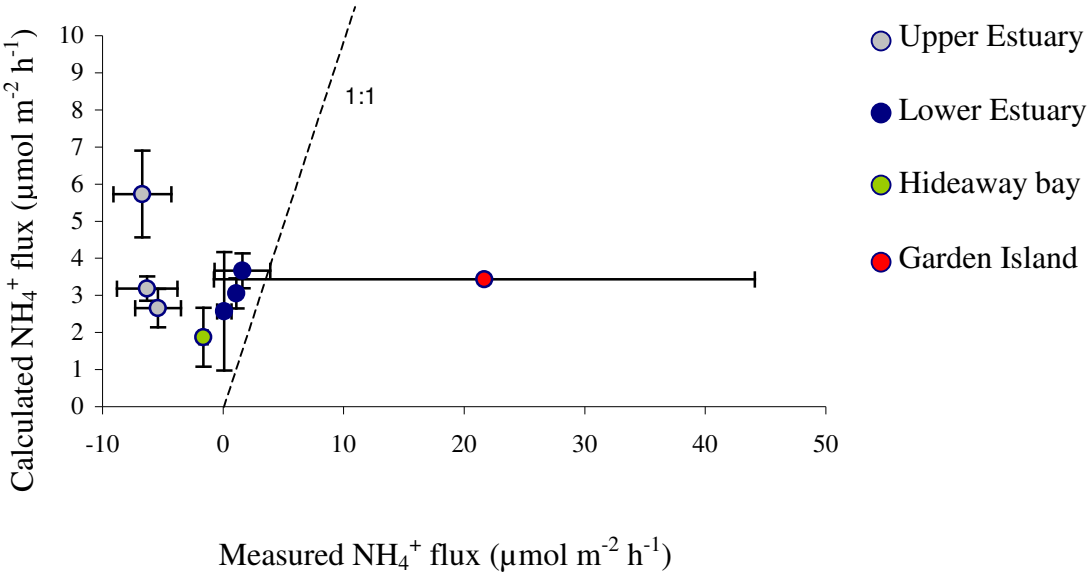


Figure 4-8 Calculated upward flux of NH_4^+ within sediment versus measured dark NH_4^+ flux at the lower and upper estuary locations in November 2004 and at Garden Island and Hideaway Bay in April 2005; line shows 1:1 relationship between modeled and measured fluxes. The error bars = SE of mean (n = 2 to 4).

4.4.5 Comparison with other estuaries

It is often difficult to compare flux rates between different studies as different techniques are practiced. However, one way to compare the importance of nutrient fluxes between environments is to compare the time it would take for the sediments to replace the standing stock of nutrients in the water column. Using a similar method to that used by Hammond et al. (1985) and Friedl et al. (1998) the standing nutrient stock in the water column was divided by the average temporal flux for different solutes at both locations (Table 4-11). In other words, the nutrient (e.g. silicate) concentration was multiplied by the depth of the water column and then divided by the daily nutrient flux. The turnover rates or replacement time in this study generally suggest that the water column nutrient concentrations are likely to be dominated by processes other then from benthic regeneration.

The turnover rates of nutrients showed a distinct temporal trend with replacement time shortest in March and November when relatively high benthic recycling is

occurring and water column nutrient concentrations are low. Comparatively, July had the longest replacement times, when benthic recycling is slow due to lower water temperatures and water column nutrient concentrations are relatively high.

Table 4-11. Replacement time (number of days) of water column nutrients by benthic fluxes. Water column nutrients and depth are taken from table 1. Benthic fluxes are taken from figures 1 and 2.

	NH_4	NO_3	PO_4^{3-}	Si
Lower Estuary				
Average depth = 30m				
March	432	76	347	140
July	625	1156	561	777
November	400	244	354	159
Upper Estuary				
Average depth = 15m				
March	254	31	191	20
July	NA	633	2778	NA
November	NA	64	NA	143

The turnover rates for nutrients in the Huon estuary are considerably longer compared with other studies. Hammond et al. (1985) found in San Francisco Bay the replacement time in the deepwater station was between 20 to 80 days for ammonia, an order of magnitude lower then this study. Callender and Hammond (1982) found that in the Potomac River Estuary, benthic ammonium fluxes were sufficient to replace ammonium water concentrations three times a day.

The Potomac River estuary also had very quick replacement times for silicate and phosphorus with timescales of 3 and 26 days respectively (Callender and Hammond, 1982), while replacement times in San Francisco Bay for silicate and phosphorus were 150 to 300 days and 250 to 1000 days respectively (Hammond et al., 1985). Contrastingly, in this study we observed replacement times between 191 and 2778 days for phosphate and 20 to 777 days for silicate, all of which indicates that the sediments are not currently a major source of nutrients in the Huon estuary.

The Potomac estuary is an example of a highly stressed river system receiving large amounts of anthropogenic nutrients. San Francisco bay is highly urbanised and probably sits between Potomac estuary and the Huon estuary in terms of

anthropogenic influence. The Huon estuary is considered to be relatively pristine in which the environment is not substantially different to pre-European arrival (Butler et al., 2000) and only receives small inputs of anthropogenic nutrients. The above observations might then suggest that in stressed systems, nutrient inputs from benthic regeneration are a major source of nutrients, while in systems with low human impact, benthic regeneration of nutrients is only a minor source of nutrients.

A key to understanding why this might be the case is to understand the prevailing sediment conditions in both stressed and un-stressed systems. Using the Huon estuary as the un-stressed example, sediments are well oxygenated and dominated by aerobic processes such as nitrification. The nitrogen, usually in the form of ammonium, liberated during remineralisation of organic matter is rapidly nitrified or reassimilated by decomposing bacteria. The nitrate produced by nitrification is then either released to the overlying water column or denitrified to N_2 and thus removed permanently from the system. Alternatively, ammonium maybe directly converted to N_2 via anaerobic ammonium oxidation. Only a few other known studies have observed this occurring (Tucker et al., 2000; Grenz et al., 2000; Hall et al., 1996). Hall et al. (1996) found similar processes occurring in sediments across the Norwegian trench of the Skagerrak (North-Eastern North Sea) between Denmark and Norway. Like the Huon estuary, this area is relatively un-impacted by human influence and the sediments are dominated by refractory organic carbon, most likely from either terrestrial origins or seafloor eroded material from the southern North Sea.

In contrast, stressed systems usually have poorly oxygenated sediments and are dominated by anaerobic processes. Ammonium becomes the dominant form of nitrogen liberated from sediments as nitrification becomes inhibited due to the reduction in oxygen. Because stressed systems have high loads of nutrients, algal blooms are frequent events over the warmer months and in turn supply the sediments with fresh labile organic matter. The bacteria in sediments rapidly decompose the algal detritus releasing ammonium and other nutrients back into the water column resupplying phytoplankton with nutrients, creating a positive feedback loop, where otherwise nutrients may have been depleted.

The relationship between bacterial respiration and ammonia release can be illustrated by the relationship between sediment oxygen consumption (used as proxy for bacterial respiration) and ammonium release. As sediment oxygen consumption increases so does ammonium release. Presumably this occurs due to the depletion of the sediment oxic layer, and inhibition of nitrification. Furthermore, dissimilatory nitrate reduction to ammonium (DNRA) may also contribute significantly to the ammonium flux (at the expense of nitrate) in sediments that become organically enriched. Christensen et al. (2000) found that DNRA increased 7-fold in sediments underneath fish cages in comparison to unimpacted sediments. Figure 4-9 shows the relationship between ammonium and oxygen fluxes across a number of near shore environments. This figure illustrates the point above that benthic regeneration of nutrients, in this case ammonium, occurs at much higher rates in eutrophic systems then in relatively un-impacted systems like the Huon estuary. Systems like Potomac river, the Northern Adriatic sea and the North-west Black Sea are heavily degraded systems due to anthropogenic inputs and the benthic regeneration of ammonium is far greater in these systems due to the likely input of labile organic matter and high benthic respiration as illustrated in the figure below.

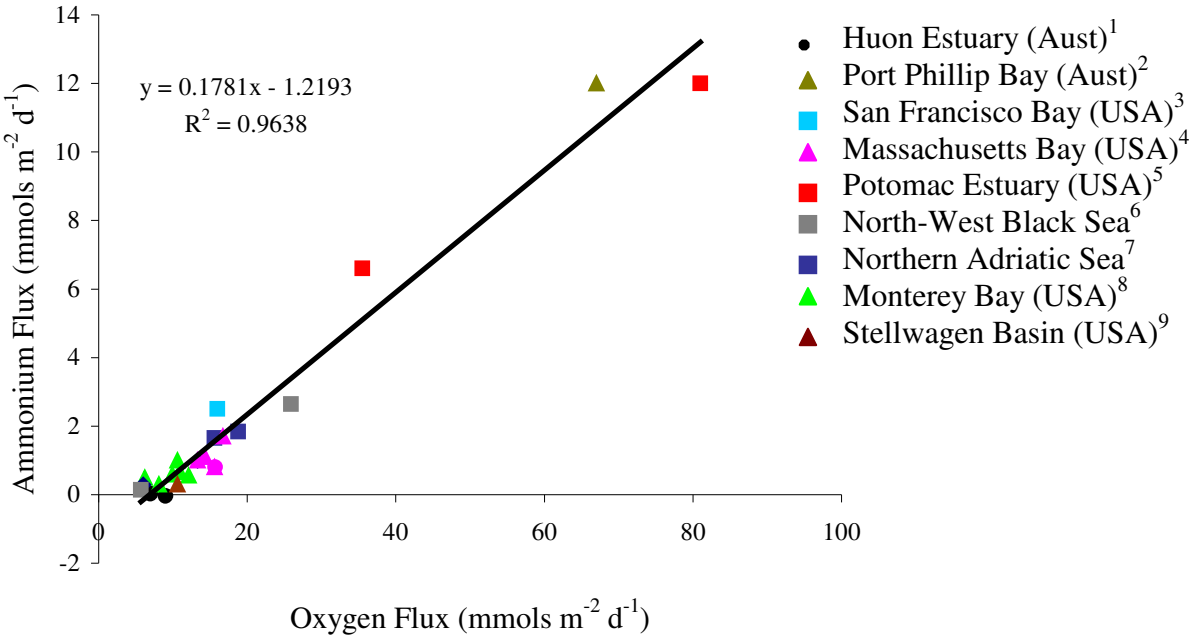


Figure 4-9. Relationship between ammonium fluxes and oxygen consumption in near shore environments. Square boxes represent eutrophic environments. Numbers in superscript refer to the following references: 1 this study; 2 Berelson et al., 1998; 3 Hammond et al., 1985; 4 Hopkinson et al., 2001; 5 Callender & Hammond, 1982; 6 Friedl et al., 1998; 7 Hammond et al., 1999; 8 Berelson et al., 2003; 9 Hopkinson et al., 2001.

4.5 Conclusions

This study has shown that benthic fluxes of nutrients are low in the Huon estuary. Average fluxes of ammonia, nitrate, phosphate and silicate were 1.3, 10.1, 1.6 and 32.5 µmol m^{-2} h^{-1} respectively.

On all occasions, the DIN flux was dominated by nitrate, which was always released from the sediment to the overlying water. Aerobic conditions were prevalent in the upper 3 – 9mm for the sediment, providing an environment conducive for aerobic processes like nitrification. Peaks of nitrate within the aerobic zone observed from nitrate pore water profiles also provided additional evidence that nitrate was been produced in the oxic zone, most likely due to nitrification. All these observations

suggest that the effluxes of nitrate and influxes of nitrite and ammonium in Huon estuary sediments are associated with intensive nitrification stimulated by the presence of relatively deep oxygenated zones. The net efflux of nitrate from the sediments suggests that they act as net regenerators of nitrogen as opposed to nitrogen assimilators.

The benthic effluxes of nitrogen however were smaller then expected from carbon oxidation rates. The low N:P ratio of benthic fluxes (approx. 3:1) indicates that processes such as denitrification may be an important nitrogen elimination process. However another potential pathway for nitrogen released during organic matter remineralisation is for the decomposing bacteria to reassimilate some of the ammonium, the rest being nitrified, due to the low nitrogen content of the organic matter been decomposed.

The relatively un-impacted sediments in this study provide a good contrast to more stressed systems in the way nutrients are recycled through sediment during organic matter remineralisation. In unstressed systems like the Huon estuary, DIN fluxes are dominated by nitrate produced via nitrification, which inturn is partially converted to N_2 via denitrification. Phosphate is also adsorbed within the sediments due to the persisting oxic conditions. Contrastingly in a stressed system, nitrification becomes inhibited due to a reduction in oxygen availability and DIN fluxes become dominated by ammonia. Denitrification efficiency is also reduced. Phosphate is released at a greater rate due to the reduction in the adsorption capacity of the sediments due to decreased oxic conditions

Chapter Five

Effect of Carbon Loading on Huon Estuary Sediments

Chapter 5. Effect of Carbon Loading on Sediment Fluxes

5.1 Introduction

In estuarine and coastal systems, sediments play an important role in remineralising particulate organic matter (POM) and therefore the cycling of nitrogen and phosphorus between the benthos and overlying water column (Sloth et al., 1995; Enoksson, 1993). Potentially, a large proportion of POM in estuarine and coastal systems reaches the sediments and, depending on its lability, is decomposed rapidly. As the carbon load increases, both the oxygen demand and nutrient cycling increases (Enoksson, 1993).

Many estuarine and coastal areas are now heavily influenced by anthropogenic inputs, such as increased nutrients from land runoff. Increased nutrient levels in the water column can lead to an increase in primary productivity and the creation of algal blooms. Ultimately systems can become eutrophic and suffer frequent algal blooms and low dissolved oxygen events.

Sediment response to increased carbon loading in eutrophic systems has been heavily studied (Farias, 2003) and the biogeochemical processes under these conditions are generally well understood. However, as most carbon loading studies have been carried out in eutrophic systems in North America and Europe, it is difficult to relate those findings back to mesotrophic systems in Australia such as the Huon estuary because of the different biogeochemical processes occurring in these sediments. Thus, this study was carried out to enhance our understanding of sediment response to carbon loading in a mesotrophic system.

This study is also important as the Huon estuary has in recent times come under increased pressure from salmon farming, leading to larger amounts of nutrients and POM entering the system (Butler et al., 2000). Previous studies have shown that as sediments decompose more organic matter, the decomposition pathway shifts from aerobic to anaerobic, and more ammonia is released to the overlying water due to inhibition of nitrification and decoupling of the nitrification/denitrification pathway (Christensen et al., 2000). Under high organic loading it has also been shown that dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium

oxidation (ANAMMOX) can also become more important (Christensen et al., 2000; Hulth et al., 2005). Therefore, understanding how the magnitude of POM flux to the sediments alters biogeochemical processes, including nutrient cycling and decomposition pathways, is vital.

It is critical for future environmental managers of the Huon estuary to understand how carbon influences nutrient cycling for the prediction of future estuarine and coastal changes and for the environmental management of estuarine and coastal systems. In regards to the Huon estuary, this knowledge can help environmental managers limit further anthropogenic inputs from fish farming activities to avoid altering the current sediment biogeochemical system.

In eutrophic systems, previous studies have shown that sediments alone can supply a substantial fraction of the nutrient requirements of phytoplankton (Dollar et al., 1991). Essentially, as phytoplankton die and deposit onto the sediments, microbial breakdown of the POM recycles fixed nitrogen back to the water column, forming a positive feedback loop for further phytoplanktonic blooms. Once a system reaches this point, it is likely to be very difficult to return to pre-eutrophic conditions even if anthropogenic inputs are curtailed.

The previous chapters of this study established that un-impacted sediments in the Huon estuary are dominated by aerobic respiration of organic carbon and that the dominant component of the nitrogen flux was nitrate produced by in situ nitrification. The aim of this study was to observe changes in sediment biogeochemical processes as the load of labile POM was increased.

The change in sediment biogeochemical functioning as POM increased was observed experimentally by adding increasing amounts of heat-killed *Spirulina* biomass to sediment cores. It was hypothesised that as the oxygen penetration depth decreased due to increased bacterial respiration, a shift would occur from sediments dominated by aerobic respiration and coupled nitrification/denitrification to sediments dominated by anaerobic respiration and high ammonium fluxes. Of particular interest was to elucidate if there was a clear point at which the system flips between these two POM

degradation pathways and whether or not this occurred as a step function or as a linear change.

To investigate these potential changes, fluxes of oxygen, carbon dioxide and nutrients at the sediment – water interface and oxygen and nutrient concentrations in the pore waters were measured in sediment core reactors. From these measurements, inferences were made about the shifts in biogeochemical processes as carbon loading concentrations increased.

5.2 Methods

5.2.1 Sampling Site and sediment collection

Sediment was collected from the Upper estuary location in early June 2005 using a small research vessel. A box corer was used to take intact sediment samples, from which an undisturbed sub-core was taken using 25-cm-long polyethylene tubes with an inner diameter of 9.7 cm. The cores were then held in an ice-cooler at in situ temperatures and were transported back to the lab within six hours of sampling. On arrival, the cores were placed into an incubation trough and brought to 15^{o}C temperature in a temperature-controlled room.

The following day, the cores were sieved through 1mm mesh to remove any macrofauna. The top 1cm of each core was removed and sieved separately. The two sediment pools were homogenized separately before being put back to the sediment reactors. Approximately 8cm of bottom layer sediment was placed into each reactor and then a further 1 cm of top layer sediment was placed over the top. This was done to help the microcosms return to 'natural' conditions as much as possible in the sense that surface microbes would be returned to similar depths. The sediment reactors were then placed back into the incubation troughs and left to re-equilibrate for a period of 3 weeks. To ensure a good exchange between the water phase of the core and the exterior seawater during the re-equilibration period, a Teflon-coated magnetic stirrer bar (5 cm length) was suspended ~5 cm above the sediment surface in each core. This was driven by an external magnet rotating at 50 – 60 rpm.

5.2.2 Carbon Loading Experiment

Prior to adding organic carbon, nutrient fluxes and oxygen consumption was measured in all reactors to ensure that the sediments had similar baseline measurements. This was also done to compare with fluxes measured in undisturbed cores from chapters 3 and 4 to examine how closely benthic respiration and nutrient cycling was re-created in the homogenised sediment cores. The carbon addition experiment only began once all cores had a similar baseline, which occurred approximately 3 weeks after re-homogenisation of the sediment cores.

A concentration series experiment was designed to assess the short-term impact of carbon loading on sediment respiration and nutrient cycling. The following concentrations (g m^{-2}) were used and randomly assigned to each reactor:

- Control – no carbon
- 3.4
- 6.7
- 10.1
- 13.5
- 20.2
- 27.0
- 33.8
- 40.5
- 54.0
- 67.5

Dried *Spirulina* powder was used as the organic carbon source. The *Spirulina* was weighed out in the appropriate amounts and then reconstituted in deionized water. The *Spirulina* was then pasteurised to remove any bacterial contamination before adding to the sediment reactors. Prior to adding the *Spirulina* to the cores, the stirrer bars were switched off and the water level in the incubation trough dropped below the top of the reactors to prevent water exchange between the reactors and the incubation trough. The *Spirulina* was then added and allowed to settle to the sediment surface over a 4-hour period. Once all the *Spirulina* had settled onto the sediment surface the stirring was switched back on and the speed adjusted so that re-suspension of the *Spirulina* did not occur. The incubation troughs were also carefully refilled to prevent re-suspension of the *Spirulina*. Throughout the experiment, the water was replaced regularly in the incubation troughs to prevent the build up of nutrients.

5.2.3 Total O_2, TCO_2 and Alkalinity and Nutrient Fluxes

Rates of nutrient exchange (ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^{3-})) as well as O_2, TCO_2 and alkalinity were measured in the dark 48 hours after adding the *Spirulina*. All flux measurements in the cores were started at the same time, so that all cores had the same exposure time to the carbon addition. This was done so that I could compare like with like.

Prior to starting the incubations the water in the incubation troughs and sediment reactors was replaced with clean water, with care taken not to resuspend the *Spirulina*. The incubations were then started by capping the cores and waiting 1 hour prior to making the first measurement. Oxygen and pH were measured by inserting electrodes into the reactor's water sampling port. Water samples for nutrient and alkalinity analysis determinations were taken from the reactor water by withdrawing 50 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. The water samples were then filtered through Acrodisc 0.45µm cellulose acetate filters into 10 ml screw-cap polypropylene containers; alkalinity samples were stored at 4°C in the dark and analysed within 2 weeks of sampling while nutrient samples were stored at -20°C and were analysed within 3 months of sampling unless otherwise stated in the text. Sediment reactor incubations were terminated prior to dissolved oxygen (DO) decreasing to 80% saturation of the starting concentration and total incubation times ranged between 3.5 to 6 hours.

The flux across the sediment-water interface was calculated as (Dalsgaard et al, 2000):

$$\text{Flux } (\mu\text{mol } l^{-1} h^{-1}) = ((T_1 - T_0) - T_w) \times V/A$$

Where:

T_0 = Concentration of analyte at start of the incubation
T_1 = Concentration of analyte at end of incubation (corrected for addition of replacement water)
T_w = Concentration change of analyte in Blank core
V = water column volume (l) and,
A = sediment surface area (m^2).

5.2.4 Oxygen Penetration Depth

At the end of the flux incubations, sub-cores were taken from the sediment reactors using 25-cm-long polyethylene tubes with an inner diameter of 4.8 cm to measure oxygen penetration depths. Before submerging the sub-cores into a glass aquarium filled with in situ water, the sediment was pushed up so that only a 1 – 2 cm gap existed between the sediment surface and the top of the core. This ensured that there was good mixing of water above the sediment surface. Mixing was achieved by continuously bubbling air through an air-stone, which created light turbulence at the water surface. Care was taken not to resuspend the *Spirulina* at the sediment surface. Bubbling air also served to maintain 100% saturation of dissolved oxygen in the aquarium water. The cores were then left in the dark overnight to re-equilibrate.

The day after sampling, oxygen microelectrodes (Clark type with a guard cathode from Unisense A/S Aarhus, Denmark) were positioned vertically above the sediment surface on a hydraulic computer-controlled micromanipulator. The microelectrode was then inserted stepwise into the sediment in 100µm intervals to measure oxygen concentration across the sediment-water interface to the bottom of the oxic zone. The sediment surface and tip of the microelectrode were observed continuously using a stereomicroscope to record when the microelectrode tip penetrated the sediment surface. This ensured the correct positioning of the measured oxygen profiles relative to the sediment-water interface and consequently the determination of the diffusive boundary layer (DBL). This method was then checked against the method described by Glud et al. (2003) in which the upper DBL boundary was determined as the intersection between the extrapolated linear O_2 gradient in the DBL and the constant O_2 value in the overlying water. The thickness of the DBL was estimated from the intersection point and the position of the sediment, which was identified from a distinct break in the concentration profile. When the two methods were in disagreement the average of the two methods was used to determine the DBL. Three to six profiles were measured in duplicate cores from each site at random positions.

The output (mV) of the microelectrode was automatically recorded onto a computer and then converted to micromolar concentrations of dissolved oxygen by comparing to Winkler titrations of the aquarium water for which the temperature, salinity and

electrode output were also recorded. The microelectrodes were calibrated in nitrogen-saturated, air-saturated and oxygen-saturated aquarium water to check the linear relationship between current output and oxygen concentration. The microelectrodes gave acceptable slopes of between 4.53 – 4.63.

5.2.5 Nutrient Pore water analysis

Once oxygen profiling was complete, the cores were then rapidly sectioned under atmospheric conditions at 0.5cm intervals from 0 to 2cm and at 1cm intervals from 2 to 5 cm, to extract pore waters for nutrient analysis. Slices were placed into centrifuge tubes and then centrifuged at 2000 rpm for 10 minutes. The water was then extracted and filtered through Acrodisc 0.4 micron filters into 10ml polystyrene tubes and frozen at -20^{o}C for analysis of NH_4^+, NO_3^- and PO_4^{3-}.

5.2.6 Analytical Methods

5.2.6.1 Ammonium

NH_4^+ was analysed using OPA derivatisation and fluorescence detection (Watson et al., 2004). The precision of the method was generally between 5–8% with a detection limit of 0.07–0.2 µM.

5.2.6.2 Nitrate + Nitrite

Nitrate was measured quantitatively reducing nitrate to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) was then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion was coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. Nitrate concentrations were obtained by subtracting nitrite values, which have been previously analyzed, from the nitrite + nitrate values (Diamond, 1999). The precision of the method was between 2 - 4% with a detection limit of 0.03 – 0.07 µM for nitrate and a precision of between 1 -12% with a detection limit of 0.005 – 0.01 µM for nitrite.

5.2.6.3 Phosphate

Phosphate was analysed by ammonium molybdate and antimony potassium tartrate reacting in an acid medium with phosphate to form an antimony-phospho-molybdate complex. This complex was reduced to an intensely blue colored complex by ascorbic acid. The color produced is proportional to the phosphate concentration in the sample (Diamond, 1998). The precision of the method was between 1 - 3% with a detection limit of 0.02 – 0.03 µM.

5.2.6.4 Alkalinity

Alkalinity was measured using a Gran titration, which was performed using an Orion 960 Autochemistry system (precision = ± 1.0%). A total of 10ml of sample at 20^{o}C was titrated with 0.01 M HCL acid. pH was measured using an Orion (91-55) pH probe connected to a PHM85 (Radiometer) pH meter (precision = ± 0.002%). TCO_2 was calculated using alkalinity, pH, salinity and temperature according to equations given by Almgren et al. (1983).

5.2.7 Statistical Analysis

The statistical analysis used SPSS version 11. Correlation and regression analysis were used to explore relationships between variables.

5.3 Results

5.3.1 Respiration Fluxes

In general, respiration fluxes (CO_2 and O_2 fluxes) increased (Figure 5-1)) as the load of carbon increased. The rate of change for oxygen penetration depth and O_2 fluxes, as carbon loading increased, was non-linear. The relationship between carbon loading and oxygen penetration and O_2 fluxes showed there was a distinct break in the relationship when carbon load exceeded 20 g C m^{-2}. Fitting two separate regressions between 0 to 20 g C m^{-2} and between 20 and 67.5 g C m^{-2} gave a better R^2 then fitting a single regression between all data points.

In contrast, CO_2 and alkalinity fluxes and the community respiratory quotient (CRQ) (CO_2:O_2 ratio) increased linearly with organic carbon loading (Figure 5-1). No distinct break in the relationship was detected for these fluxes and the CRQ ratio as carbon loading increased. Regression analysis showed that a single fitted linear regression gave a strong relationship between carbon loading and CO_2 fluxes, alkalinity fluxes and CRQ ratio with R^2 of 0.95, 0.71 and 0.81 respectively.

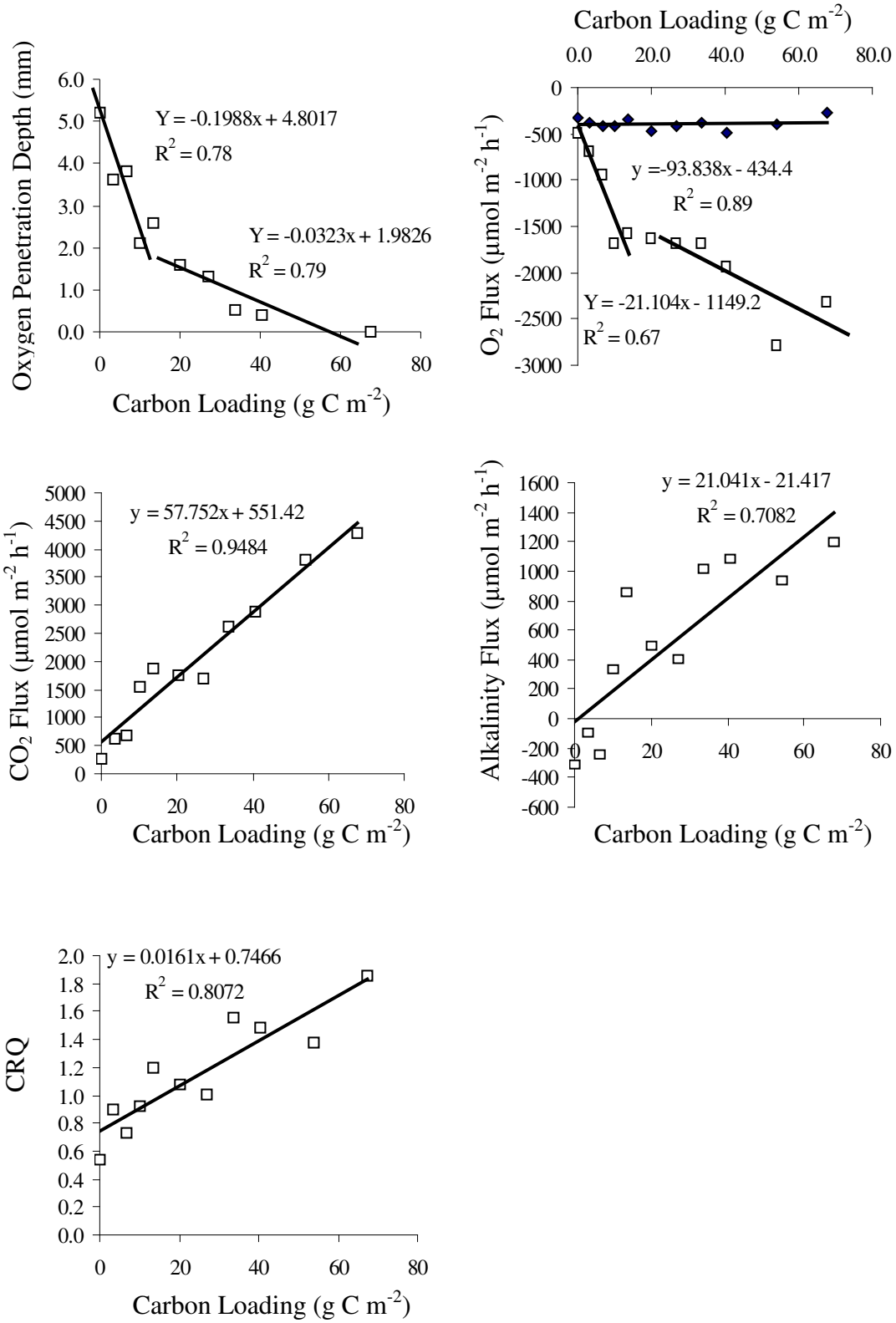


Figure 5.1. The effect of carbon loading on sediment metabolism. a) Oxygen penetration depth (mm); b) Total oxygen flux (µmol m^{-2} h^{-1}); c) CO_2 flux (µmol m^{-2} h^{-1}); d) alkalinity flux (µmol m^{-2} h^{-1}) and e) community respiratory quotient (CRQ). ◆Pre carbon loading □ Post carbon loading.

5.3.2 Nutrient Fluxes

Ammonium fluxes (Figure 5-2) showed a similar trend as observed for respiration fluxes. Ammonium fluxes ranged between 20 and 680μmol m^{-2} h^{-1} with a rapid linear increase in ammonia fluxes as carbon loading increased to 20 g C m^{-2}. This relationship demonstrated a strong connection between ammonia release and carbon loading (r^2 = 0.99). Above 20 g C m-2 the relationship was much weaker (r^2 = 0.27).

Nitrate (Figure 2b) was released to the overlying water in the untreated reactor, but was taken up by the sediments in all treated reactors. Regression analysis indicated that a relatively weak relationship (r^2 = 0.51) existed between nitrate flux and carbon loading although the general trend was for sediments to increase nitrate uptake as carbon loading increased.

Nitrite (Figure 5-2) was initially taken up by the sediments, but was generally released in treated sediments. However, regression analysis indicated only a very weak (r^2 = 0.20) relationship between nitrite flux and carbon loading. Phosphate fluxes were always released to the water column, however no trend was observed between phosphate release and carbon loading. Regression analysis showed a poor relationship (r^2 = 0.10) between phosphate fluxes and carbon loading.

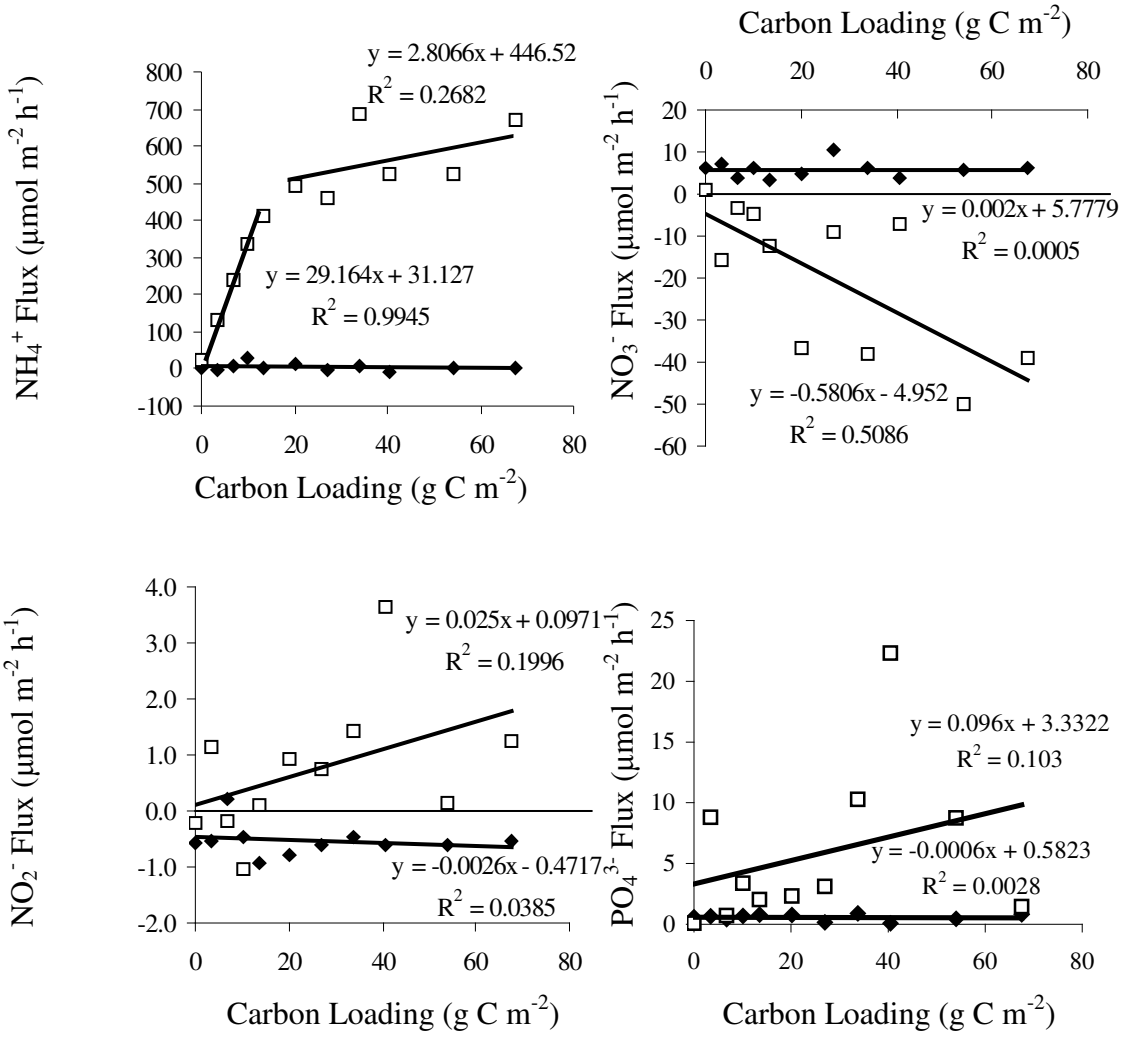


Figure 5-2. The effect of carbon loading on nutrient fluxes at the sediment – water interface a) Ammonia flux; b) Nitrate flux; c) Nitrite flux and d) Phosphate. ◆ Pre carbon loading □ Post carbon loading.

5.3.3 Porewater Nutrients

In general, ammonium concentrations increased with depth in reactors with carbon loadings < 20 g C m^{-2}. Reactors with carbon loadings ≥ 20 g C m^{-2} had ammonium concentrations that decreased with depth (Figure 5-3). Ammonium pore water concentrations in the untreated core were 2µM at the surface and increased to 125µM at 4cm depth. In contrast, ammonium concentrations were 2271µM at the surface and decreased down to 143µM at 5cm depth in the reactor with the highest amount of carbon loading (67.6 g C m^{-2}).

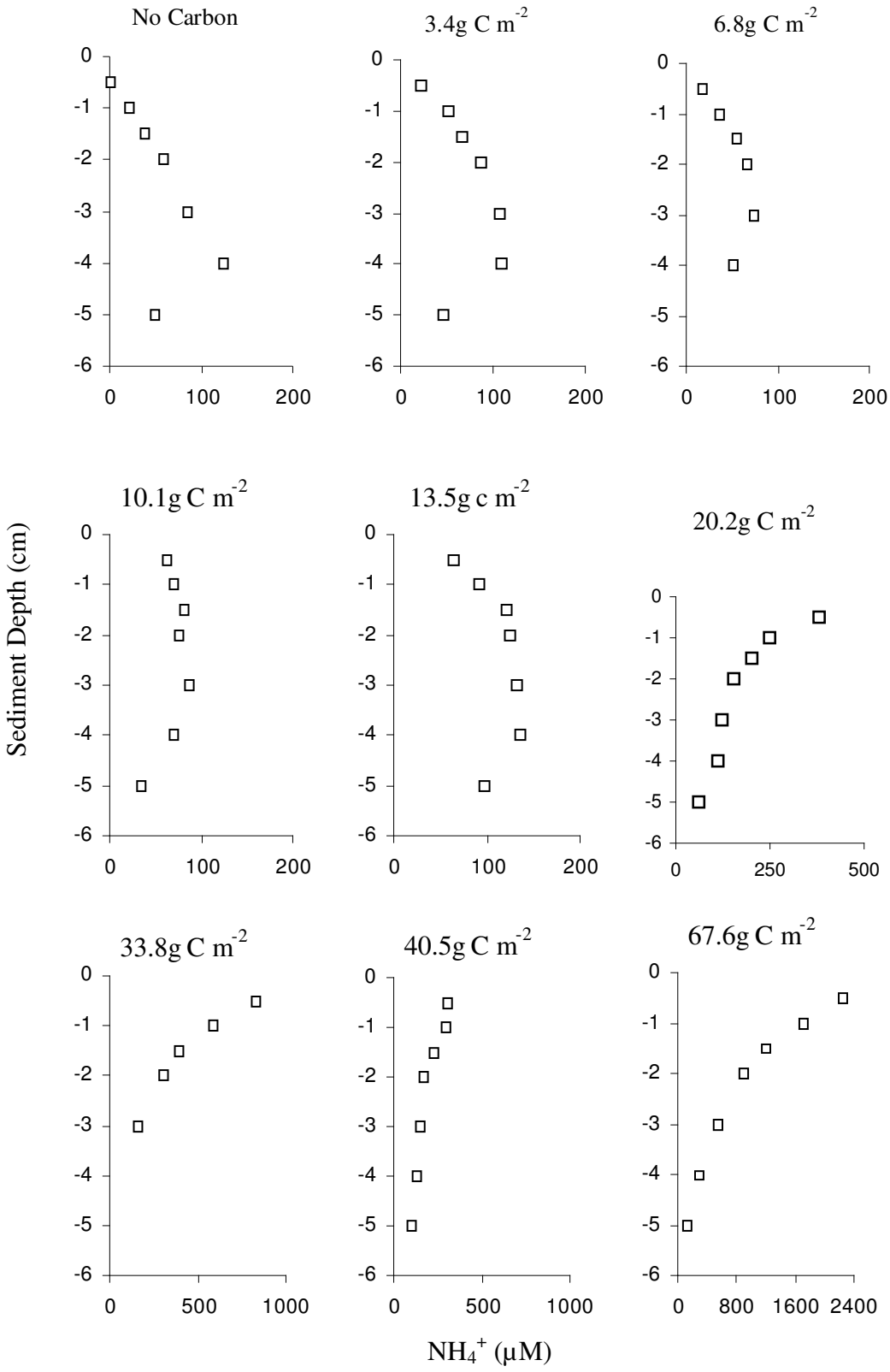


Figure 5-3. The effect of carbon loading on ammonium concentrations (µM) in sediment pore waters. Note that the horizontal scale is different for different carbon loading concentrations due to large differences in concentrations at different loading rates.

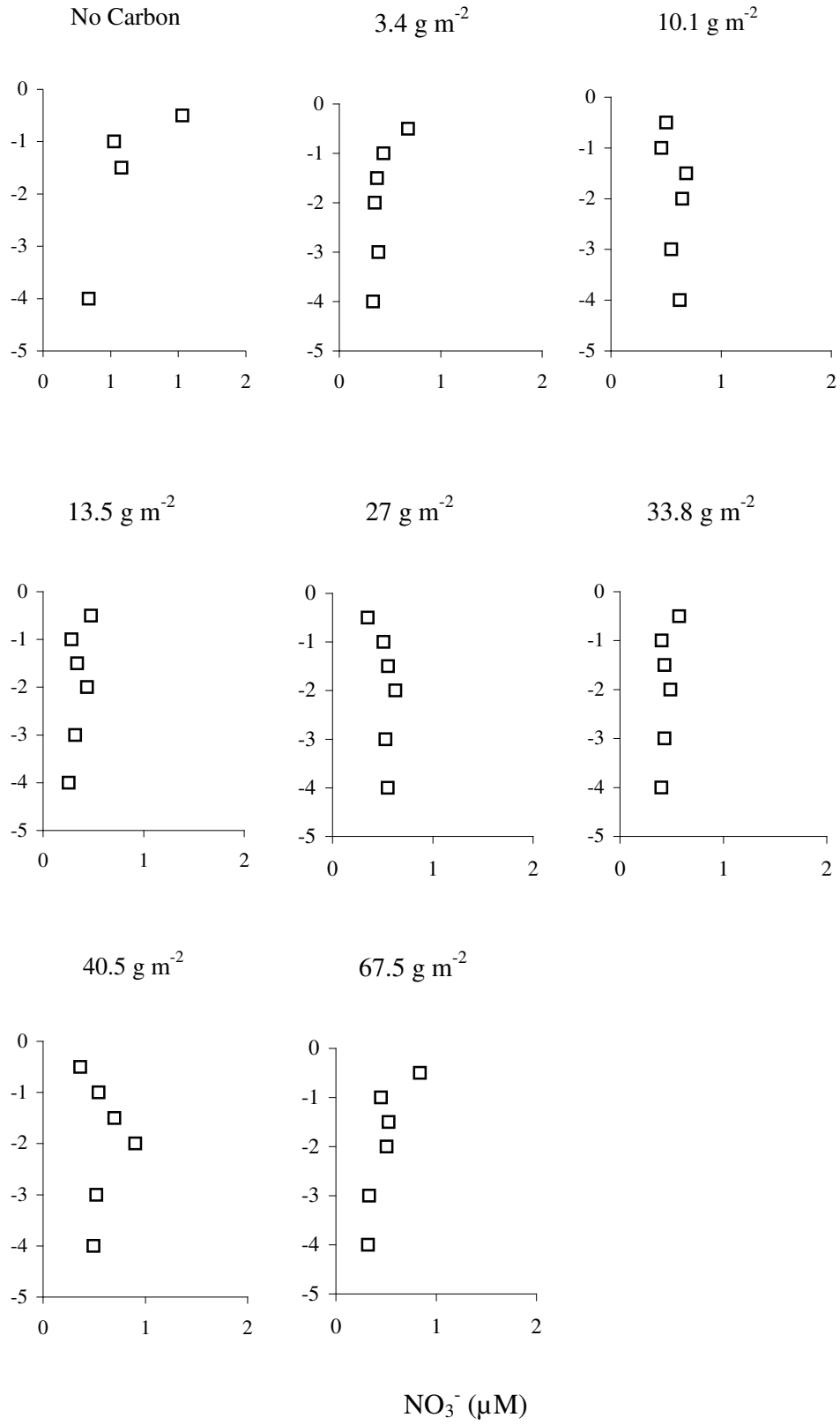


Figure 5-4. The effect of carbon loading on nitrate pore water concentrations (µM).

Nitrate pore waters were generally low in all sediment reactors and no obvious trend was discerned in regards to nitrate concentrations and carbon loading. The untreated reactor showed the greatest peak of nitrate in the top 1cm of sediment, which correlated well with the measured nitrate efflux for the untreated core in figure 5-2b.

5.4 Discussion

5.4.1 Fluxes before carbon addition

Prior to loading the reactors with carbon, the sediments were incubated and fluxes of oxygen, ammonium, nitrate, nitrite, and phosphate were measured. This was done to ensure that all reactors had similar baseline measurements prior to treatment and to compare with fluxes from the field study to elucidate if the homogenisation process had a major effect on either sediment respiration or nutrient cycling.

Table 5-1 compares the results for nutrient fluxes and oxygen consumption in homogenised sediments with the data from intact cores described in chapters 3 and 4. It can be seen that sediment-water flux rates for nutrients and for oxygen consumption closely mimicked the rates observed from undisturbed intact cores. In particular, oxygen consumption and penetration depth, nitrite and phosphate fluxes in the homogenised cores closely resembled the observed fluxes in undisturbed intact cores from the field study. Ammonium and nitrate fluxes diverged most from the fluxes in the intact cores with elevated ammonia fluxes and lower nitrate fluxes in the homogenised cores. However, as with intact cores, nitrate was still the dominant component of the DIN flux, indicating that nitrification was occurring (Enoksson, 1993). The lower nitrate fluxes may have been due to the disturbance of ammonia-oxidizing bacteria during the mixing process, hence reducing the nitrification potential of the sediments. This resulted in a net efflux of ammonium, rather then a net uptake of ammonium as observed from intact cores from the upper estuary sites of the field study.

The O_2:DIN ratio (Table 5-1) was also very similar between the homogenised and intact cores. In chapter 4, it was concluded from CO_2:DIN ratios that the majority of

nitrogen liberated during organic matter remineralisation was either denitrified to di-nitrogen gas or reassimilated by decomposing bacteria. Assuming that oxygen reflects organic matter remineralisation in this instance, given the O_2:DIN ratios between homogenised and intact cores are almost identical, similar conclusions can be made about the homogenised cores. That is, the high O_2:DIN ratio (38.9) indicates the shortfall in DIN relative to respiration maybe due to the temporary or permanent removal of nitrogen liberated during organic decomposition by nitrification and subsequent denitrification or reassimilated by the decomposing bacteria. The fluxes of nitrate from the sediments to the water column suggests that nitrification is occurring in the sediments, and it therefore likely that some of the nitrate produced during this process is denitrified. In support of this, Macleod et al. (2004) observed coupled nitrification-denitrification in sediments of a nearby bay, which displayed very similar biogeochemical characteristics as to the sediments in this study.

Importantly, the homogenisation process did not appear to have a dramatic impact on carbon and nutrient cycling processes for this experiment. This vindicates the approach taken and also allows us to make some inferences about how sediments in 'natural' conditions may respond to carbon loading events.

Table 5-1. Comparison of fluxes from undisturbed cores from results in chapter 3 & 4 with fluxes from homogenised cores in the present chapter. Fluxes given as means ± SE in µmols m^{-2} h^{-1}. Oxygen penetration depth is measured in millimetres (mm).

Fluxes	Fluxes from undisturbed cores (chapter 3 & 4)[1]	Fluxes from cores in this chapter[2]
Oxygen	-398.2 ± 25.5	-392.1 ± 18.5
Ammonia	-1.7 ± 1.5	4.8 ± 2.9
Nitrate	11.3 ± 1.1	5.8 ± 0.6
Nitrite	-0.5 ± 0.1	-0.5 ± 0.1
Phosphate	0.8 ± 0.2	0.6 ± 0.1
Oxygen penetration depth	5.0 ± 0.5	5.2[3]
O:DIN	39.9	38.9

[1]Results are averages of all flux measurements in the upper estuary including all cores sampled in March, July and November 2004

[2]Results are the average of all the cores used in this experiment prior to carbon addition

[3]Result is from 1 core not loaded with carbon. Average for all cores could not be obtained as oxygen penetration depth was only measured at the end of the carbon loading experiment due to the need to take a sub-core from the reactors in order to carry out the oxygen penetration measurements

5.4.2 Fluxes after carbon loading

Following the addition of Spirulina, there was a rapid increase in the flux rates of most analytes, which agrees with previous findings by Enoksson (1993) and Garber (1984). The fluxes for CO_2 and alkalinity increased linearly with carbon loading, as did the CRQ, however fluxes of ammonium and oxygen appeared to show a step change in the flux rate at additions of carbon >20.2 g C m^{-2}. At this quantity of carbon loading, the flux rate whilst still increasing, slowed significantly. The fluxes of nitrite and phosphate showed little correlation with increasing carbon loading.

It is proposed from these findings that sediments became saturated with carbon at the surface and the ability of the sediments to decompose more carbon aerobically was limited due to the rapid consumption of oxygen. Oxygen penetration into the sediments at this point was less than 2 mm. This may suggest that at the point at which the step change in oxygen flux rates occurred, sediment metabolism was switching from predominantly aerobic respiration to predominantly anaerobic respiration.

This may explain why CO_2 fluxes did not display a step change in flux rate, whilst oxygen fluxes did. Both aerobic and anaerobic respiration produce CO_2 as end products and therefore CO_2 should continue to increase at a linear rate as carbon loading increases regardless of the type of respiration occurring. The CRQ ratios indicate that as carbon loading increased so did the proportion of anaerobic respiration and it therefore follows that CO_2 fluxes should continue to increase as well. Alkalinity fluxes also increased at a linear rate, with sulphate reduction (a form of anaerobic respiration) the most likely source of the alkalinity (Berelson et al., 1998), which also provides extra support to the proposition that anaerobic respiration became the dominant form of metabolism as carbon loadings increased.

Unexpectedly however, ammonium fluxes did not increase linearly with CO_2 fluxes. If the production of CO_2 increases linearly with carbon loading, than it could be expected that ammonium fluxes would increase linearly as well, because ammonium is produced through both aerobic and anaerobic forms of respiration. However, the results from this experiment showed ammonium fluxes initially increase linearly with

carbon loading, but that the flux decreases at the point that metabolism switches to anaerobic respiration.

One possible reason why ammonium flux rates slow down at this point would be anaerobic ammonium oxidation (ANAMMOX), whereby ammonium is oxidised under anaerobic conditions. According to ANAMMOX stoichiometry, ammonium is oxidised using nitrite as the electron acceptor and inorganic carbon as the carbon source.

$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$ (Reginatto et al, 2005)

The stoichiometry for ANAMMOX suggests that only a small amount of inorganic carbon is used for every ammonium ion oxidised (0.066:1). This could explain why CO_2 continued to increase linearly while ammonium did not. At the point where the step change in the ammonium flux rate occurs, the sediments have become almost anaerobic providing an environment potentially conducive to ANAMMOX. Therefore it is possible that at this point, ANAMMOX is responsible for consuming some of the ammonium produced, and because the ANAMMOX reaction uses only a small amount of inorganic carbon, ANAMMOX should not greatly affect the CO_2 flux rate, but should cause a decline in the ammonium flux rate. It is therefore proposed that ANAMMOX is responsible for the step change in ammonium flux rate at the point where the sediment is switching from aerobic to anaerobic respiration.

In table 5-2 below, all results have been aggregated into four discrete categories: zero, low (3.4 – 6.8), medium (10.1 – 27) and high (33.8 – 67.6) rates of carbon loading to summarise the changes in biogeochemical processes under different carbon loading scenarios. With zero carbon loading, the sediment behaved in much the same way as was observed in intact sediment cores from the field study. The sediments were characterised by a relatively deep oxygen penetration depth of 5.2mm and low CRQ ratio of 0.6 indicating the dominance of aerobic respiration and the likely consumption of oxygen due to nitrification. The deep oxic zone also provided an environment conducive for nitrification to occur. The nitrate efflux, as discussed earlier, provides evidence of nitrification. Oxidation of sulphides is also an important

consumer of oxygen in marine sediments and could also be responsible for the low CRQ ratio in this case. Modelled oxygen consumption profiles in chapter 3 often showed a peak of oxygen consumption occurring at the oxic/anoxic interface and is possibly due to the oxidation of sulphides. The consumption of alkalinity by the sediments in this scenario further supports nitrification and possible sulphide oxidation, as both these processes consume alkalinity.

The addition of carbon causes a rapid response from the sediments in which bacteria begin to remineralise the added carbon. Because bacteria consume oxygen during the decomposition of organic matter, the depth of oxygen penetration was reduced. Under low carbon loading (< 6.8g m^{-2}) the oxic zone was reduced from 5.2 mm to 3.7mm. This reduction in oxic zone has an impact on both carbon and nitrogen cycling pathways. The CRQ ratio has now risen to 0.8, and while this indicates that aerobic respiration is still the dominant carbon remineralisation pathway, the influence of anaerobic respiration is increasing or it could be that nitrification is decreasing. It is also possible that there is a lag time between the addition of carbon and the stimulation of anaerobic respiration. The smaller oxic zone also begins to reduce nitrification, with the result that nitrification/denitrification becomes uncoupled and direct denitrification becomes more important. That is, denitrification supported by the uptake of nitrate by the sediments. Nitrite has also switched from being taken up by the sediments to being released.

This trend increases going into the medium-loading scenario. Under this scenario, aerobic conditions have further degraded as observed through the increased depletion of the oxic zone to 1.9mm and the CRQ ratio continuing to rise to 1.1. Nitrate fluxes into the sediment increased and ammonium fluxes out of the sediments increased rapidly. This suggests that the demand for nitrate is now considerably exceeding supply. The demand could either becoming from direct denitrification or dissimilatory nitrate reduction to ammonium (DNRA). Studies by Macleod et al. (2004) and Christensen et al. (2000) have shown that both direct denitrification and DNRA become more important as sediments become enriched with organic carbon. However the efficiency of denitrification decreases as organic carbon increases (Macleod et al., 2004). Alkalinity fluxes also rapidly increase and were directed out of the sediment into the water column, compared to the low scenario where alkalinity was consumed

by the sediment. The increase in alkalinity is most likely due to sulphate reduction, which is an anaerobic process, and therefore supports the suggestion that anaerobic respiration becomes the dominant form of benthic metabolism as carbon loading increases.

Under the high loading scenario in Table 5-2, anaerobic respiration is now the dominant metabolic process (CRQ = 1.6), with the oxic zone almost depleted and ammonium and phosphate fluxes at their highest levels. Alkalinity fluxes out of the sediment have also continued to increase, indicating increased activity by sulphate reducers.

Table 5-2. The effect of increasing carbon loading on sediment biogeochemical processes. The flux rates are in µmols m^{-2} h^{-1}.

g C M^{-2}	O_2 Pen	O_2	CO_2	Alk	CRQ	NH_4^+	NO_2^-	NO_3^-	DIN	PO_4^{3-}	CO_2:DIN
Zero	5.2	-483	259	-319	0.6	24	-0.2	0.8	24	0.1	10.6
Low	3.7	-807	650	-172	0.8	183	0.5	-9.5	174	4.7	3.7
Medium	1.9	-1640	1719	514	1.1	425	0.2	-15.6	410	2.7	4.2
High	0.3	-2177	3404	1049	1.6	602	1.6	-33.6	570	10.7	6.0

Low = 3.4 - 6.8; medium 10.1 – 27.0; High 33.8 – 67.6 g C m^{-2}.

5.4.3 Comparison with other Studies

The findings in this study agree well with previous findings. Benthic respiration has been found to dramatically increase with the addition of organic matter in laboratory experiments (Sloth et al., 1995; Enoksson, 1993) in sediments directly below fish cages (Macleod et al., 2004; Christensen et al., 2000; Hargrave et al., 1997; Holmer & Kristensen, 1992) and in sediments receiving 'fresh' algal material during an algal bloom (Grenz et al., 2000).

Nutrient fluxes increased during these studies, as did the increase in anaerobic respiration, such as sulphate reduction. Macleod et al. (2000) observed that during the early part of the fish-stocking period there was a net efflux of nitrogen from sediments under cages as nitrate and N_2. This indicated that the nitrogen deposited as part of the organic matter was remineralised through nitrification and denitrification. However, as the organic carbon load built up towards the end of the stocking cycle

(after 9 months), nitrogen was predominantly lost from the sediments as ammonia and the sediments became a net sink for nitrate, as was witnessed experimentally here. Christensen et al. (2000) found that DNRA became an important process in sediments that are undergoing organic enrichment. They found that DNRA was 7-fold greater than in sediments that had not undergone organic enrichment. It is therefore likely that the DNRA is responsible for some of the nitrate uptake and ammonium release in this experiment.

In general then, the effect of organic loading on nitrogen cycling results in the possible decoupling of nitrification and denitrification and increase in DNRA. Under minimal or low organic carbon loading, nitrification is responsible for cycling nitrogen released during organic matter degradation, which in turn supplies denitrification. However as organic carbon loading increases, respiration rates increase, and oxygen becomes depleted in the sediments and nitrification is reduced. Due to the reduction in nitrification, the nitrification-denitrification pathway is slowly decoupled. Nitrification can no longer supply all the demand for nitrate, which has increased due to the increase in denitrification and possible occurrence of DNRA, and therefore nitrate is drawn down from the water column. Furthermore, Caffrey et al. (1993) showed that as organic matter loading increased, the ratio of nitrification to denitrification decreased from 1.55 to 0.05, indicating that the importance of nitrification as the nitrate supply for denitrification decreased as organic carbon addition increased.

5.5 Conclusions

This experiment showed the initial response of sediment to a pulse of labile carbon. The results showed that the short-term response was related to the amount of carbon added. As the carbon concentration increased, the oxic zone decreased due to increased bacterial respiration, and the degradation pathway shifted from aerobic to anaerobic respiration as evidenced by the increasing values of the CRQ and increase in alkalinity fluxes to the overlying water column, most likely due to increased activity by sulphate reducers. This resulted in a rapid increase of nutrients released into the water column. While the time succession of bacterial processes was not studied, they could perhaps be modelled from the data in this study as the

concentration series in effect mimics the build up of carbon over time, with the impact on biogeochemical processes in this study comparable to observations of sediment response to algal blooms (Grenz et al., 2000) or fish farm waste (Macleod et al., 2004) in natural systems over time.

It is recognized however that the results from this study have come from simplified sediment systems. In natural systems, the response to organic loading is much more complex due to the influence of benthic fauna, different states of organic substrates and physical forcing such as tidal and river flows. This experiment does however show the importance of organic loading in determining the rates at which inorganic nitrogen and phosphate are released to the overlying water column and the effect organic loading has on sediment nitrification and denitrification.

Further work on this matter should investigate the effect of organic loading on specific processes such as sulphate reduction, nitrification, denitrification and DNRA. Additionally, experiments should be set up to determine the response of benthic infauna indigenous to the Huon estuary to organic loading. These studies are vital to understanding a more complete picture of the response of Huon estuary sediments to organic loading.

Chapter Six

General Discussion

Chapter 6. General Discussion

6.1 Introduction

The rapid growth of the Huon estuary aquaculture industry in Tasmania has lead to the need for sustainable development in order to maintain a healthy and viable industry. To enable this to happen, system-wide ecological models have been developed by the CSIRO to assist this process. A major part of these models has been to focus on the sources and sinks of nutrients in the estuary. The research undertaken in this thesis relates to the ecological role of sediments within the estuary, with a particular emphasis on benthic respiration and nutrient cycling processes and the main drivers (i.e. temperature, organic carbon, bioturbation etc) of these processes.

The first part of this chapter discusses the construction of conceptual models of benthic respiration and nutrient cycling combining the results from the first 3 research chapters of this thesis. Both the underlying assumptions and the knowledge gaps of the models are discussed. The models are then used as a reference to the remaining discussion, which discusses the role of the source of organic carbon to the sediment, and how the quality of this organic carbon regulates benthic respiration and nutrient cycling. I then discuss the effect of organic carbon loading on nutrient cycling and a final section on the ecological significance of nutrient cycling to the broader Huon estuary environment. I have also used this chapter to discuss the knowledge gaps arising from this study and suggest future research to close these gaps.

6.2 Conceptual nutrient cycling model for sediments in the Huon estuary

A conceptual model and budget of benthic respiration and nutrient cycling is shown for each study location in Figure 6.1a & b. Benthic respiration in the models was based on TCO_2 flux rates presented in chapter 3. Nutrient cycling was based on sediment-water nutrient flux rates presented in chapter 4. The amount of ammonium mineralisation during organic matter decomposition was based on carbon oxidation rates, which were obtained from TCO_2 flux rates from chapter 3, and the stoichiometric relationship between carbon and nitrogen of the organic matter

undergoing remineralisation. The total DIN mineralised during carbon oxidation was predicted by the following equation (Geoscience Australia, 2007):

$TDINp = C_{ox} \times (N:C)$

Where C_{ox} is benthic carbon dioxide flux, N:C is the stoichiometric relationship of the decomposing organic matter and TDINp is the total DIN predicted to be released. This equation follows the stoichiometric reaction for aerobic respiration:

$$106(CH_2O)16(NH_3) + (H_3PO_4) + 106O_2 \longrightarrow 106CO_2 + 16NH_3 + PO_4$$

For the purpose of the conceptual models I have assumed that the organic matter undergoing remineralisation is a mixture of both marine and terrestrial carbon. As an estimate of the average C:N ratio of the organic matter undergoing remineralisation, I have used the average C:N ratio's measured for both the Lower and Upper estuaries in Chapter 2. Therefore the C:N ratios used to model remineralisation and therefore ammonification and denitrification were 11.1 and 17.2 at the lower and upper estuary locations respectively.

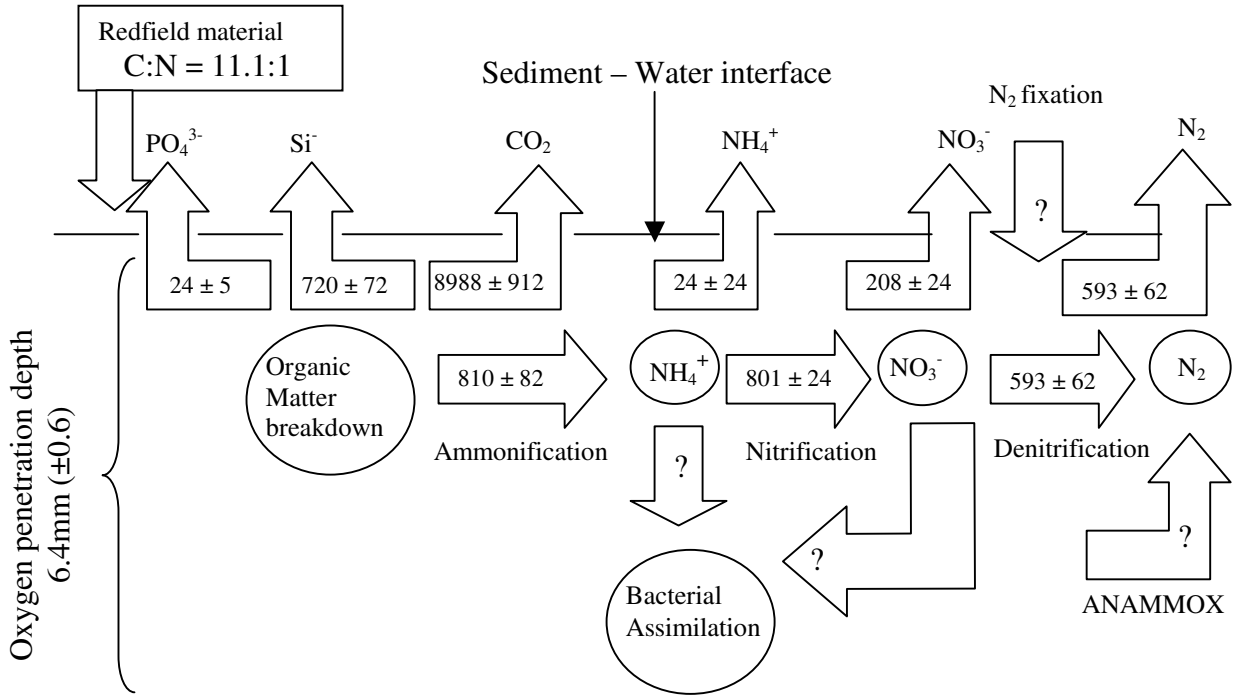
I have assumed that refractory terrestrial organic matter being deposited onto the sea floor is remineralised based on evidence from a study in Young Sound, North East Greenland where it was found that benthic carbon degradation was driven to a large extent by terrestrial sources of organic matter (Glud et al., 2000). Chapter 2 in this study showed that terrestrial sources of organic carbon was the dominant source of carbon at both sampling locations, and that only a small amount of marine organic matter was present at any time during the sampling periods. Therefore given the study by Glud et al. (2000) and the dominant presence of terrestrial organic carbon in Huon estuary sediments, it seems plausible that terrestrial sources of organic matter play an important role in driving carbon degradation in the Huon estuary. However, to get a better understanding of the types of carbon being remineralised, future studies should focus on direct measurements of the types of organic carbon reaching the sea floor, sedimentation rates and carbon burial rates.

Denitrification was predicted from the calculated rates shown in figure 4-7 (Chapter 4). The N_2 flux was then calculated using the following biochemical reaction:

$$106(CH_2O)\ 16(NH_3) + H_3PO_4 + 94NO_3^- = 106CO_2 + H_3PO_4 + 177H_20 + 55.2N_2$$

The nitrification rate was estimated by adding the denitrification rate together with the nitrate efflux (Seitzinger, 1987). The nitrate efflux is assumed to be the product of nitrification as discussed in this chapter 3. Phosphate and silicate fluxes were based on the sediment-water fluxes presented in chapter 3.

A: Lower estuary



B: Upper estuary

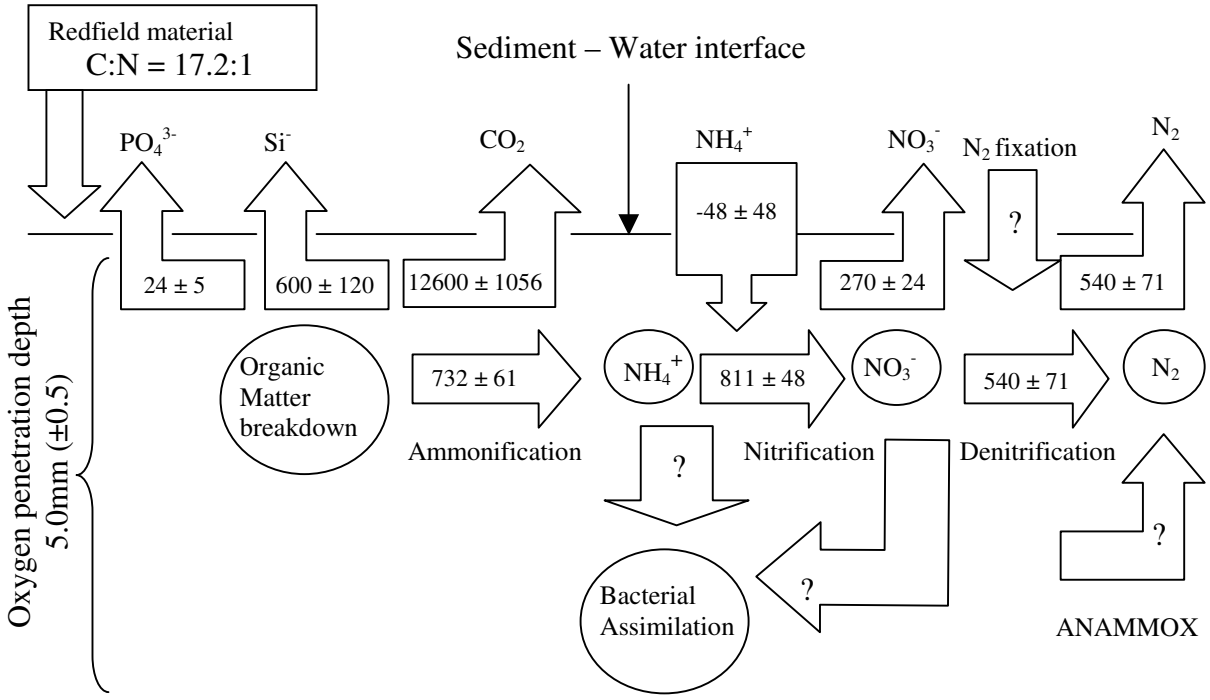


Figure 6-1 Respiration and nutrient models for A: Lower and B: Upper Estuary. All numbers given are the annual average flux rates with units of µmol m^{-2} d^{-1}. Numbers in italics represent the standard error of the mean.

One of the key assumptions made in the conceptual models is that all the ammonium mineralised during the oxidation of organic carbon is converted to nitrate via nitrification. The proposal that nitrification is occurring in the sediments is based upon a number of findings from this study. In chapter 3 it was established that the sediments had an oxidised zone between 3 and 9mm through out the study period. It is well established in the literature that oxidised sediments provide favourable environments for nitrification to occur (Hulth et al., 2005). Analysis of nitrate porewater profiles (Figure 4-4, Chapter 4) showed that peaks of nitrate occurred in the oxidised zone of the sediments, which suggests that ammonium mineralised during organic carbon decomposition was been oxidised to nitrite and then nitrate by nitrification. Additional to the results presented in this study, ammonium-oxidising bacteria were also detected in sediments at Stringers Cove, which is near the Huon estuary (Figure 1-3). Bissett (2004) analysed sediments for bacterial diversity and also specifically sought to identify the ammonia-oxidising community diversity. In that study, it was found that ammonia-oxidising bacteria (AOB) populations were always present in high numbers irrespective of organic carbon loading.

Therefore, the evidence suggests that nitrification is occurring in Huon estuary sediments. However, whether or not nitrification is responsible for consuming most of the ammonium produced in the sediments is debatable. A number of knowledge gaps were identified due to the difficulty in interpreting metabolic processes from sediment-water fluxes derived from core incubations and porewater profiles. In the models, I have assumed that the amount of ammonium mineralised during organic matter decomposition was based on carbon oxidation rates, which were obtained from TCO_2 flux rates from chapter 3, and the stoichiometric relationship between carbon and nitrogen of the organic matter undergoing remineralisation. In balancing the models I assigned the remaining ammonium, not released to the overlying water column, to nitrification. However, as shown in the conceptual models this ignores the possibility that the ammonium liberated during organic carbon mineralisation is reassimilated by bacteria (Lomstein et al., 1998) or directly converted to N_2 via ANAMMOX (Thamdrup and Dalsgaard, 2002; Trimmer et al., 2003). Lomstein et al. (1998) found that 86% of ammonium mineralised was reassimilated into microbial biomass.

Table 6-1 compares the validity of the assumptions made in the conceptual models (i.e. 0% bacterial assimilation) against calculations incorporating 86% bacterial assimilation of ammonium as found by Lomstein et al. (1998) and 43 %, half the bacterial assimilation of ammonium as found by Lomstein et al. (1998). The results of incorporating bacterial assimilation into the conceptual models, reduces the amount of both nitrification and denitrification, as would be expected. Bacterial nitrogen assimilation of 86% however is too high as all the remaining ammonium not assimilated by bacteria is nitrified and released to the overlying water and denitrification flux rates are negative, which is not plausible. Bacterial assimilation at 43% creates a more balanced equation, and results in 20 - 31% of ammonium mineralised being denitrified, which is more in line with denitrification rates measured at Stringers Cove (Macleod et al., 2004).

Table 6-1. Summary of a) ammonification (as calculated by C_{ox} x N:C with C_{ox} obtained from TCO_2 flux rates and N:C assuming a Redfield ratio of 1:6.625), b) bacterial assimilation using rates of 0% as used in conceptual models, 86% as found by Lomstein et al. (1998) and 43% half the bacterial assimilation found by Lomstein et al. (1998), c) nitrification (estimated by adding denitrification rate with nitrate efflux), d) nitrate efflux (as measure in core incubations in chapter 4), and e) denitrification (as calculated in chapter 4 for the conceptual models assuming 0% bacterial assimilation; where I have assumed 43 and 86% bacterial assimilation, denitrification is the difference between nitrification and nitrate efflux). All units are in µmol m^{-2} d^{-1}.

	Ammonification (C_{ox} x N:C) a	Bacterial assimilation b	Nitrification c	Nitrate efflux d	Denitrification e
Lower estuary conceptual model	810	0	801	208	593
Upper estuary conceptual model	732	0	811	270	540
Lower estuary conceptual model	810	86% assimilation 697	a-b 113	208	c-d -95
Upper estuary conceptual model	732	86% assimilation 630	a-b 102	270	c-d -168
Lower estuary conceptual model	810	43% assimilation 348	a-b 462	208	c-d 254
Upper estuary conceptual model	732	43% assimilation 315	a-b 417	270	c-d 147

The denitrification rates measured by Macleod et al. (2004), using the isotope pairing technique, ranged between 192 and 456 µmol m^{-2} h^{-1} in unimpacted sediments at Stringers Cove adjacent to the Huon estuary. The surface sediments in Stringers Cove have similar organic matter content, oxygen penetration depth and DIN, nitrate and ammonium fluxes to those measured in the Huon estuary here. The denitrification rates estimated in the 43% bacterial assimilation scenario (147 – 254 µmol m-2 h-1) are around the low end of rates measured by Macleod et al. (2004), while the denitrification rates estimated in this study assuming 0% bacterial assimilation (540 –

593 µmol m-2 h-1) are higher than the rates measured by Macleod et al. 2004). Therefore it is likely that assuming a 0% bacterial assimilation rate as I have done in the conceptual models is likely to overestimate both the nitrification and denitrification rates and therefore future research should involve measuring bacterial nitrogen assimilation rates.

The conceptual models have also ignored the alternative nitrogen cycling pathways of ANAMMOX and DNRA as they were not measured in this study. DNRA however is unlikely to be important in these sediments, as it is usually only detected in sediments that have undergone organic enrichment, because it typically involves anaerobic forms of respiration, rather than aerobic respiration (Christensen et al., 2000). ANAMMOX however has been detected both in marine (Thamdrup and Dalsgaard, 2002) and estuarine sediments (Trimmer et al., 2003) although its relative importance to the total N_2 flux can range between 2 and 70% (Hulth et al., 2005). It is not possible to distinguish the relative importance of denitrification and ANAMMOX in this study, and therefore it is impossible to say at this time what impact ANAMMOX might have on the conceptual models. Future research of nitrogen cycling in Huon estuary sediments should therefore aim to measure both denitrification and ANAMMOX and therefore determine their relative importance.

N_2 fixation has also been detected in marine sediments and is especially important in heterotrophic marine sediments where the availability of organic carbon is low. It has been shown in low carbon-availability heterotrophic sediments that N_2 flux is reversed due to the reduction in denitrification and increase in N_2 fixation and therefore sediments become a net source of nitrogen rather than a sink (Fulweiler et al., 2007). Given that the sediments in this study have very small amounts of labile carbon and denitrification is likely to be small, based on direct measurements by Macleod et al, (2004), it is possible that N_2 fixation maybe occurring in Huon estuary sediments.

In summary, the conceptual models suggest that most of the ammonium mineralised during organic carbon degradation is processed via coupled nitrification-denitrification, where nitrification takes place in the upper oxic zone of the sediments and denitrification in the sub-oxic zone just below the oxic/sub-oxic interface (Hulth

et al., 2005). The observations from sediment-water fluxes and stoichiometric ratios between carbon, nitrogen and phosphate fluxes tend to support the coupled nitrification-denitrification pathway for processing remineralised nitrogen. However, as I have discussed, a number of important gaps have being identified in the conceptual models, in particular the role of bacterial assimilation and ANAMMOX. Therefore what is needed next, are studies that directly measure some of the key processes at the same time to improve the conceptual understanding of sediment processes. For example it would be instructive to be able to measure ammonification, bacterial assimilation, nitrification, denitrification and ANAMMOX, at the same time.

6.3 The influence of organic carbon on benthic respiration in the Huon Estuary

No previous studies in the Huon estuary have attempted to relate the sources of organic matter to sediment metabolism, with the exception of a study carried out by Cook et al. (2004) in the intertidal zone of the estuary. Therefore, one of the main aims from this study was to identify the sources of the organic matter in sub-tidal sediments, and to evaluate how the 'quality' of this organic matter influences respiration and nutrient cycling.

Research from chapter 2 established that sediments at the study locations are rich in organic carbon (4.1 to 8.8% of dry weight) with the organic carbon coming from a range of sources including terrestrial, bacterial, algal and marine animal matter. However, terrestrial organic matter was the dominant source. Lipid biomarker analysis showed that the terrestrial organic matter was from the same source at both study locations, however the proportion of the terrestrial organic carbon to total organic carbon was lower at the lower estuary stations (55.7 to 58.6%) compared to the upper estuary (72.2 to 75.3%).

The main sources of terrestrial organic carbon are most likely from terrestrial runoff from the upper catchment via discharge from the Huon River (Butler et al., 2000). The upper catchment is largely intact and relatively pristine, with the vegetation of the catchment characterised by alpine (austral-montane), temperate rain forest and

Sclerophyll forests (Eucalyptus spp.) with only 5.6% of the catchment currently cleared. Therefore, anthropogenic sources of carbon and nutrients, is likely to be low. The lower catchment is characterised by a small population with, the main industry being agriculture, in the form of horticulture and extensive livestock grazing. Therefore large sources of anthropogenic inputs of carbon and nutrients from activities such as sewage treatment, intensive agriculture and large-scale industry (e.g. manufacturing industries) are low also.

The relatively pristine nature of the environment surrounding the Huon estuary is therefore important in regulating the quality of organic matter entering the Huon estuary and also phytoplankton production within the estuary. Because anthropogenic sources of carbon and nutrients are low, the water quality is high in the Huon estuary, with both nitrogen and phosphate concentrations low throughout the year. The low nutrients combined with the highly coloured nature of the water column (due to CDOM and tannins from the upper catchment) serve to limit phytoplankton production rates. The Huon Estuary Study found phytoplankton growth to be only moderate with (geometric) mean and median chlorophyll *a* values of about 1 mg chl *a* m^{-3} and only exceeding 4 mg chl a m^{-3} about 10% of the time (Butler et al., 2000).

The high inputs of terrestrial organic carbon, low anthropogenic nutrient inputs and moderate phytoplankton growth in the estuary, therefore has important consequences for benthic respiration in the Huon estuary, which were generally low, ranging between 228 and 840 μmols m^{-2} h^{-1} CO_2. To gauge the magnitude of the respiration rates and organic carbon content measured in this study, they were compared to a number of other studies previously undertaken in a range of different sedimentary environments including intertidal zones, shallow coastal sediments, deep coastal sediments and deep-sea sediments. Table 6-2 shows that Huon estuary sediments have the highest concentrations of organic carbon (4.1 to 8.8% DW) of the reviewed studies, with the exception of Plum Island Sound (USA) (10.3%). However, respiration rates in the Huon estuary (228 to 840 μmols m^{-2} h^{-1} CO_2) were much lower than in sediments found in intertidal zones and shallow coastal environments (Table 6-2) where the presence of labile organic carbon in the form of decaying benthic algal material, together with higher temperatures, is likely to drive the higher respiration

rates in these environments (see discussion below about microphytobenthos in the Huon estuary).

Table 6-2 shows that the respiration rates in this study were more comparable with the rates observed in deeper coastal sites and deep-sea sediments such as Monterey Bay in California and the sediments of the Baltic Sea. A major contrast between the sediments in this study and the sediments of Monterey Bay and the Baltic Sea however is the organic carbon contents. The Huon estuary ranged between 4.1 and 8.8% while the Baltic Sea sediments ranged between 1.0 and 3.6%, and Monterey Bay had organic carbon contents of 0.4%, which is10-fold lower than compared with sediments of this study. The large differences in respiration rates relative to total organic carbon between these environments, suggests that the fraction of bio-available carbon is much lower in the Huon estuary than either Monterey bay or the Baltic Sea.

To assess the bioavailability of organic carbon in different sedimentary environments, respiration rates (as measured by CO_2) were normalised to organic carbon contents. Figure 6-2 shows that the Huon estuary sediments from this study had the lowest rates of respiration normalised to organic carbon compared to other environments. This indicates that most of the organic carbon in the sediments of the Huon estuary is not labile and therefore is refractory to biodegradation, which inturn drives the low respiration rates.

Table 6-2 Summary of % organic carbon, CO_2 & O_2 fluxes (µmols m^{-2} h^{-1}) and oxygen penetration depth reported in benthic systems from a range of different systems. (The average was calculated for a range of values by averaging both the minimum values and maximum values. The result was to have a range based on the average minimum values and maximum values obtained for each type of sedimentary location)

Location	Temperature (^oC)	Salinity (‰)	% Organic Carbon	CO_2 Flux	O_2 Flux	O_2 Penetration Depth (mm)	Reference
Intertidal Sediments							
Huon Estuary, Tasmania	5.0 – 21.0	4 - 35	1.0 – 7.0	375 - 6875	500 - 2583		Cook et al 2004
Flax Pond and Long Island Sound, New York	4.0 – 21.5		2.0 – 4.0	1375 - 6667	958 – 5708	0.9 – 2..5	Mackin & Swider 1989
Bangrong Mangrove Forest, Phuket Island				1317 - 2596	642 - 1688		Kristensen et al 2000
Tagus River, Portugal		3 - 37			250 - 5000	0.4 – 1.8	Cabrita & Brotas 2000
River Great Ouse, England	4.5 – 22.0		0.1 – 2.2		500 - 7000		Trimmer et al 1998
River Colne, England	1.5 – 24.0	2 – 34	0.4 – 3.7		1000 - 8000		Dong et al 2000
Average	**3.8 – 22.1**	**3 - 35**	**0.9 – 4.2**	**1022 - 5379**	**642 - 4997**	**0.6 – 2.2**	
Shallow Coastal Sediments (<20m)							
Port Phillip Bay, Victoria	10 .0– 13.0			750 - 5000	792 - 4250		Berelson et al 1998
Golfe de Fos, Mediterranean Sea					533 - 1308	3.0 – 15.0	Rabouille et al 2003
Boston Harbour, Massachusetts	1.0 – 18.0		0.1 – 6.1	417 - 7708	292 - 9167		Giblin et al 1997
San Francisco Bay, California	12.0 – 17.0		1..3 – 1..5	417 - 1375	458 - 917		Hammond et al 1985
Galveston Bay, Texas	12.4 – 30.1	1 - 20	0.5 – 1.6		408 - 708		Warnken et al 2000
Northern New South Wales Estuaries, (Aust)	14.0 – 31.0	8 – 35	0.1 – 6.2	37 - 2254	519 - 1545		Ferguson et al 2003
Cherrystone Inlet, Chesapeake Bay	6.5 – 32.4	14 - 23			18 - 3844		Reay et al 1995
Plum Island Sound,	3.6 – 28.1	0 – 32	0.2 – 10.3	125 – 21,667	250 – 18,041		Hopkinson et al 1999
Douro River Estuary, Portugal	5.5 – 24.0	5 – 19			353 - 2013		Margalhaes et al 2002
Elkhorn Slough	7.0 – 30.0	0 - 66			304 - 4479	1.0 – 6.5	Caffrey et al 2002
Average	**8.0 – 24.8**	**5 - 33**	**0.4 – 5.1**	**349 - 7601**	**393 - 4627**	**1.5 – 11.0**	
Deep Coastal Sediments (21 – 100m)							
Continental Shelf, Eastern Massachusetts	1.5 – 12.0		0.4 – 3.4	308 - 2070	258 - 1254		Hopkinson et al 1999
Northern Adriatic Sea	18.0 – 22.0			508 - 1050	220 - 829		Hammond et al 1999
North-Western Black Sea					237 - 1079		Friedrich et al 2002
Monterey Bay, California		4.0 – 8.0	0.4	196 - 742	208 - 563		Berelson et al 2003
Western Irish Sea	11.5		0.1 – 0.8		229 - 852		Trimmer et al 2003
Southern Kattegat, Baltic Sea	5.3 – 12.7	25 - 32			320 - 539	1.9 – 9.1	Rasmussen & Jorgensen 1992
Kattegat, Denmark	5.3 – 7.0	19 - 34	0.3 – 3.6	138 - 1300	235 - 965	3.3 – 6.0	Rysgaard et al 2001
Average	**7.0 – 12.7**	**25 - 34**	**1.2 – 4.2**	**288 - 1291**	**244 - 869**	**2.6 – 7.6**	
Huon Estuary, Tasmania	**8.0 – 16.0**	**30 - 35**	**4.1 – 8.8**	**228 - 840**	**151 - 592**	**3.1 – 9.0**	**This study**
Deep Sea Sediments (>100m)							
Svalbard, Artic Sea	-1.9 – 2.8	35	0.8 – 2.1		77 - 467	5.0 - ≥59	Hulth et al 1994
Skagerrak, Baltic Sea	~5.0		1.0 – 3.0	538 - 604	326 - 399		Arnosti & Holmer 2003
Pacific Ocean (off Central Chile)	1.7 – 5.6			12.5 - 763	58 - 654	6.5 – 129.7	Glud et al 1999
Skagerrak, Baltic Sea	5.5 – 7.0	34 – 35	1.8 – 3.2	200 - 444	173 - 523	5.0 – 19.0	Rysgaard et al 2001
Northern Aegean Sea	13.5 – 16.6	39	0.2 – 0.6	35 - 450	48 - 289		Stahl et al 2004
Average	**4.7 – 7.4**	**36**	**1.0 – 2.2**	**196 – 565**	**136 - 466**	**5.5 – 69.2**	

An interesting comparison can be made between this study and the study carried out on the inter-tidal area of the Huon estuary by Cook et al. (2004). Like this study, Cook et al. (2004) found that the intertidal sediments were dominated by refractory terrestrial organic carbon. However, although microalgal sources were low, they found that microphytobenthos-derived material was the main driver of bacterial production via consumption of extracellular carbohydrates produced by the microphytobenthos.

The presence of microphytobenthos, and thus supply of extracellular carbohydrates, provides the key difference between the high respiration rates of the inter-tidal sediments compared with the low respiration rates of the sub-tidal sediments from this study. It was concluded earlier in the thesis (chapter 3) that microphytobenthos were unlikely to be present in sub-tidal sediments, largely due to light limitation at the sediment surface. The main source of labile microalgae material that is supplied to the sub-tidal sediments is therefore produced in the water column of the estuary, which generally has only moderate phytoplankton levels (Butler et al., 2000). Thus due to the combination of the likely absence of microphytobenthos and only moderate production rates of phytoplankton in the water column, only small amounts of labile organic carbon reach the surface of the sub-tidal sediments. Therefore sediment respiration rates are much smaller in sub-tidal sediments compared with inter-tidal sediments as is shown in Figure 6-2.

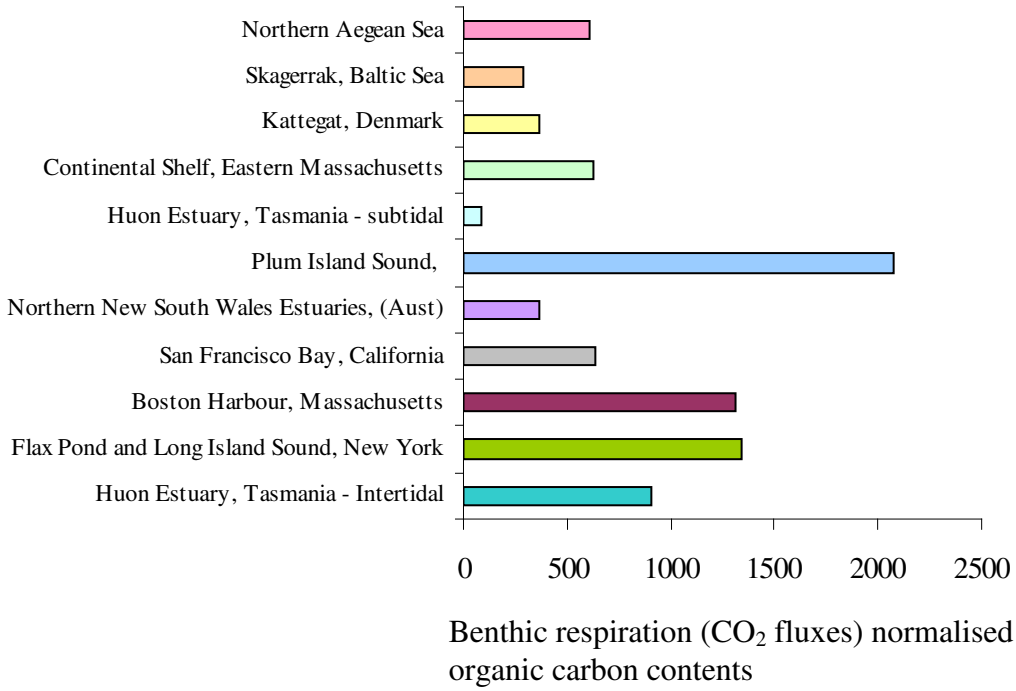


Figure 6-2 Benthic respiration (as measured by CO_2 fluxes (µmols m^{-2} h^{-1})) normalised to % organic carbon contents. The data represents the average of the range of CO_2 fluxes and organic carbon contents for each environment given in Table 6-1.

6-4 How does sediment respiration and nutrient cycling respond to organic carbon perturbation

A major threat to the pristine nature of the Huon estuary however is the emergence of the aquaculture industry, namely salmon farming. The fish farms are important point sources of nutrients (Table 6-3) to the estuary with inputs coming from leaching of food, fish excretion and remineralisation of uneaten food and faeces in the sediments. The HES (Butler et al., 2000) suggested that nutrient inputs from the fish farming industry have significantly increased chlorophyll and DIN levels in the estuary. According to the HES model, doubling 1997 finfish-farming production could lead to increased frequency or density of summer blooms, while quadrupling loads would put the system on the brink of N saturation, and would substantially increase the risk of

prolonged blooms. Increasing loads by a factor of 10 would completely change the nature of the system, producing elevated DIN and large blooms throughout summer (Butler et al., 2000). Since, the conclusion of the HES, new data collected on Chlorophyll *a* levels between 2002 and 2004 have shown that chlorophyll *a* levels have increased significantly since the time of the HES (Thompson and Parslow, 2005). Therefore increasing levels of phytoplankton in the water column may lead to the organic enrichment of the sediments, which inturn may feed nutrients back into the water column further exacerbating the nutrient enrichment of the water column.

The HES models were developed however in the absence of information in regards to sediment biogeochemical processes and therefore little is known about the potential response of the sediment system to increased loads of organic matter. This study has now helped to fill that gap by establishing baseline flux measurements of analytes associated with respiration and nutrient cycling in unimpacted sediments and also undertook an experiment where sediments were loaded with labile organic carbon to examine the changes in respiration, nutrient fluxes and porewater concentrations.

The organic carbon loading experiment in chapter 5 showed that sediments in the Huon estuary are very responsive to organic enrichment, whereby organic matter is rapidly decomposed releasing large amounts of nutrients. The results from chapter 5 showed that as carbon load increased, oxygen consumption increased in the sediments and the oxygen pool was reduced. The reduction in oxygen availability is likely to reduce the ability of the ammonium-oxidising bacteria to convert ammonium to nitrate, which leads to an increase in the flux of biologically available ammonium to the water column. The reduction in nitrification relative to ammonification in the sediments results in the decoupling of nitrification and denitrification and direct denitrification becomes more important. This is shown in the sediment – water fluxes where nitrate fluxes are reversed from being effluxes to the water column when nitrification is the dominant process to been taken up by the sediments under heavy organic loading, presumably due to the demand from denitrification.

The results in chapter 5 mirrored results from the fallowing project at a fish farm at Stringers Cove (Macleod et al., 2004). They found that nitrogen in sediments under the fish cage was predominantly lost in the form of ammonium to the water column as

organic carbon built up, and also found that the sediments became a net sink for nitrate. Macleod et al. (2004) found that although denitrification rates increased to a point where, with high organic loading, it was removing approximately ten times the amount of nitrogen entering the sediment as nitrate this was still only 5% compared to that lost as ammonium. This is the converse of what they observed in unimpacted sediments, in which denitrification was strongly coupled with nitrification.

Therefore, the conceptual functioning of sediments is very different under increasing organic carbon loading compared to the conceptual models outlined in section 6.1. As organic carbon increases ammonification increases and oxygen is decreased because of increased respiration. As oxygen decreases, nitrate maybe used as a terminal electron acceptor in anaerobic respiration, possibly denitrification, but also to dissimilatory nitrate reduction to ammonium (DNRA). Christensen et al. (2000) found that DNRA increased 7-fold in sediments under fish cages compared to un-impacted sediments. The other possibility is ANAMMOX, but irrespective of the rates of both denitrification and ANAMMOX, ammonia is the major form of nitrogen released under high organic carbon. Hence, either or both of DNRA or diffusive ammonium fluxes directly from ammonification are drivers of very high amounts of ammonium released to the water column.

This switch to release of ammonium from the sediments has the potential to change the nitrogen profile of the water column from one that is normally dominated by nitrate concentrations to an environment dominated by ammonium concentrations. Such a change to the nitrate:ammonium ratio in the water column may impact on phytoplankton ecology of the estuary by shifting the phytoplankton composition to more toxic species such as *Gymnodinium catenatum* (Butler et al., 2000). Blooms of *G. catenatum* can have serious effects on the local aquaculture industry, in particular the shellfish industry. In the past, *G. catenatum* blooms have caused closures of shellfish farms in the Huon Estuary for periods of up to nine months (Butler et al., 2000).

The HES showed that high concentrations of ammonium in the bottom phase of the water column maybe a key condition in supporting a bloom of *G. catenatum.* An aspect of their biology that supports this hypothesis is their ability to vertically

migrate diurnally. Migration to the bottom waters during the night enables uptake of ammonium released from the sediments to support photosynthesis in the surface waters the following day. Being flagellate gives *G. catenatum* a competitive advantage over diatoms for getting at this ammonia. Therefore, maintenance of the nitrification-denitrification system in the sediments is seen as important to the long-term sustainable use of the estuary for aquaculture.

6-5 Ecological significance of Benthic Nutrient Fluxes in the Huon Estuary

One of the key aims of this study was to address the knowledge gap from the Huon Estuary Study (HES): specifically to ascertain whether or not the source of nitrogen emanating from the bottom waters was nutrient regeneration in sediments. A second aim was then to evaluate the importance of benthic nutrient regeneration to the broader Huon estuary system and provide data to help calibrate the 3D nutrient models developed by the CSIRO.

While this study can not rule out the potential for sediments to provide ammonium to the water column during summer, as no field campaign was conducted during a summer algal bloom period, this study can suggest that, in unimpacted areas (which constitute the vast proportion of the estuarine area), deposited nitrogen is successfully recycled via the nitrification-denitrification pathway as shown in the conceptual models and that on many occasions the sediments acted as net sinks of ammonium; or that ammonium is reassimilated into microbial biomass or directly lost as N_2 via ANAMMOX. Either way, sources of ammonium and nutrients in general, are retained in the sediments or converted to N_2 and therefore no longer biologically available in the water column.

In order to put into context the significance of the sediments as a source of nutrients, I have compared annual nutrient inputs from sediments with inputs from other sources calculated in the HES. All the data for each nutrient from the 3 sampling trips in 2004 were pooled and an average annual flux calculated. The average annual flux was then

multiplied by the surface area of the estuary floor (77.4 km^2), excluding the 5% associated with intertidal areas, to get an estimate of estuary-wide nutrient inputs from un-impacted sub-tidal sediments. It is noted that the extrapolated Huon estuary inputs from sediments are only approximate as they are based on only a few laboratory measurements. However, sediment biogeochemistry results in the HES, suggest that apart from small regions of sand, mud (i.e. sediment grain size < 63 microns) comprised almost the entire estuary floor in its middle and lower reaches. Therefore, the extrapolation of the results from this study to the whole estuary is not unreasonable for a first estimation of the importance of nutrients from benthic regeneration.

Using these estimates, the extrapolation shows that the sediments are only minor sources of both nitrogen and phosphate compared to other sources, which supports my argument that sediments have low respiration rates and potentially significant denitrification and/ or ANAMMOX rates. The major source of nitrogen to the Huon estuary comes from the marine system (Butler et al., 2000) (Table 6-3), which is approximately 8 times higher then the Huon River, the next highest source. Marine waters are also the major source of phosphate to the estuary, about 9 times higher then the Huon River, the next most prominent source. The sediments provide about 32 tonnes of inorganic phosphate the third most important source, but approximately 2 orders of magnitude below marine sources. Overall the sediments are providing an N:P ratio of 3, well below "Redfield ratio" of 16:1, the ratio that phytoplankton assimilate N and P. This means that phytoplankton must obtain nitrogen from sources other than what is generated by the sediments to meet their nutritional requirements.

Table 6-3 Annual loadings of nutrients to the Huon estuary (tonnes)

Source	TDN	NO_3	NH_4	TDP	Reference
Huon River	966	51	23	142	Wild-Allen et al. (2004)
Kermandie River	32	9	8	1	Wild-Allen et al. (2004)
STP outfalls	28	-	-	9	Butler et al. (2000)
Fish farms	268	-	-	25	Wild-Allen et al. (2004)
Atmospheric	18	-	-	-	Butler et al. (2000)
Marine	7654			1272	Butler et al. (2000)
Sediments	96	91	12	32	Current study

Note: In order to compare with the nutrient fluxes from this study, table 6-3 only reports dissolved nutrients

Overall, the sediments are relatively minor contributors of nutrients to the broader Huon estuary. The sediments are net contributors of ammonium overall, but showed on many occasions that they can act as sinks for ammonium as well. Furthermore, ammonium inputs from the sediments are small with the Huon River and fish farming industry providing larger contributions. The sediments provide 7 to 8 times more nitrate to the water column than ammonium presumably due to intensive nitrification, however it is still dwarfed by the marine inputs of nitrate. The sediments also provide phosphate to the water column, however once again this is only minor in comparison to sources from the ocean and the Huon River.

Nitrification and denitrification within the sediments may therefore provide an important ecological service to the broader Huon estuary environment. On many occasions during this study, the sediments were a sink for ammonium and a source of nitrate presumably due to intensive nitrification. In fact, the highest ammonium uptake rates and nitrate effluxes to the water column occurred during the November sampling campaign at the Upper estuary sites, when ammonium concentrations in the water column were at their highest concentration (=3.1 μM). This suggests that the rate-limiting step for nitrification was ammonium production in the sediments and the AOB obtained ammonium from the water column to meet their requirements.

In light of these findings, it is proposed that nitrification and subsequent denitrification in the sediments plays an important role in regulating the amount of nitrogen for biological uptake in the water column. Ammonium is initially drawn down from the water column by the AOB, which convert the ammonium to nitrate. The nitrate is then either denitrified to di-nitrogen gas and no longer available for biological uptake or released to the water column.

6-6 Conclusions

This study has shown that benthic fluxes of nutrients into the water column are low in the Huon estuary. The relatively low rates of respiration, due to the refractory nature of the organic carbon, are thought to be the key to this finding. In the Huon estuary context low nutrient fluxes and respiration rates, along with the normal functioning of

the nitrification-denitrification pathway can be considered as key indicators of a healthy sediment ecosystem.

Organic loading of these sediments with labile carbon has shown that the nitrification-denitrification pathway is susceptible to being overwhelmed, due to increased respiration and reduction in the pool of oxygen in the sediments. This results in large amounts of ammonium being released into the water column and therefore makes the estuary more susceptible to blooms of algae, of which toxic dinoflagellates such as *G. catenatum* are especially important. Therefore maintenance of 'healthy' sediments is seen as important to the long-term sustainable use of the estuary.

This study has provided insights into how sediments function in relatively pristine coastal environments, in particular what the sediments are not doing at the moment (such as releasing large amounts of ammonium), but what they could be doing if labile organic carbon is increased. The findings have provided a good contrast to sediments that exist in environments exposed to increasing anthropogenic influence and provide a snapshot of how heavily polluted systems may once have functioned. This study has therefore added to the growing literature of how coastal sediments recycle carbon and nitrogen and their importance to local and global carbon and nitrogen cycles.

Finally this thesis has resolved a number of the original aims of this study which included identifying the major sources of organic carbon, estimating both rates and main drivers of benthic respiration and nutrient cycling, identifying the key processes that underpin respiration and nutrient cycling such as aerobic respiration and nitrification and to observe the response of the sediments to organic enrichment. However a number of important knowledge gaps have been identified from this study that should be included in future research undertaken in the Huon estuary. To build on the foundations laid by this study, the next step would be to carry out a set of carefully designed experiments in regards to both benthic respiration and nutrient cycling. In particular, an experiment that simultaneously measures bacterial nitrogen assimilation, ANAMMOX, denitrification and nitrification would be useful to gain a better understanding of the nitrogen cycle. These experiments should be carried out under both unimpacted and impacted sediments as while I have shown that organic

carbon loading alters sediment metabolism which is consistent with the literature, at this stage it is still unknown as to exactly how benthic metabolism would be controlled if farming does greatly increase. Furthermore, how these individual processes (i.e. nitrification, ANAMMOX, denitrification and DNRA) collectively influence nutrient fluxes from the sediment under unimpacted and impacted conditions needs to be elucidated.

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