Investigating The Links Between Faunal Activity And Organic Geochemistry In Continental Margin Sediments: Tracer Studies Across The Arabian Sea Oxygen Minimum Zone

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Doctor of Philosophy
University of Edinburgh
2005

Declaration

I, Clare Woulds declare that the work in this thesis, presented for the degree of PhD, is all my own, except where otherwise stated.

Abstract

The cycling and burial of organic matter (OM) in marine sediments is the main driver for all seafloor processes and is important in the biogeochemical cycles of C, other bioelements, and trace metals. Factors including sedimentation rate, oxygen exposure, interactions with mineral surfaces, and OM supply rate and reactivity, affect the efficiency with which sedimentary OM becomes buried. Fauna also influence OM burial efficiency, through activities that lead to sediment mixing and ventilation, alteration and re-mineralisation of OM, and stimulation of microbial activity. Uncertainty remains, however, as to the interactions and relative importance of the factors that determine OM burial, and, of these, faunal processes are the least well described or quantified. Questions remain regarding the way in which faunal activity, oxygen availability and OM supply interact and influence each other, and OM burial efficiency. Previous studies of faunal processes have mostly been limited to microcosm experiments involving single species of fauna, and, critically, studies of whole communities under *in situ* conditions have been particularly rare.

The continental margin of the Arabian Sea exhibits sedimentary OM enrichments roughly coincident with a mid-water oxygen minimum zone, which have contributed to an ongoing debate over OM distributional controls. OM distributions across the Arabian Sea's margins are only partially explained by patterns of oxygen availability, and the link between fauna and sediment geochemistry in this region has not been explored.

The objectives of this study were to investigate the role that fauna play in short-term OM processing, how this varies with OM supply and quality, oxygen availability and faunal community structure, and how faunal activity is linked to sediment organic geochemistry. This was achieved through experiments and organic geochemical sampling, at sites spanning the OMZ on the Pakistan margin. The steep gradients in OM quantity and quality, oxygen, and faunal communities, and seasonal changes in OM supply, provided an exceptional natural setting to assess these relationships.

Incubation studies were conducted on intact sediments containing whole faunal communities, aboard ship and *in situ* and using a novel system to maintain ambient

oxygen levels. ^{13}C-labelled algae were added to the sediments and traced into organisms, sediments, and respired pools. The resulting carbon budgets are some of the most complete to date, and allowed direct comparison among an unprecedented range of site conditions. Where macrofauna and higher-quality OM were present, OM uptake by fauna was greater; at one site macrofaunal uptake equalled total respiration, illustrating the key significance of fauna. Oxygen had a threshold effect on the faunal groups responsible for OM processing, with foraminifera and macrofauna dominating OM processing below and above the threshold, respectively. Notably, a new technique was developed for the quantitative tracing of labelled amino acids, which allowed the first molecular level tracing of OM in whole community and *in situ* experiments, and provided among the first direct links between faunal digestive activity and sediment OM composition. Compound-selective assimilation and OM alteration were observed, the patterns of which were taxon-specific.

The pigment and carbohydrate contents of sediments showed that OM on the Pakistan margin was of generally low quality (degraded), despite the presence of the OMZ. Abundances of these biochemicals were partially controlled by oxygen availability, but other important factors included the degree of OM decay during passage through the water column. Links between faunal biomass and sediment organic geochemistry were weak. However, higher faunal abundances were generally associated with lower concentrations, which could be the result of faunal burrowing activities and sediment ventilation.

This is a detailed study of the interaction of faunal activity and sediment organic geochemistry, across sites exhibiting a wide range of biogeochemical conditions. It shows how both organic matter dynamics and faunal activity influence each other, and are influenced by oxygen availability, and provides new molecular level information regarding the functioning of benthic ecosystems in sediment C-cycling.

Acknowledgements

First and foremost I would like to thank Greg Cowie for his unstinting help and support, and for allowing me the freedom to work as I chose. I am also deeply indebted to Steve Mowbray for his teaching and assistance in the laboratory, and to Matt Schwartz for being a great friend and co-worker.

I have received valuable and welcome advice (and data) at all stages of my work from Lisa Levin and Jack Middelburg, and this is greatly appreciated.

This project was made possible by the extremely hard work of all the scientists who sailed on cruises CD 145, CD 146, CD 150 and CD 151. I would particularly like to thank Kate Larkin, Andy Gooday, Rachel Jeffreys, Lisa Levin and Christine Whitcraft for spending long hours extracting fauna from the ^{13}C tracing experiments, and also Henrik Andersson and Sandra Vandewiele, both for their help with running the experiments at sea, and also for being such efficient and sharing collaborators thereafter. I am indebted to Oli Peppe, Eric Breuer and Willie Thompson for running the landers and corers, and also to Gareth Law, Sue McKinley, and everyone else with whom I sailed. Many thanks to Brian Bett and the shipboard parties of cruises CD 145 and CD 150.

Thanks also to the captain, officers, engineers and crew of the RRS Charles Darwin, and to the SOC engineers, all of who helped to make our cruises successful. Thanks also to our agents in Muscat, Inchcape Shipping Agents.

In addition to those already mentioned, I would like to thank Peter Lamont, John Gage, Stephanie Schumacher, Eric Breuer and Gareth Law for sharing their data with me.

Also thanks go to Lisa Levin and Peter Lamont for educating me in benthic biology.

I carried out pigment analyses at NIOO in The Netherlands, and this was greatly facilitated by Jack Middelburg. While I was there, Marco Houtekamer and Jan Sinke, who processed all my chromatograms, made sure the work ran very smoothly. The modelling of pigment data was accomplished with a model sent to me by Tim Brand, and benefited from the valuable advice of Karline Soetaert.

Carbon isotopic analyses were carried out principally by Charlie Scrimgeour at the Scottish Crop Research Institute, who was always most helpful, and sent me data almost instantaneously. Further ^{13}C analyses were carried out by Robert Michener and Dave Harris. Jen Gonzalez was also incredibly helpful in organising catalogues of faunal samples, somehow between us we sorted them out!

I have received help, advice and correspondence from Tom Preston, Ming-Yi Sun, Cindy Lee, Neal Blair, Leon Moodley, Hidetaka Nomaki, Helen Kettle, Mark Teece, Ralph Goericke and Rick Keil. They have all been very willing to help me, even though I contacted most of them out of the blue.

Thanks also to Ann Mennim, Jim Smith and Alan Pike for general help with laboratory work and equipment in Edinburgh.

I would like to thank my fellow students at Edinburgh for discussion and fun over the last three years, Bryne Ngwenya for wide ranging advice, and especially Janette Tourney for her moral support, chocolate, and statistics notes.

Thanks also to my advisor, Godfrey Fitton, and my examiners Ursula Witte and Bryne Ngwenya.

I would also like to thank my family for having been supportive of, and interested in what I have been doing.

Most of all I would like to thank my wonderful fiancé Paul, who has been supportive, encouraging and tolerant as required.

This work was funded by NERC and the Leverhulme Trust. Travel to NIOO and to conferences was made possible by grants from the William Dickson Travel Fund and the University of Edinburgh Small Project Grant committee.

Throughout my PhD I have been based at the School of GeoSciences, at The University of Edinburgh.

Contents

Chapter 4: Short term processing of organic carbon by benthic organisms across the Pakistan Margin: Spatial and seasonal variations 73

CHAPTER 1
Introduction

1.1 Introduction To The Carbon Cycle

The global carbon (C) cycle consists of surface reactive reservoirs, and a sub-surface geological loop. Surface reservoirs include terrestrial and aquatic biota, the ocean and the atmosphere, and are connected by fluxes such as photosynthesis, respiration, gas exchange and riverine transport. The geological loop includes reservoirs in carbonate and oil bearing rocks and in coal measures. In the ocean, inorganic carbon is exchanged with the atmosphere, and carbon is cycled between inorganic dissolved forms and organic matter (OM) in food chains (Fig. 1.1).

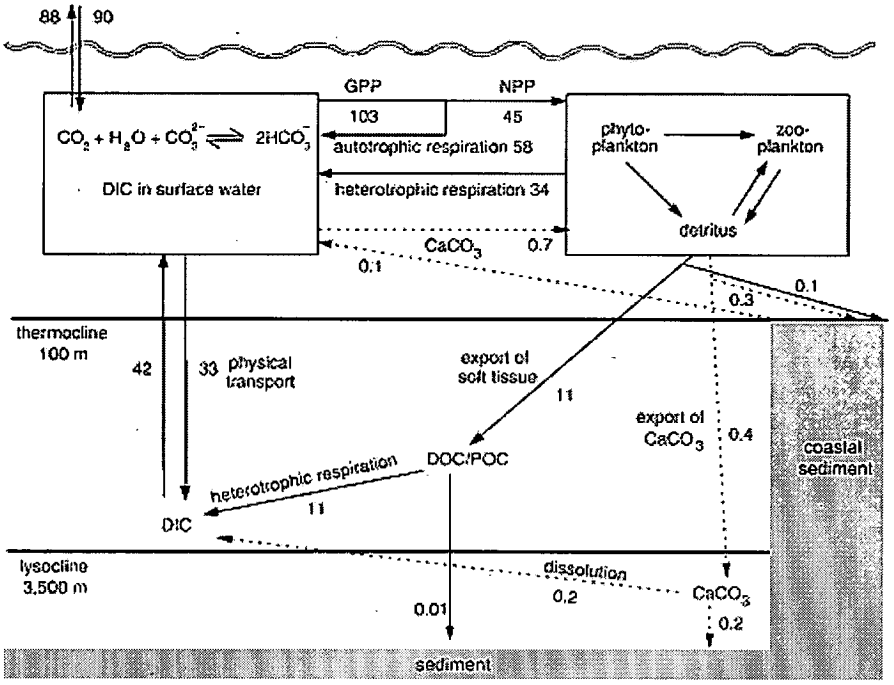


Figure 1.1. Carbon cycling in the ocean, from Prentice et al. (2001). Fluxes are in PgC/yr.

The burial of OM in seafloor sediments represents a flux of carbon out of the surface reactive reservoirs (which play a part in short-term climate change), into the geological loop of the cycle, where it is removed from atmospheric contact for millions of years. While this flux is not large (~ 0.01 Pg y^{-1}, compared to, for example, the 103 Pg y^{-1} of carbon transformed from dissolved inorganic carbon to

OM by photosynthesis in the oceans, Prentice et al., 2001), there are only two other processes, the formation of carbonate rocks, and burial of soils, that sequester carbon on geological timescales. The former of these is small relative to the burial of OM in marine sediments, but the latter is relatively large (Prentice et al., 2001). It is the burial of OM in sediments, however, that is related to the ocean nutrient budget, and to changes in the sizes of ocean and atmosphere carbon pools during past short-term climate fluctuations.

The majority of OM burial in marine sediments occurs in estuaries and deltas (130 Mt y^{-1}), followed by continental shelves underlying highly productive waters such as Walvis Bay and the Chilean and Arabian margins, which collectively account for 14 Mt y^{-1}. These are followed in significance by carbonate sands (7.4 Mt y^{-1}) and then deep ocean sediments (5.7 Mt y^{-1}) (Berner, 1982). Continental margin sediments contain a disproportionate fraction of the global pool of sedimentary OM, due the relatively shallow, productive waters they underlie (DeMaison and Moore, 1980) (Fig. 1.2).

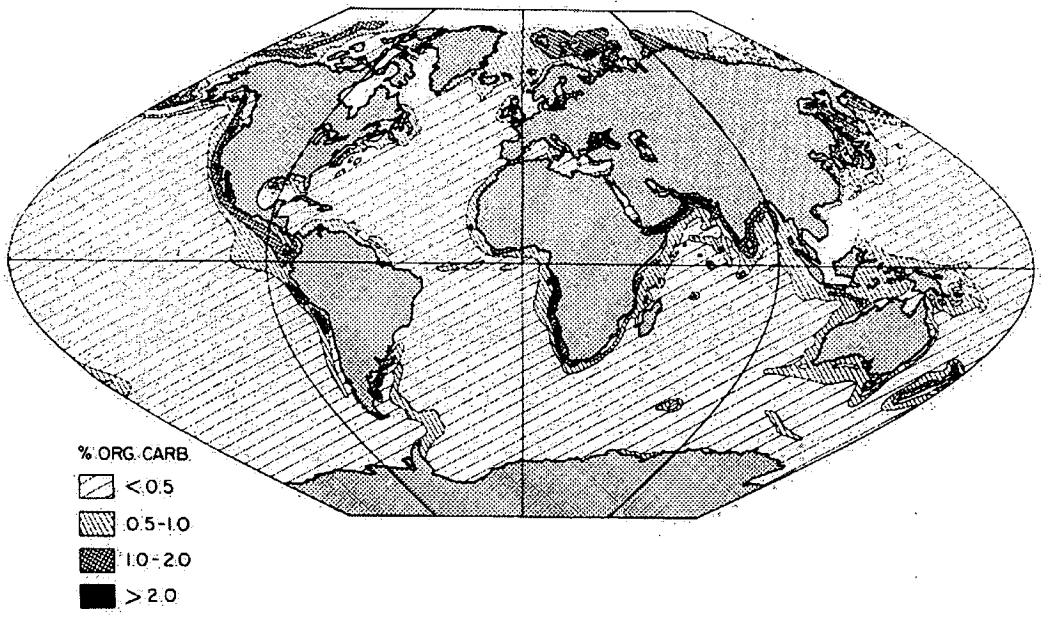


Figure 1.2. The distribution of organic carbon in marine sediments, from DeMaison and Moore (1980).

Vigorous recycling of OM in both the upper and lower portions of the water column results in typically less than 1 % of the OM produced in the photic zone being

deposited in the underlying sediment (Prahl et al, 2000; Hamanaka et al., 2002). Once part of the sediment, OM is subject to a complex interaction of processes (sedimentary C cycling), with two alternative ultimate fates; burial and incorporation into sedimentary rocks, or remineralisation and return to the ocean as dissolved inorganic carbon. Before these fates are reached however, sedimentary OM fuels the benthic food chain, and is thus ingested, assimilated, egested, solubilised, consumed by bacteria, and mixed through the sediment. A brief account of sediment C cycling is given here, in order to introduce this project, and previous work in the field is more fully summarised in the following sections.

The proportion of OM delivered to the sediment that is eventually buried is known as burial efficiency, and is determined by the balance of decay and preservation processes. A considerable amount of previous study has addressed the question of whether the supply of OM to the sediment (largely determined by productivity) or the concentration of dissolved oxygen in bottom waters is responsible for the occurrence of OM rich sediments (e.g. Paropkari et al., 1992). Similarly, other workers have endeavoured to show whether the burial efficiency of OM is principally determined by oxygen exposure, or various preservation factors including sorptive protection, sedimentation rate and hydrodynamic related OM supply (e.g. Mayer, 1994; Hedges and Kiel, 1995).

The availability of oxygen and of reactive OM (as a food supply) are the two factors most influential in determining the size, composition and function of the faunal communities that populate marine sediments (e.g. Smith et al., 2000; Levin et al., 2000; Cook et al., 2000). Many types of organism are represented in marine benthic communities, ranging from bacteria, through nematodes and foraminifera, to annelids, molluscs, crustaceans and holothurians. Among this myriad of organisms is found a wide variety of feeding strategies, diets and life habits. Fauna gain their food by filter feeding, by selectively or non-selectively processing sediment at the surface or at depth, or by predating on other animals. These different feeding strategies in turn lead to a wide variety of gut architectures, chemistries and digestion efficiencies. Fauna may live in (infauna), or on the surface of the sediment (epifauna), and can be sessile, live in tubes, or constantly generate new burrows.

Deposit feeders may live head-up or head-down, and may flush their borrows with bottom water with varying intensity.

Therefore the abundance and behaviour of fauna in sediments is strongly influenced by oxygen and OM availability, and in turn, through bioturbation, irrigation, digestion and microbial stimulation, fauna influence the cycling and burial of OM. Thus the OM a sediment contains, and the fauna it supports are intimately linked through feedback mechanisms.

While the roles of factors such as O2 exposure and sorptive processes in determining C-cycling and burial in marine sediments have been relatively well studied, the role of fauna remains poorly characterised and quantified. While the influences of faunal activities on C cycling are intuitively important, uncertainties remain as to the relative impacts of the various faunal classes and processes, and as to the details, mechanisms and exact effects of those processes. As a result of this, faunal processes are poorly represented in benthic process models.

The general aim of this study was to investigate the interactions between benthic fauna and environmental conditions, and the role these play in shaping the organic geochemistry of, and OM cycling through, continental margin sediments.

1.2 The Arabian Sea Project

This study was a part of a broader international project focused on the benthic biogeochemistry of Arabian Sea sediments across the Pakistan margin. The project was a multi-disciplinary effort to understand the interaction of seafloor biology and geochemistry from a dynamic, process-driven viewpoint. All aspects of sediment geochemistry and biology across the Pakistan margin were surveyed. In addition, experiments were conducted to allow direct observation of biogeochemical fluxes and microbial processes. These included benthic nutrient, gas, dissolved organic matter and trace-metal flux determinations, oxygen consumption rate determinations, and OM tracing studies, which were conducted both on recovered samples and *in situ*, using benthic lander technology.

The overall objectives of the Arabian Sea project were:

- To concentrate on the interaction of geochemical and biological processes.

- To survey the benthic biology and geochemistry of an oxygen minimum zone (OMZ).
- To study the factors controlling the benthic community, its behaviour and trophic dynamics.
- To measure solute fluxes to and from the sediment, including those of trace metals and dissolved gasses and organic carbon (DOC), in order to describe the cycling of these across an OMZ.
- To investigate microbial process dynamics across an OMZ, and the role these play in C cycling.
- To investigate sedimentary carbon cycling and burial in relation to faunal processes and low oxygen conditions.
- To quantify the role of fauna in sedimentary C cycling.
- To study the response of the above to a monsoon-induced seasonal pulse of OM to the sediment.
- To investigate the occurrence of chemeoautotrophic strategies of anaerobic microorganisms.

In order to achieve these objectives, sampling and experimentation were carried out, in contrasting pre- and post-monsoon seasons, on the Pakistan margin of the Arabian Sea, which exhibits a pronounced midwater OMZ, and which will be further described in chapter 2.

Because the work performed in this study was part of the Arabian Sea project, studies of OM processing by the benthic community were supported by a full biological survey of the margin, and will contribute results to the construction of benthic process models. Similarly, the organic geochemical analyses carried out in this study (of sediment pigments and carbohydrates) benefited from the availability of sediment weight percentage organic carbon (%Corg) and $\delta^{13}C$ data, and complemented and were complemented by lipid, amino acid, lignin and other data produced by other parties. Therefore, some interpretations made as part of this study draw upon data produced by other members of the project.

While this study is intended to stand alone, the eventual aim of the Arabian Sea project is that all organic geochemical, oxygen dynamic, benthic flux, radioisotope, biological process and faunal survey data will be drawn together to allow a thorough assessment of the reciprocal influences and interactions among benthic communities, oxygen exposure times, OM quantity and quality, and C burial efficiency. Therefore, the findings of this study will also contribute to synthetic studies not included here, and thus the Arabian Sea data set may allow progress to be made in some of the fields described in the following sections.

1.3 The Benthic Community

Benthic fauna are broadly divided into groups based on size. Microorganisms are the smallest, meiofauna are defined as organisms with a diameter of 63-300 μm, and are followed by macrofauna (300μm-1cm diameter), and megafauna (diameter > 1 cm).

The microbial class comprises aerobic and anaerobic bacteria, yeast, fungi, protozoa and small metazoa, and is often found to constitute as much as 95% of the biomass in seafloor sediments (Boetius and Lochte, 2000; Moodley et al, 2002; Witte et al, 2003b).

The meiofauna can be divided into metazoans, usually dominated by nematode worms, and protists, consisting largely of foraminifera.

The macrofaunal communities of deep-sea sediments are usually dominated by crustaceans (30-50%) and polychaetes (40-80%). Molluscs, holothurians, ophuiroids, astroides, crinoids, hexactinellids, anthozoa, and hydroids also feature, some of which also fall into the megafauna class (Nybakken, 1993).

With increasing water depths, the average size of fauna tends to decrease, such that abundant macrofaunal communities are found mostly on the upper portions of continental slopes, and deeper sites are dominated by meiofauna and bacteria (Pinet, 1992; Nybakken, 1993).

Roughly 80% of the benthic community are deposit feeders, which ingest sediment (with varying levels of selectivity) and digest the OM it contains. Filter feeders are rare, although are observed to flourish where suspended matter is abundant (Heip et al., 2001). Strict herbivores and carnivores are also rare, and omnivory and scavenging are the dominant feeding types.

1.3.1 Controlling Factors

The availability of oxygen and of reactive OM have been found to be the dominant factors controlling benthic community composition, population size and behaviour (e.g. Smith et al., 2000; Levin et al., 2000; Cook et al., 2000). Other factors that may contribute include sediment particle size and sorting, water depth, temperature, hydrodynamic forcing, resuspension/deposition and hydrostatic pressure (Pinet, 1992; Flach et al., 1998; Levin et al, 2000).

Of the two main factors, studies on the Oman margin and in other locations have suggested that the availability of fresh, reactive OM exerts the dominant control on macrofaunal abundance (Rowe et al., 1991; Smith et al., 2000; Levin et al., 2000; Cook et al., 2000; Duniveld et al, 2001). Macrofaunal abundance has been shown to correlate with the flux of OM to the sediment to such an extent that the former has been suggested as a proxy for the latter (Duineveld et al., 2001). This has also been found to be the case for microbial populations (Boetius and Lochte, 2000). Further to this, studies contrasting the relationships of faunal communities with sediment OM quantity and reactivity have found that OM reactivity is more important to the benthos (DeMaster et al., 1994; Heip et al., 2001).

Nilsson and Rosenberg (1994) demonstrated experimentally the effect of enforced hypoxia on the behaviour of benthic organisms. They found that increased oxygenation prompted burrowing and irrigation activity, while hypoxia resulted in decreased diversity, as organisms migrated upwards, left the sediment, or died. The degree to which oxygen availability controls benthic community abundance and composition has been found to be taxon-dependent; for example, echinoderms have been observed to be less tolerant of low oxygen conditions than bivalves or polychaetes (Nilsson and Rosenberg, 1994).

The availability of oxygen has also been observed to have a limiting effect on species diversity (Rosenberg, 2001). Notably, while decreasing oxygen availability has been found to cause steadily decreasing species diversity, faunal abundance and biomass are affected in a non-linear way. The general decrease is interrupted by a peak at low oxygen concentrations, which may be a function of parallel changes in the availability of OM (Rosenberg, 2001) (Fig. 1.3).

Continental margins affected by midwater oxygen minimum zones (OMZs) (e.g. off Peru, Namiba, Oman, Pakistan), exhibit steep oxygen concentration gradients, and thus sites with sharply contrasting oxygen availability. The faunal distributions on such margins are distinctly different from the general decrease in faunal size with increasing depth shown by oxygenated margins, and these differences may be attributed to variations in the availability of oxygen.

Macrofaunal abundances are typically minimal at the cores of OMZs, in some cases leading to a total absence and laminated sediments (e.g. the Pakistan margin, Peter Lamont pers. comm.), and this clearly indicates a significant control on their abundance by oxygen availability.

Dense, high biomass, low diversity communities have been found at OM rich sites at the lower boundaries of OMZs worldwide, where only a few, specially adapted species can survive (Meadows et al., 2000; Levin, 2003). These must coincide with the low oxygen peak in biomass suggested by Rosenberg (2001), and support the suggestion that food supply (and OM reactivity) is a dominant control on macrofaunal abundance, as they imply that fauna can adapt to low-oxygen environments in order to take advantage of abundant food. They further suggest that oxygen availability only influences faunal abundance and function at very low or borderline/threshold levels (e.g. <0.3 ml/l) (Levin et al, 2000). Similar observations have been made for nematodes, for which oxygen levels of 0.13 ml/l were not low enough to prevent their abundance being governed by the bioavailability of OM (Cook et al., 2000).

In a cross-margin study of sediments on the Oman margin of the Arabian Sea, which spanned a mid-water oxygen minimum zone, oxygen was observed to exert a relatively weak control on benthic community behaviour, as no relationships were observed between burrow depth and oxygen availability (Smith et al., 2000). However, work on the same margin found that bottom-water dissolved oxygen concentrations influenced the occurrence of surface versus sub-surface feeding behaviour (Levin et al., 2000), and also produced a morphological adaptation in a species of polychaete, which exhibited larger respiratory surface areas where oxygen was scarce (Lamont and Gage, 2000).

Thus oxygen availability has been shown to influence the abundance, behaviour and diversity of benthic communities, but so long as just sufficient oxygen is present, faunal abundance appears to be influenced to a greater extent by the availability of reactive OM.

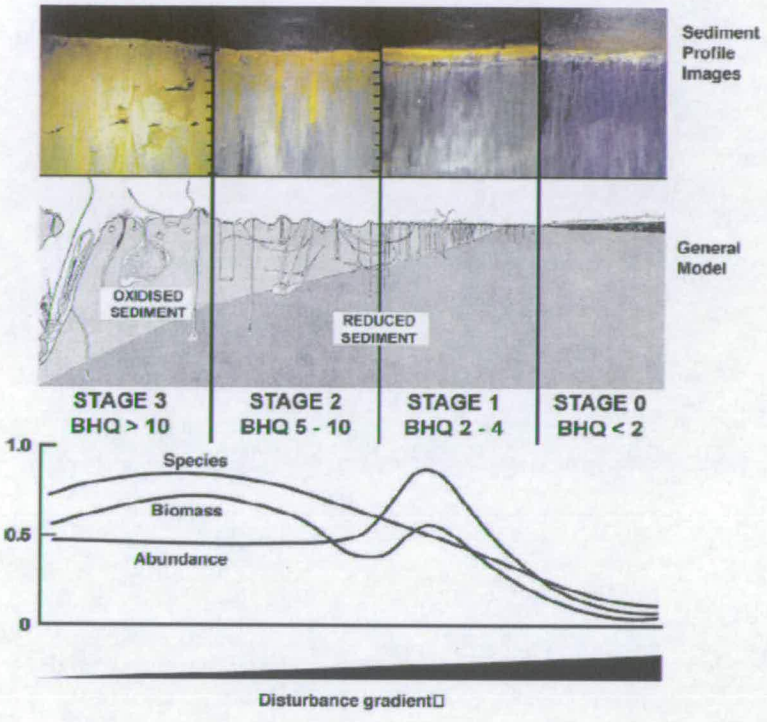


Fig. 1.3. Changes in the abundance, diversity and behaviour of benthic macrofauna as oxygen levels fall, from Rosenberg (2001).

1.3.2 Faunal Communities of Arabian Sea Margin Sediments

The Arabian Sea features one of the largest volumes of oxygen depleted water on Earth, as a layer between depths of ~150m and 1000m. This is the result of intense seasonal productivity, and limited midwater ventilation. Where it impinges on the continental margin, steep oxygen gradients are enforced on the sediments, causing considerable cross-margin variations in OM availability, OM reactivity, and faunal communities. The Oman margin of the Arabian Sea has been extensively studied, and the Pakistan margin, where productivity is less intense, but oxygen concentrations are lower, has been less well characterised. The Arabian Sea is described in depth in chapter 2

Studies on the Oman Margin, at depths spanning the OMZ have found the macrofauna communities to be dominated by polychaetes. Spionids and cirratulids

were the dominant families in low oxygen conditions. Molluscs and crustaceans were rare, but more abundant at deeper, oxygenated sites. Intriguingly, the macrofaunal biomass on this margin was higher within the OMZ, where more reactive OM was present but where oxygen was scarce ($<0.5 ml L^{-1}$), than below it (Levin et al, 2000). Nematode and foraminiferal biomass also followed this pattern, and these groups also showed reduced diversity within the OMZ (Cook et al., 2000; Gooday et al., 2000). Among the foraminifera, calcareous species dominated the community within the OMZ, and gave way to agglutinated forms at deeper sites, below the OMZ (Gooday et al., 2000).

Sampling performed during this Arabian Sea project found variations in the benthic community across the Pakistan margin, which paralleled variations in the availabilities of oxygen and OM. While foraminifera were found at all sites across the margin, macrofauna were absent from locations with the lowest oxygen concentrations (~ 300-700 m depth). At sites with sufficient oxygen, polychaetes dominated the macrofauna. Crustaceans were also relatively abundant, and their relative abundance increased at deeper sites. Molluscs, and occasionally echinoderms, were also found. The dominant polychaetes varied across the margin, but the two main families were the Cirratulidae, and at sites in the OMZ lower boundary zone, a species of amphinomid named *Linopherus sp.* (Peter Lamont, pers. comm.). The main contrast with the Oman margin was the absence of macrofauna from the most hypoxic sites, where sediments were consequently laminated.

Foraminifera overwhelmingly dominated the meiofaunal populations, and metazoan meiofauna (nematodes) were very rare. Calcareous foraminifera dominated at shallower sites, while deep sites exhibited agglutinated varieties (Kate Larkin and Stefanie Schumacher, pers. comm.), and this is consistent with findings on the Oman margin (Gooday et al., 2000).

Bacterial mats have been observed on the Pakistan margin, in the core of the OMZ, and in the northeast of the region, where there are tectonically driven sulphide seeps. These were found to be formed by the bacterium *Thioploca*, a chemoautotroph with which both sulphide oxidation and sulphate reduction are associated (Schmaljohann et al, 2001). Bacterial mats have also been observed at other low-oxygen and

sulphidic sites, including *Beggiatoa* mats found in the Gulf of California (Guezennec et al, 1996).

Further comparisons of the faunal communities on the Oman and Pakistan margins cannot yet be made, as the Pakistan margin survey is not yet complete.

1.4 Organic Matter Decay

1.4.1 Modelling

Sedimentary OM decays progressively with time as it becomes buried in the sediment. This occurs through heterotrophic respiration, utilising a progression of terminal electron acceptors (Hedges and Keil, 1995, for a review of OM decay and preservation; Hartnett and Devol, 2003). Decay over time produces the widely observed exponential decrease in sediment %Corg with increasing depth in the sediment (Fig. 1.4). The shape of this typical profile (an exponential decrease in %Corg with depth, to a non-zero asymptote concentration, interrupted by a sub-surface peak) led initial attempts at modelling the decay of OM to consider it as a first order reaction, where the rate of decay was determined by the amount of OM present, and its decay constant (Berner, 1982). Subsequent studies showed, however, that as decay progresses, the biochemical composition of OM is altered, and thus so is its decay constant. Simultaneously, the same OM becomes buried, and, as it moves downward through the sediment, it comes into contact with different conditions (for example, oxygen availability is typically reduced).

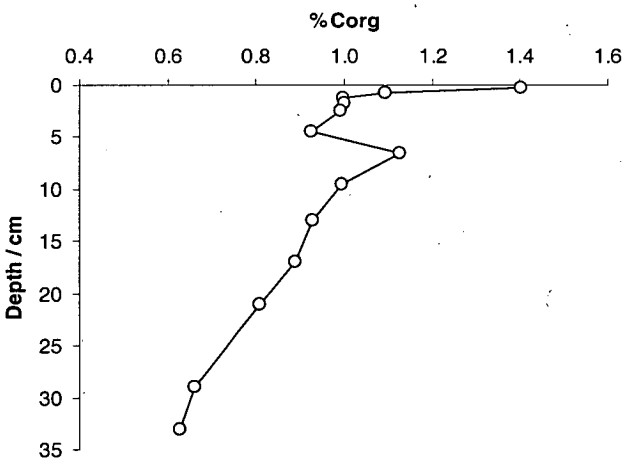


Figure 1.4. A typical downcore profile of sediment %Corg, showing an exponential decrease, interrupted by a sub-surface peak (from 1850m water depth on the Pakistan margin, courtesy of Greg Cowie).

These alterations also change the reactivity of OM, thus a single decay constant is not necessarily appropriate.

This led to the development of the multi-G approach, in which OM is considered as consisting of several fractions, each with a different reactivity (Jorgensen, 1978). Most studies that have used a multi-G approach to the modelling of OM decay (e.g. Shankle et al., 2002) have found that OM decay can be described by three (or sometimes two) OM fractions of differing reactivity; one that dominates at the sediment surface and is of maximal reactivity, a second relatively unreactive component, and, finally an unreactive component that constitutes the background asymptote concentration that becomes buried. Middelburg (1989) developed the multi-G model into a continuous-G model, in which the decay constant of OM is considered to change continuously during decay. This model is probably closer to actuality, but may be more difficult to use.

The modelling of OM decay is further complicated by faunal activity, which mixes reactive OM to depths to which it may not otherwise survive, and creates sub-surface peaks (Fig. 1.4), through non-local transport.

1.4.2 Selectivity of OM decay

Processes causing OM alteration within the water column, including bacterial decay and grazing by zooplankton, have been found to be compound-selective, and thus to determine the composition of OM reaching the sediment (e.g. Ittekkot et al., 1984 a, b; Cowie and Hedges, 1996; Lee et al., 2000). Previously observed compositional alterations include the preferential loss of fatty acids and amino acids over bulk OM, the loss of glucose and ribose from the suite of aldosese (e.g. Henrichs and Farrington, 1987; Hamanaka et al, 2002), and the preferential preservation of cell-wall amino acids and carbohydrates vs. intracellular components, (Cowie and Hedges, 1996). In the water column, pigments have been shown to be the most reactive component of OM, generally followed by the lipids and amino acids, total N, carbohydrates, and lastly total C (Lee et al., 2000). At the other end of the reactivity scale, Cowie et al. (1992) observed that relatively recalcitrant components, such as lignin, became relatively enriched in the sediment through preferential preservation during sinking.

In the sediments, selective decay continues, but the relative reactivities of OM components vary with setting, due to differing OM sources and water-column

histories, and decay profiles are not always apparent due to masking of downcore decay profiles by bioturbation.

Selective decay in the sediment has also been observed within biochemical classes. Several studies have found that the suite of amino acids changes in favour of the relatively unreactive glycine, serine, aspartic acid and threonine, with preferential losses of glutamic acid, alanine, leucine, iso-leucine, phenylalanine, and tyrosine, with progressive decay (e.g. Henrichs and Farrington, 1987; Cowie et al., 1992; Horsfall and Wolff, 1997; Dauwe and Middelburg, 1998). It has been suggested that the preferentially preserved amino acids are associated with phytoplankton cells walls, which afford them some protection from decay (Cowie et al, 1992, Thomas and Blair, 2002) (a continuation of the process seen in zooplankton guts and the water column). In addition to selective decay and preservation of amino acids, the non-protein amino acids β-alanine and γ-amino butyric acid have been found to be decay products of aspartic and glutamic acids respectively, and therefore indicative of state of decay (Cowie and Hedges, 1984 a; Hedges et al., 1999). Dauwe and Middelburg (1998) made the first wide-ranging and rigorous examination of diagenetic trends in amino acid composition to develop an OM degradation index (DI) that could be applied to OM in natural marine samples ranging from fresh, intact organisms to heavily oxidised deep-sea sediments. This was done by subjecting amino acid mole percentage data to principal component analysis (PCA). The first principle component was associated with depletion/enrichment of cell-wall versus intracellular amino acids in fresh versus heavily degraded samples, respectively. Various marine sediment samples produced scores on this first principle component axis that showed differences in degradation state consistent with other indices (e.g. non-protein amino acid and hexosamine contents, intact protein content, and direct determinations of OM decay rates in slurry incubations).

Among the carbohydrates it has been observed that glucose and ribose decay most rapidly, and that xylose and galactose are preferentially preserved. Rhamnose and fucose have also often been observed to accumulate, and may be produced by bacteria during decay (Hedges et al., 1988; Cowie et al., 1992; Hernes et al., 1996; Opsahl and Benner, 1999; Hedges et al., 1999; D'souza et al., 2003). Once again, it is thought that the compounds that are preferentially preserved are associated with

cell walls, and those that are rapidly lost are associated with the cell contents (Ittekkot and Degens, 1982; Ittekkot et al., 1984 b; Cowie et al., 1992). A similar tendency for cell wall components to be preserved has also been observed among lipids (Sun, 2000).

The occurrence of selective decay suggests that the biochemical composition of sedimentary OM will be partially dependant on its degradation state, and this will also determine its reactivity, its potential to become buried, and its utility to the benthic community as a source of food.

1.4.3 Preserved OM Characteristics

Observations have shown that the eventual residue of OM alteration and selective decay is typically ^{13}C- and aliphatic-rich, N-poor, recalcitrant OM, a large proportion (~75+%) of which is molecularly uncharacterisable (Hedges et al., 2000). Although this fraction evades characterisation, NMR analysis has revealed that a significant proportion of the nitrogen is present in the form of amine groups (Zang et al., 2000). Results have indicated that whole proteins can become encapsulated in hydrophobic regions of humic material, and have led to the suggestion that refractory organic nitrogen is not only present as (possibly altered) amines, but as intact amino acids and proteins (Zang et al., 2000).

The exact mechanisms for the formation of refractory OM are still debated. Geopolymerisation, a term used to describe abiotic condensation reactions between low-molecular-weight by-products of decay, has often been offered as a possible mechanism, and is partially supported by observations in the laboratory of the abiotic formation of glucose-amino acid melanoidins (Hedges, 1978) and other field evidence. Abiotic organic-organic reactions have also been suggested as contributing to the old, refractory and uncharacterisable pool of dissolved organic carbon (DOC) in marine sediments (Keil and Kirchman, 1994). Recent studies, however, have clearly demonstrated that selective preservation of certain biochemicals, rather than random abiotic condensation reactions, are a contributing mechanism in the formation of refractory humic material (Hedges et al, 2000). The relative importance of the different formation pathways, and the exact nature of

preserved OM remain, however, largely unknown. They may, in the future, be elucidated using the detailed biochemical techniques employed in this study.

1.4.4 Non-Faunal Factors Affecting OM Preservation

A considerable amount of research has been dedicated to the question of what determines the burial efficiency of OM in seafloor sediments. Factors, including oxygen availability, sedimentation rate, primary productivity, water depth, sediment sorptive/hydrodynamic processes, and OM reactivity have been investigated as controls on burial efficiency, and the findings are outlined below.

1.4.4.1 Sediment Texture and Sorptive Effects

Fine-grained sediments have often been observed to have higher %Corg contents than coarse-grained sediments (e.g. Keil et al., 1998 and references therein). A suggested mechanism for this has been that organic debris has similar hydrodynamic properties to fine-grained mineral material, and thus tends to accumulate in the same locations (Keil et al., 1998). It has also been shown, however, that the majority of OM in most marine sediments is intimately linked to the sediment surface (Mayer, 1994). This appears to be a sorptive process, and fine-grained sediments are more OM-rich due to their larger specific surface areas (Mayer, 1994). Moreover, for wide ranges of grain sizes, a constant ratio of C content to sediment surface area of 0.5-1mg C/m^2 has been observed (Hedges and Keil, 1995;Keil et al, 1998). This led to the "monolayer hypothesis", in which it was proposed that OM is present as a single molecule thick coating on mineral grains, and this has been supported by the observation that the "monolayer equivalent" OM loading tends to define the downcore asymptote OM concentration in many sediment cores (Mayer 1994). Sorptive association with a mineral surface may afford OM protection from decay processes by restricting access for enzymes through the positioning of OM in pores, or through the occupation of key functional groups in bonding.

Although it has now been shown that OM associates with mineral surfaces in a patchy, rather than "monolayer" way, collective results indicate that sorption, and processes that control the delivery of mineral surface area (and thus OM) to the sediments, are important factors determining sedimentary OM distributions and, potentially, burial efficiency (Hedges and Keil, 1995).

1.4.4.2 Sedimentation Rate

Rapid sedimentation has been observed to enhance carbon burial efficiency in marine sediments. Henrichs and Reeburgh (1987) showed this in a plot of burial efficiency against sediment accumulation rate, including data from many different environments, which revealed a positive correlation, with a slope that was independent of oxygen concentration. High sedimentation rates can, however, also act to decrease sediment %Corg through dilution of OM with terrigenous material (Paropkari et al., 1992). The mechanisms by which sedimentation rate influences C burial efficiency are thought to be linked to the way it controls the time OM spends near the diagenetically active sediment-water interface, and thus its exposure to bacteria and various oxidants (Canfield, 1994). This is further developed with the concept of oxygen exposure time (see below).

1.4.4.3 Oxygen Availability

It has been generally observed that sediments deposited under low oxygen conditions exhibit high %Corg, and burial efficiencies. This has been found to be the case in organic-rich sediments underlying modern anoxic water masses including the Baltic and Black Seas, Lake Tanganyika and the continental margins of Southwest Africa, South America and Mexico, and the Arabian Sea (e.g. DeMaison and Moore, 1980; Cowie et al., 1999; Meadows et al., 2000; Hartnett and Devol, 2003). This is thought to be because lack of oxygen forces decay to proceed along less energetically favourable paths via alternative electron acceptors (O_2, followed by NO_3^-, Fe^{3+} and MnO_2, and SO_4^{2-}), ultimately leading to enhanced OM preservation, due either to generally decreased OM decay rates or to preferential preservation of oxygen sensitive components. Evidence for the latter mechanism has been found in turbidites on the Madeira Abyssal Plain, where an oxidation front was observed, burning down through anoxic deposits, causing an ~ 80 % reduction in sediment %Corg that was previously preserved in the absence of oxygen (Buckley, 1988; Wilson, 1985; Cowie et al., 1995).

Organic matter decay rate combines with burial rate to determine burial efficiency. Comparisons of sedimentary OM decay rates under aerobic and anaerobic conditions have not been conclusive as to the effect of oxygen on burial efficiency, due to timescale constraints. Selected studies have shown that decay rates of various

biochemicals were more rapid in the presence of oxygen than in it absence (e.g. Sun et al., 1993b; Sun, 2000). Also, and notably, fluctuating redox conditions, likely to typify conditions in sediments irrigated by faunal activities, have also been found to generate OM decay rates significantly greater than their anoxic equivalents, and not greatly different from their fully oxygenated equivalents (Aller, 1994; Sun et al, 2002).

Other field and experimental studies, however, have suggested that the relationship between oxygen availability and OM preservation is not straightforward, and that other factors also play roles, possibly dominant ones, in determining C burial efficiency (e.g. Henrichs and Farrington, 1987; Calvert et al., 1995).

Incubation studies have often shown little measurable or systematic difference in OM decay rates under aerobic and anaerobic conditions (Kristensen and Blackburn, 1987), and Kristensen (2000) observed that the presence of oxygen only increased decay rates for pre-aged, or relatively degraded OM. Also, Cowie and Hedges (1992) showed no difference in the burial efficiency of C, N, carbohydrates, amino acids or lignin in studies at two coastal sites that differed only in bottom-water oxygen concentration. Similarly, in the Gulf of California, sediments within and outside the OMZ were found to have the same %Corg and hydrogen index values (a measure of OM freshness or quality). Also, the regional %Corg maximum was slightly below the lower boundary of the OMZ. In the Arabian Sea, on the Oman and Pakistan margins, maximal %Corg values were also found to extend outside the zone of lowest bottom-water oxygen concentrations (Cowie et al., 1999; Levin et al, 2000; Suthhof et al, 2000).

Thus, while oxygen depletion generally appears to be associated with increased sedimentary OM content, the relationship, and the notion that it is due to enhanced preservation of OM in the absence of oxygen, is not straightforward, and other factors clearly exert significant influences.

1.4.4.4 Summary

It is now recognised that the factors that are most influential in controlling OM burial efficiency (oxygen availability, primary production and OM supply rate, sedimentation rate and surface area/sorption) are intimately linked, and do not act in isolation. Notably, the influence of all these factors can be viewed to be exerted

through a single parameter - oxygen exposure time (OET) - which is a measure of the time for which OM is exposed to oxygen, in the water column and in sediment porewaters, before being buried (Canfield, 1994). Increased primary production tends to enhance the rain rate of OM to the sediments. So too will a greater flux of mineral surface area, which carries with it a loading of sorbed OM. The increased OM flux in turn increases the sediment biological oxygen demand, and reduces the depth in the sediment to which oxygen penetrates, which, together with a high sedimentation rate, reduces OET. Such conditions of high OM supply rates and high sedimentation rates tend to occur in relatively productive, shallow settings, proximal to continents. The OM that reaches the sediments in such settings tends to be relatively fresh, and therefore decays at similar rates and to similar extents under oxic and anoxic conditions. Consequently, factors determining

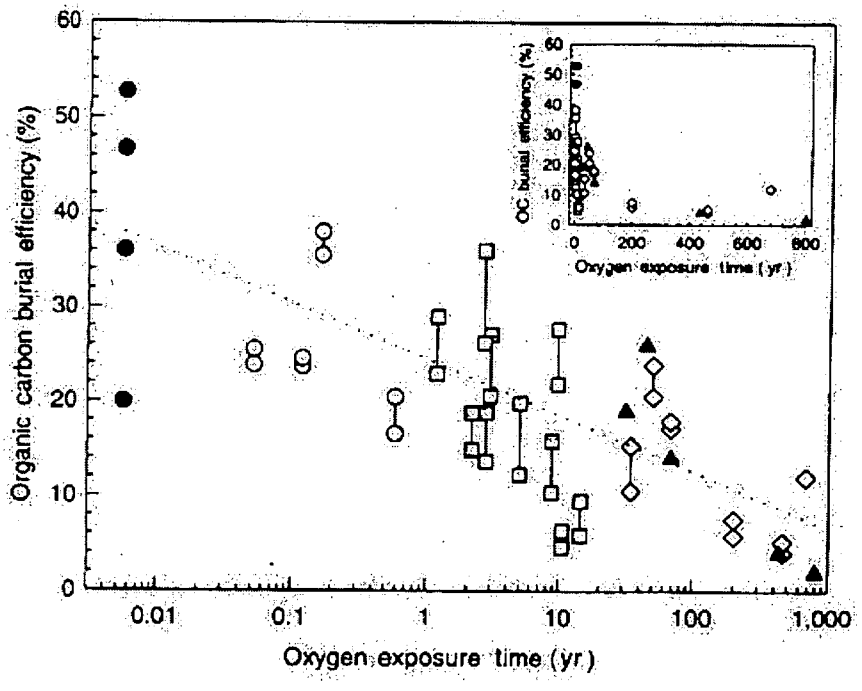


Figure 1.5. The relationship between carbon burial efficiency and oxygen exposure time (OET). Note the logarithmic x-axis. OET is plotted on a linear scale in the inset graph. From Hartnett et al. (1998).

OM supply (primary production, sedimentation rate, sediment texture) are likely to be the primary factors in determining sedimentary OM distributions in these settings, which are thus observed to show highly variable C burial efficiencies (Fig. 1.5).

Conversely, in deep ocean settings, OM delivery and sedimentation rates are low (due to lower productivity, the length of the water column, and distance from land). Oxygen exposure times are consequently relatively long, and the more refractory OM that survives passage through the water column, and is deposited at the benthic interface, tends to show significant differences in preservation under aerobic and anaerobic conditions (Hulthe et al., 1998). In these settings, preservation rather than supply becomes the dominant control on sedimentary OM distributions, as the long OETs ultimately overcome the effects of sorptive preservation (Fig. 1.5 and Hedges and Keil, 1995).

Canfield (1994) suggested that the sedimentation rate dividing these two scenarios is roughly 0.04 g cm^{-2} y^{-1}. Hedges and Keil (1995) proposed that the transition from sorptive preservation control that dominates in most shelf and slope sediments to the OET control observed on the continental rise and abyssal plain, typically occurs somewhere between 2 and 3km depth.

In summary, oxygen exposure time is a parameter that takes into account the important factors that influence burial efficiency. The correlation between OET and burial efficiency has been shown to hold for a wide range of seafloor settings, but breaks down at low OETs, suggesting that there are other factors, which remain unaccounted for (Fig. 1.5) (Canfield, 1994; Hartnett et al., 1998).

1.5 Organic Geochemistry

As highlighted above, the Arabian Sea margins have provided fuel to debates regarding the degree to which oxygen exposure and other parameters influence the OM content of sediments (e.g. Paropkari et al., 1992; Pedersen et al., 1992; Calvert et al., 1995). This has been due to the fact that maximal OM concentrations have been observed not to fully coincide with oxygen minima, but rather to occur at and below the lower boundary of the OMZ (e.g. Cowie et al., 1999; Shankle et al., 2000). More recently, detailed biochemical analyses have been performed on Pakistan margin sediments in an attempt to further this debate. Amino acid and lipid data have shown that while OM within the OMZ may be less degraded that that outside it, bottom water oxygen concentrations are not the only factor controlling the distribution and composition of OM on the margin (Schulte et al., 2000; Suthhof et

al., 2000), and other factors such as sedimentation rate, biological winnowing and cross-margin transport also play a part (Schulz et al., 1996; Smallwood et al., 1999). Thus questions remain regarding the interaction of oxygen, sediment OM content and composition, OM supply, sedimentation rate, and various biological parameters. The current Arabian Sea project, through a comprehensive survey of the Pakistan margin, aimed to address some of these issues.

In this study the pigment and carbohydrate contents of Pakistan margin sediments were investigated. This data will be used in conjunction with characterisations of sedimentary %Corg, $\delta^{13}C$, C/N, lipids, amino acids, lignin and faunal communities, as well as site OM fluxes, OETs and burial efficiencies, in order to thoroughly asses OM dynamics across an OMZ.

1.5.1 Pigments

Pigments are mostly produced by plants for light collection, and to fulfil other roles associated with photosynthesis. The dominant pigments in the marine environment are chlorophyll-a, its decay products, the pheopigments, and accessory pigments, including chlorophylls b and c, xanthophylls and carotenes. Pigments are generally very reactive when compared to other biochemical classes (Lee et al., 2000). Their short decay half-lives have led to their use as tracers of biomixing processes over the timescale of days to weeks, and as indices of OM supply and quality in marine sediments (Pfannkuche et al., 2000), and their inventories can be used to estimate the total flux rate of OM to the sediment (e.g. Boon and Duineveld, 1998). A fraction of the pigments, however, has been observed to be relatively refractory (Stephens et al., 1997, Chen et al., 2005), and this facilitates the use of pigments and their decay products as indices of paleoproductivity over timescales of hundreds of thousands of years (e.g. Harris et al., 1996; Dahl et al., 2004).

The suites of primary and accessory pigments present in a sediment or water sample have been found to be potentially indicative of the phytoplankton source of OM (e.g. fucoxanthin is a diatom biomarker, Bianchi et al., 1996; Jeffrey and Vesk, 1997). In addition, various chlorophyll decay products have been shown to be indicative of zooplankton grazing, and some of these are even species-specific (Shuman and Lorenzen, 1975; Louda et al., 1998; Goericke et al., 1999; Bianchi et al, 2000; Squier

et al. 2002). Further to this, sediment microcosm studies have shown that pigment decay rates are accelerated in the presence of burrowing fauna and oxygen (Sun et al., 1993a; Sun and Dai, 2005).

Thus, a knowledge of the abundance and composition of pigments in sedimentary OM, together with a survey of faunal communities, has the potential to reveal a range of facts about benthic OM source, diagenetic history and reactivity, and about relationships between sedimentary OM, faunal processes and preservational controls. For further details see chapter 6.

1.5.2 Carbohydrates

In contrast to the pigments, carbohydrates have not always been observed to be particularly reactive, and they may form significant proportions of preserved OM. Although they have generally been observed to be more reactive than bulk OM in the water column (e.g. Ittekkot et al., 1984 a, b; Cowie et al., 1992), in the sediments they have been observed to be less reactive, and sometimes even preferentially preserved (Hernes et al., 1996; Danovaro et al., 2001). The aldose composition of natural samples has been shown to be indicative of terrestrial versus marine OM sources (Cowie and Hedges, 1984a).

Selective decay is also typically observed among the carbohydrates. Aldoses principally associated with algal cell contents, namely glucose and ribose, have been observed to be the most reactive in marine environments, and those more associated with cell walls the least reactive (e.g. Cowie and Hedges, 1984a; Hedges et al, 1988; Hamilton and Hedges, 1988). Also, rhamnose and fucose typically show preferential preservation, possibly due to production as by products of microbial decay (e.g. Opsahl and Benner, 1999).

Due to the decay patterns described above, sedimentary carbohydrate suites have the potential to be indicative of OM freshness and potential food quality. Thus, the carbohydrates represent a contrast to the pigments, and provide a further, possibly supporting, indicator of OM quality or freshness, and how this relates to environmental OM control factors and faunal activity.

1.6 The Effects Of Faunal Activities on Sedimentary OM Cycling

Examination of Figure 1.5 shows considerable scatter in the data at short OETs (Hartnett et al., 1998). Thus, in settings where OET is less than ~ 0.01 years, other factors must be particularly influential in determining burial efficiency. It is suggested that much of the variability in OM burial efficiency at low OETs may be related to benthic faunal activities.

Fauna have been shown to influence OM cycling and burial through the re-location of OM, sediment ventilation, the formation of microenvironments, ingestion and digestion, and microbial stimulation (Aller, 1982), therefore the abundance and composition of benthic faunal communities, and the environmental factors that influence these, are likely to have an impact on sedimentary OM cycling. The current knowledge of these processes is described below.

1.6.1 Bioturbation

Fauna have been observed to transport OM through the sediment and between redox zones during ingestion, egestion at the surface or below, horizontal ploughing, and non-local transport (hoeing) down burrows.

These processes have been directly observed during *in situ* isotope labelling experiments, where sub-surface label peaks were found, indicating non-local mixing and the storage of fresh OM at depth (Blair et al., 1996; Levin et al, 1997). In microcosm labelling experiments, deeper label penetration was observed in microcosms containing macrofauna than those that did not (Sun et al., 1999; Thomas and Blair, 2002). Different organisms exhibit different patterns of movement and burrowing (e.g. organisms vary in their maximum burrowing depth and rate, and in burrow shape and size, and whether they excrete at the surface or at depth). Therefore the nature of sediment mixing has been observed to be dependent on the taxa present (and sometimes on only one dominant taxon) at a site (DeMaster et al., 1994).

Bioturbation has classically been modelled as a simple diffusive process. The problems with this approach are that it cannot represent either the unidirectional nature of biomixing (upwards or downwards, depending on the taxon), selective

ingestion, or non-local transport. In addition, bioturbation is spatially heterogeneous, and can occur on a larger scale than the approach allows (Aller, 1982; Wheatcroft, 1992; Smith et al, 1993; Blair et al., 1996). Models have been developed to address these shortcomings (in particular, to account for non-local mixing), but they are not yet widely used (Soetaert et al., 1996).

The effect of bioturbation (i.e. sediment mixing) alone on OM burial efficiency is unclear. On one hand, biomixing can transport fresh, reactive OM to sediment depths that are depleted in oxygen and have lower porosity and microbial abundance, and this could serve to lessen the rate and extent of decay (Kristensen and Blackburn, 1987). Bioturbation may also uplift buried, relatively recalcitrant OM above the redox front, thus producing oscillating redox conditions, and extending OET, leading to further decay. Thus, it has been suggested, head-down conveyor belt feeders that ingest at depth and egest at the surface are likely to increase net OM decay rates (Wheatcroft, 1992), whereas reverse conveyor belt feeders may have the opposite effect.

In addition to simple mixing, fauna have also been observed to physically affect the OM contents of sediments through sediment capture and biodeposition by filter feeders (Heip et al., 2001), and loss of OM caused by megafaunal bioturbation and winnowing (Smallwood et al, 1999).

It is therefore unclear whether bioturbation acts to increase or decrease OM burial efficiency, and the answer to this is likely to be specific to the benthic community and conditions of any given setting (Sun et al, 2002). The net effects may depend on the relative importance of organisms with different feeding modes, as well as factors such as the amount and quality of OM delivered to the sediments and the depth of sedimentary redox boundaries relative to burrowing depths.

1.6.2 Irrigation

Burrowing organisms actively flush their burrows with fresh bottom water, drawing reactants (including dissolved oxygen) into the sediment, and flushing out waste products (Sun et al., 1999). It has been suggested that this can increase the volume of oxygenated sediment threefold (thus extending OET and increasing total decay) (e.g. Kristensen, 2000; Sun et al, 2002). Some fauna have been observed to pump

bottom water through their burrows periodically (in fact, most fauna probably work in this way) leading to fluctuating sediment redox conditions, which have also been generally shown to facilitate OM decay (e.g. Aller, 1994).

Modelling of the effects of irrigation on the availability of respiratory electron acceptors has led to the suggestion that an increased density of burrows leads to increased rates of remineralisation (Aller and Aller,1998). Observations in microcosms and natural sediments have also shown that irrigation leads to increased decay rates, both of bulk OM, and of individual biochemicals (Sun et al., 1999; Sun, 2000). These decay rate increases have been attributed both to increased oxygen availability (Sun et al., 1999), and also to increased microbial activity, and processes such as denitrification and ammonification (Kristensen and Blackburn, 1987; Aller and Aller, 1998). Thus, irrigation re-exposes sub-surface, relatively refractory OM to oxygen and other respiratory electron acceptors, and could increase net OM remineralisation, by as much as 47% (Kristensen, 2000).

The processes of bioturbation and irrigation are linked, as they are both results of burrowing activity, but may have different effects on OM preservation. Sun et al (2002) found that purely physical (artificial) sediment mixing promoted OM preservation by burying reactive OM below the redox front. Natural bioturbation, however, increased OM decay rates due to the co-occurrence of irrigation.

1.6.3 Microbial Stimulation

It is generally thought that, due to their overwhelming dominance of the benthic community biomass, microoganisms are responsible for the majority of OM remineralisation (e.g. Moodley et al., 2002; Moodley et al., 2005). However, the actions of larger fauna have been observed to stimulate microbial activity, and so increase overall decay rates, and thus also have an additional indirect effect on OM cycling.

One mechanism of microbial stimulation is the introduction of respiratory electron acceptors into the sediment, and removal of by products by irrigation, which allows microbial respiration to proceed unhindered (Sun et al, 2002). In addition, burrowing activities constantly expose fresh OM surfaces for microbial attack (Aller, 1982).

Macrofauna, through bioturbation, secretion of burrow linings, and the hoeing and storage of fresh organic detritus in burrow chambers, also introduce fresh, reactive OM at depth in the sediment, where it would otherwise not penetrate (Levin et al, 1997). This results in high densities of meio and microfauna in burrow walls (some of which are then 'harvested' by the macrofauna) (Levin et al, 1997; Cook et al, 2000), which tends to increase net decay; and also in anoxic hotspots where increased preservation might be expected. The introduction of fresh OM at depth also stimulates cometabolism of otherwise recalcitrant, buried OM (Canfield, 1994).

Macrofauna have also been seen to actively graze on the microbial community, maintaining it in a state of exponential growth, and causing an overall invigoration of the sediment C cycle (Sun et al., 1999).

Thus, in general, the stimulation of microbial processes by macrofaunal activities has been found to produce an increase in overall OM decay.

1.6.4 Selective Ingestion

Comparisons of the composition of faunal gut contents with that of the sediments on which they feed have revealed them to be enriched in chlorophyll-a (Miller et al., 2000), organic nitrogen, proteins, lipids, carbohydrates and radionucleides (DeMaster et al, 1994), suggesting that some animals are capable of actively selecting food-rich particles for ingestion, rather than randomly processing bulk sediment.

Evidence has been found for particle selection on the basis of several criteria. It has been suggested that some fauna are able to select on the basis of particle composition, and thus seek out fresh OM (e.g. Blair et al., 1996; Miller et al., 2000). Smith et al (1993), after comparing bioturbation coefficients derived from two different radioisotopes (^{210}Pb and ^{234}Th), suggested that particles were selected for ingestion and biological mixing on the basis of their age. The age-dependent mixing hypothesis is supported by observations of high ^{234}Th concentrations in animal guts (Lauerman et al., 1997), and of seasonal bioturbation prompted by pulsed OM delivery. Some fauna have also been shown to preferentially ingest and transport fine-grained particles (Wheatcroft, 1992). This is consistent with observations that

fine-grained sediments tend to have relatively high OM contents, and also high densities of burrowing fauna (Mayer, 1994).

Selective ingestion as a feeding strategy is only practised by a certain, but as yet unknown, fraction of taxa (Fauchald and Jumars, 1979; Thomas and Blair, 2002). It is likely however to maximise the proportion of sedimentary OM that is subjected to faunal uptake and gut passage, and would thus enhance the role of macrofauna in sedimentary OM processing.

1.6.5 Digestion

Some megafauna have been observed to consume 0.4-25 times their dry body weight in sediment each day, suggesting (by extrapolation to the whole benthic community) that 30% or more of fresh OM will only be available to the rest of the faunal community after megafaunal or macrofaunal gut passage (Miller et al., 2000; Witte et al., 2003b). Thus, the biochemical alteration of OM during digestion is likely to be a significant process governing the quality of OM available to the rest of the benthic community, the organic geochemistry of sediments, and the reactivity of OM during microbial decay.

Digestion and assimilation are compound-selective processes. On a bulk level, two very different taxa (copepods and holothurians, pelagic and benthic respectively) have been observed to preferentially assimilate nitrogenous compounds from bulk OM (Cowie and Hedges, 1996; Miller et al., 1998). In the copepod feeding study, amino acids were most efficiently assimilated (83% assimilated), followed by sugars (73%), and pigments (26-37%), and uncharacterised OM was preferentially egested (Cowie and Hedges, 1996).

On a more detailed level, a feeding study with polychaetes has shown that among the amino acids, glycine, aspartic and glutamic acids and lysine were preferentially assimilated, while serine, threonine and glycine were enriched in faecal pellets (Thomas and Blair, 2002). Among the aldoses, glucose, arabinose and ribose have been shown to be more efficiently assimilated by copepods than xylose, mannose and galactose (Cowie and Hedges, 1996). As with decay processes that have been shown to be compound-selective, these amino acid and aldose assimilation patterns are thought to be related to cell structure, in that compounds associated with cell walls

and frustules appear to be less accessible to, and/or are afforded some protection from digestive enzymes (Cowie and Hedges, 1996). Preferential assimilation has also been observed among the lipids (preferential assimilation of fatty acids, and egestion of phytol, Sun et al., 1999), alongside the production of new compounds (*iso*-15:0 fatty acid and a C_{16} alcohol) not present in the diet (Bradshaw et al., 1991;Sun et al., 1999). Thus, egested OM will be depleted in useful and/or limiting biochemicals, and may show lower bulk reactivity than fresh OM.

Gut passage also affects the physical state of OM, breaking up clusters, dissociating it from clays, and packaging it into faecal pellets. Therefore, while excreted OM may be of relatively low food quality, it may be made more susceptible to enzymatic attack, hence the relatively high oxygen consumption rates sometimes observed in faecal pellets (Aller, 1982).

As with all faunal processes, the effects of digestion on OM biochemistry are likely to be taxon-specific, as gut chemistry (enzymes and surfactants) and architecture have been shown to vary among taxa, depending on food preferences and feeding strategies (Penry, 1989; Mayer et al., 1997).

Detailed studies of the effects of digestion on sediment composition and OM reactivity have been rare, and the complexities arising from species-specific effects are yet to be addressed.

1.7 Previous Studies of Faunal OM Processing

A serious limitation of much of the work described above is that the majority of it was conducted in microcosms, where only one species, and one faunal process were considered at a time. Such studies cannot provide an holistic view of OM processing by whole benthic communities in their natural settings. The work described below was an attempt to redress this shortcoming.

A few experimental studies have been carried out in a range of seafloor environments, from estuaries to the ocean floor, which have aimed to construct carbon budgets for intact sediments and their benthic communities, and so quantify the role of fauna in short-term C-cycling. The studies mostly involved tracing artificially added algal detritus by means of a stable isotopic label (^{13}C), and have provided direct observations of rapid and significant uptake, processing and transport

of fresh OM by foraminifera, and bacteria (Middelburg et al., 2000; Moodley et al., 2000, 2002), and by macrofauna (DeMaster et al., 1994; Levin et al., 1997; Witte et al., 2003 a, b).

Moodley et al (2002), in a deep-sea study (over ~2 days), found that the foraminifera took up the largest amount of label (29% of that recovered), and the macrofauna and megafauna (3.5%) the smallest. Bacteria constituted up to 95% of the biomass, but contained only 22% of the total processed label, suggesting they were not particularly active in the uptake of fresh OM, and may have been more significant in the remineralisation of relatively refractory OM (Moodley et al, 2002; Witte et al, 2003b). In contrast, Witte et al. (2003 a, b), in similar settings, found that the macrofauna dominated OM uptake in the short term (over several days), and foraminiferal and bacterial uptake only became dominant after 1-2 weeks. Thus, some studies reveal differences in faunal OM processing among sites, but others have shown remarkably uniform patterns between estuarine and deep-sea settings (Moodley et al., 2005). The variations in conditions between sites that bring about these similarities and differences in the path and short-term fate of OM remain unclear.

The largest C sink in isotope labelling studies is usually found to be remineralised dissolved inorganic carbon (DIC) (constituting 30-70 % of processed C, Middelburg et al., 2000; Moodley et al., 2002; Witte et al., 2003 a). It has often been assumed that due to their overwhelming dominance of the biomass, bacteria are responsible for the majority of this. Heip et al. (2001) investigated the question by assigning responsibility for sediment community oxygen consumption (SCOC) between the macrofauna and meiofauna, based on their biomass, and independently measured metabolic rates. The remainder was attributed to bacteria. Despite some bacterial biomass being shown to be dormant, the authors concluded that in the deep sea, bacteria dominate respiration. The domination of OM cycling by small organisms is in agreement with observations that they have faster gut turnover times and / or metabolic rates than larger fauna (Mahaut et al., 1995), and tend to constitute larger proportions of the benthic biomass in deep settings. At a continental shelf site, however, Heip et al. (2001) found the benthic biomass to be dominated by microfauna, but estimated that ~50% of SCOC was carried out by the macrofauna

and megafauna. Thus, in some settings it cannot be assumed that bacteria dominate OM remineralisation, and the macrofauna may play a greater role in C cycling than has been previously thought. In addition the macrofauna may also play vital indirect roles through burrowing, irrigation, and microbial stimulation.

The studies described above do not allow direct comparisons to be made of the functioning of fauna in OM processing between contrasting environments, thus the reasons for the observed variation in the faunal processing and fate of OM between sites remain unclear. Moodley et al. (2005) showed experimentally that reduced faunal OM processing in the deep sea compared to estuarine settings was due to the difference in temperature. It remains to be seen, however, what the effects of differences in faunal community, OM and oxygen availability are.

1.8 Summary and Project Rationale

The burial of organic carbon in continental margin sediments is one of only a few processes by which carbon becomes sequestered for geological timescales, and an understanding of factors governing this burial process is important to our understanding of the global carbon cycle.

Oxygen exposure time, as influenced by bottom-water dissolved oxygen concentration, sedimentation rate and primary productivity, and other factors such as sorptive protection, is thought to be a primary influence on the burial efficiency of organic carbon.

Fauna are responsible for the processes of bioturbation, irrigation, ingestion and microbial stimulation, many of which seem to increase OM decay rates, in part through their influence on oxygen exposure. The significance of these processes in determining the short-term and long-term fate of OM in marine sediments, with full faunal communities, has not been extensively studied, and the overall effect of benthic faunal activity on OM burial efficiency is the least well understood or quantified aspect of C cycling in marine sediments. Faunal processes have been investigated experimentally, but usually in vitro, and a holistic description of the role of fauna in OM cycling thus is still to be completed. Previous studies of faunal processes have usually been conducted using single species of fauna. Thus, potential inhibiting or enhancing interactions between fractions of the benthic community

have not been accounted for. A small number of full-community feeding studies have been conducted, and have produced a variety of results. These have not allowed direct, systematic comparisons to be made between sites with contrasting environmental conditions and communities, and reasons for differences in the short-term fate of OM among sites therefore remain unclear. The description and quantification of faunal processes in OM cycling is further hampered by their species-specific nature, which, coupled with the fact that no two sites exhibit exactly the same community, have encouraged single-species studies, and thus far rendered generalisations unobtainable.

The least well-characterised role of fauna in C-cycling is the biochemical alteration of OM that results from gut passage and digestion. This has been studied for several biochemical classes, each in isolation, and never using whole-community feeding experiments. Furthermore, direct links have not been made between faunal processes, especially digestion, and the organic geochemistry of the sediments in which the fauna live. A wider understanding of the decay and preservation of OM in marine sediments would also be facilitated by a molecular-level approach.

The study carried out for this PhD project was designed to expand our knowledge of the interactions between fauna and OM cycling through the sediments in which they live, and the impacts of fauna on sedimentary organic content and composition.

Experiments and sampling were designed with the gaps in current knowledge in mind. Therefore, the interaction between faunal processes and sediment organic geochemistry was investigated using whole-community pulse-chase ^{13}C tracer experiments, allowing the tracking of C through the sediment and faunal community at bulk and molecular levels. These, together with sediment sampling, were carried out at sites displaying large contrasts in sedimentary OM content, faunal communities and bottom-water oxygen concentrations. In addition, this study was conducted as part of a comprehensive characterisation of the ecology and geochemistry of the study sites. Therefore, the results became part of a wider description of biogeochemical processes in continental margin sediments.

1.9 Research Questions and Hypotheses

This study aimed to address the following specific questions;

- What is the short-term fate of OM in Pakistan margin sediments, and to what degree are fauna involved in determining that fate?
- Which classes of fauna are most active in early OM processing, and how does this vary among sites with contrasting organic contents, oxygen availability, temperature, depth and faunal communities?
- In what way does macrofaunal gut passage alter the amino acid composition of OM, is this species dependent, and can it be linked to amino acid suite changes observed in the sediment?
- How do the abundances of carbohydrates and pigments vary across the Pakistan margin, and how does this relate to oxygen availability, total organic carbon, OM quality and faunal community composition and activity?
- What is the quality of OM on the Pakistan margin, and how is that related to site conditions and faunal factors?

This study aimed to test the following hypotheses;

- Faunal processing of OM is a significant part of sedimentary OM cycling, and its intensity varies with location depending on the faunal community present.
- The abundance and behaviour of fauna, and therefore their role in OM cycling, is strongly influenced by site oxygen and OM availability.
- Benthic community composition has a strong influence over the type and nature of faunal processes that are most influential at any site.
- Bacteria and foraminifera dominate the overall processing of OM by the fauna, but the macrofauna accomplish considerable uptake and processing at some sites.
- The alteration of the amino acid suite of OM during gut passage is relatively subtle, as most forms of life require similar amino acid balances.
- The effects of burrowing fauna combine with oxygen exposure time and other factors such as OM supply, to control the OM content and composition of Pakistan margin sediments.

1.10 Structure of Research

This study was conducted on the Pakistan Margin of the Arabian Sea at sites along an offshore transect, spanning the depths where an intense OMZ impinged on the continental slope. Sampling and experimentation included the following;

- Megacores and multicores were sectioned and preserved for carbohydrate and pigment analysis.
- Megacores were incubated aboard ship with ^{13}C tracer added in the form of labelled algal detritus. The short-term movement (2-5 days) of the tracer into sediment, porewater, overlying water, bacteria and different faunal classes was measured.
- Similar isotope labelling studies were conducted *in situ* using an autonomous benthic research platform (benthic lander) with an incubation chamber.
- This work was all set against a wider program of geochemical and biological surveys, and determinations of the rates of microbial processes, sediment accumulation and mixing rates, and the benthic fluxes of dissolved nutrients, gases, trace metals, and organic matter.

CHAPTER 2

Methods

2.1 Introduction To The Field Area

The aims of the Arabian Sea project were, broadly, to investigate the interaction of biological and geochemical processes at the seafloor, and to study how that interaction depends on key variables such as dissolved oxygen (DO) concentration, organic matter (OM) supply, and faunal community abundance and composition. This study had the particular aim of focusing on the interaction between faunal processes and the organic geochemistry of, and OM cycling in, seafloor sediments in a range of settings.

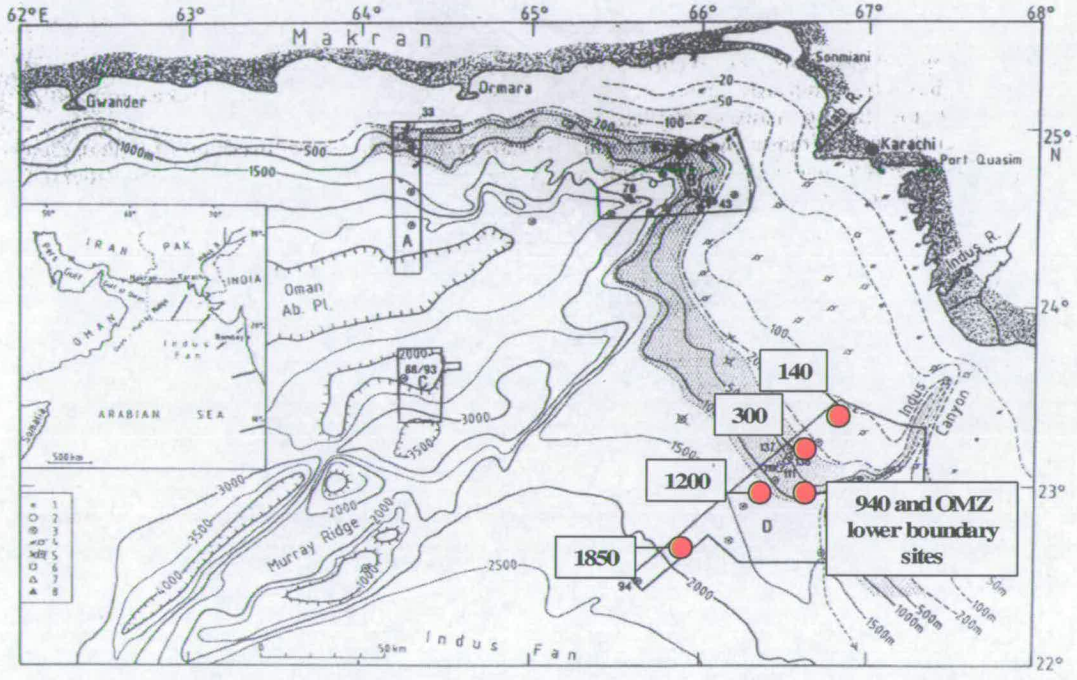


Figure 2.1. Map showing location of study sites 140m, 300m, 940m, 1200, and 1850m (in order of distance from shore). The additional OMZ lower boundary sites were along the same transect as the 940m site (slightly to the SE of the main transect). Adapted from Schulz et al. (1996)

The Arabian Sea exhibits one of the largest volumes of oxygen depleted water in the world (Helly and Levin, 2004), between depths of ~150m and 1000m. Where this oxygen minimum zone (OMZ) impinges on the continental margin it creates steep cross-margin gradients in oxygen availability, OM quality and quantity, and benthic faunal community abundance and composition (Cowie, 2005, and references therein). The Arabian Sea continental margin therefore provides a natural laboratory for the study of benthic biogeochemical processes over a wide range of benthic

environments. By studying sites above, within, and below the OMZ, the effects of varying oxygen and OM availability on the function of fauna in sediment OM cycling, and on sediment organic geochemistry can be investigated. In addition, the Arabian Sea is subject to a monsoonal climate, which produces biannual upwelling and productivity, resulting in a seasonally pulsed supply of OM to the sediments (Haake et al., 1993a, Lee et al., 1998). Thus by visiting the area before and after a monsoon, the response of the benthic community to an increase in OM availability can be studied.

The Arabian Sea OMZ is most intense on the eastern side of the basin (Cowie, 2005). This region is not as intensively studied as the Oman margin, but was visited in 1993 during the SONNE-90 cruise, and at that time a geochemical and sedimentological characterisation was completed (e.g. Cowie et al., 1999, Kiel and Cowie, 1999, Schulte et al., 2000).

The Pakistan margin of the Arabian Sea was chosen for this study on the basis that it is less well characterised than, and distinct from, the Oman margin in terms of oxygen availability, de-nitrification rates, monsoon, upwelling and productivity intensity and sediment structure, and that, although it has been previously studied, its biology and functioning are still relatively unknown (Cowie, 2005). A transect line across the Pakistan margin was chosen in an area where the bathymetry was thought to be suitable for the deployment of benthic landers (Fig. 2.1).

2.1.1 Monsoons and Upwelling

The Arabian Sea is affected by monsoon winds, driven by seasonally varying temperatures over the Indian Subcontinent and Indian Ocean. In the summer, rising air over India draws in air from the SW, producing the SW monsoon from June to September. As the land cools in autumn, high-pressure forms and the winds reverse, producing NE winds from November to March. Intermonsoon periods are, by contrast, very calm. Both NE and SW monsoon winds drive offshore surface currents that result in seasonal upwelling, which is more intense in the Western Arabian Sea, where monsoon winds are strongest (Haake et al., 1993a). This upwelling of nutrient-rich water stimulates phytoplankton blooms, which result in

pulses of OM to the sediment below (Haake et al., 1993a). The plankton bloom resulting from the SW monsoon of 2003 is shown on Figure 2.2.

Primary productivity has been found to vary across the Arabian Sea, from 200-454 gC m^{-2} y^{-1} off Oman, through 180-369 gC m^{-2} y^{-1} in the north and on the eastern margin, to 30-105 gC m^{-2} y^{-1} in the central region (Pfannkuche et al., 2000, Suthhof et al, 2000, and references therein). Productivity on the Pakistan margin is therefore of intermediate magnitude when compared to that off Oman and further offshore. Observations have shown that plankton abundance reaches peak values at the start of each monsoon, as mixing reaches a critical depth. After this, productivity spreads out over greater depth, before the thermocline shallows again at the end of the monsoon, concentrating production, and producing a second peak in productivity indicators (Prahl et al., 2000). The Arabian Sea phytoplankton community has been found to be composed largely of diatoms, with significant contributions also from prymnesiophytes, prochlorophytes and cyanobacteria (Tarran et al., 1999, Barlow et al., 1999). The dominant group of phytoplankton is observed to change in a succession through the monsoons, and diatoms also flourish during intermonsoon periods.

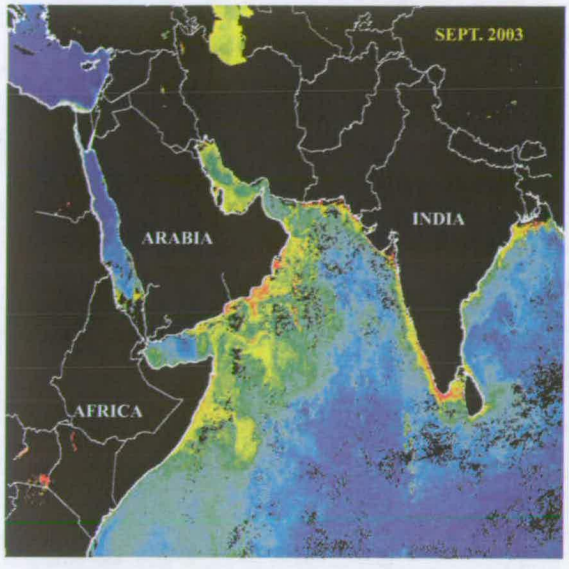


Figure 2.2. Satellite image of the chlorophyll bloom during the summer monsoon 2003, from http://www.bigelow.org/climatechange/ArabianBloom_2003.htm. Warm colours are high concentrations.

The two monsoons are not of equal magnitude. Haake et al. (1993a) observed that maximal windspeeds and particle fluxes, and minimal sea surface temperatures associated with monsoon-induced upwelling, were more pronounced for the summer, SW monsoon. These seasonal variations were more pronounced in the West of the region (Haake et al., 1993a). The monsoonal increase in particle flux is observed to be accompanied by increases in the fluxes of carbonate, biogenic opal, organic carbon and lithogenic material to the sediment, thus the monsoon causes an increase

in wind deposition of terrestrial material, as well as an increase in local primary production (Haake et al., 1993a).

2.1.2 Oceanography and the Oxygen Minimum Zone

The Arabian Sea does not have the subtropical-cell-type circulation of the Atlantic and Pacific that creates equatorial upwelling. Instead, it has a cross-equatorial cell, with upwelling at the northerly coasts, and southwards surface transport of water to southern hemisphere subduction areas (Schott et al., 2002). During monsoon-driven upwelling, low-salinity water is replenished by flow from the south along the Somali and North Equatorial currents. High salinity water (Arabian Sea Water) is formed within the basin through evaporation and cooling during the NE monsoon, and this sinks, but remains relatively shallow. The only replenishment of water below this level is through relatively small flows of Red Sea and Persian Gulf high salinity water from the north, and a flow of old, and thus oxygen poor water from the south (Morrison et al., 1998, 1999).

The Arabian Sea exhibits an intense midwater OMZ (with dissolved oxygen concentrations $<0.5 mlL^{-1}$, and often $<0.1 mlL^{-1}$) between roughly 150m and 1000m depth, which prevails throughout the year. Factors that contribute to the existence of the OMZ include stagnation, lack of ventilation, and heterotrophic consumption of OM from the intense, monsoon-induced plankton blooms. Intensification of the OMZ during the monsoons is only slight, suggesting that consumption of sinking organic matter is not the only reason for its existence (Sarma, 2002). The tracing of CFC's in seawater has shown that the residence time of water in the OMZ is not abnormally long (~ 10 years, Olson et al., 1993), thus stagnation is not solely to blame either. It is thought that the existence and relative invariance of the OMZ are due to a combination of factors. Modest water replenishment at mid-depths by relatively low oxygen water maintains the OMZ during intermonsoon periods, and during monsoons, intense OM consumption cancels out the effects of ventilation by flow from the south and wave action (Sarma, 2002, Olson et al., 1993).

The Arabian Sea OMZ varies spatially in its intensity. It is observed to thicken northwards (Olson et al., 1993), and is more intense, with greater amounts of water column de-nitrification, towards the east (Naqvi, 1987, Naqvi et al., 2000).

2.1.3 Pakistan Margin Sediment Geochemistry

Sediments on the Pakistan margin vary from laminated to bioturbated, depending on the availability of dissolved oxygen (DO), and the consequent faunal community (Schulz et al., 1996). Geochemical and carbon isotopic data (the presence of terrestrial lignins and plant waxes, and $\delta^{13}C$ values that decrease offshore) suggest that OM on the Pakistan margin is of predominantly marine origin (Cowie et al., 1999, Schulte et al., 2000, Calvert et al., 1995).

Weight percentages of organic carbon (%Corg) vary between ~ 1.5 and 4.5 %. Maximal values are coincident not with the low oxygen core of the OMZ, but with its lower boundary, and slightly deeper, in bioturbated sediments (Cowie et al., 1999, Calvert et al., 1995). While some authors have claimed that there is a (possibly weak) causal relationship between limited oxygen availability and high sediment %Corg, and organic carbon to surface area ratios (Kiel and Cowie, 1999, Cowie et al., 1999, Schulte et al., 2000), others have cited the spatial mismatch in low oxygen and high %Corg, and the invariance in hydrogen index across the margin, in order to refute this relationship (Calvert et al., 1995). The latter authors claimed instead that the %Corg of Pakistan margin sediments was controlled by OM supply from surface waters, dilution in the sediment, sediment texture, and downslope movement.

Hydrogen index was found to be invariant across the Pakistan margin, implying that the quality or freshness of sedimentary organic matter was also roughly constant (Calvert et al., 1995). Suthhof et al. (2000) found that the %Corg maximum coincided with maximal organic carbon normalised yields of total amino acids, and that mole percentages of tyrosine were enhanced at low oxygen sites. They contested however that these features were not due to fresh OM being preserved in the absence of oxygen, but did consider that the abundance of tyrosine at low oxygen sites may have been due to redox-related preservation. Schulte et al. (2000) found that while some lipids were enriched in OMZ sediments (e.g. phytol, n-alcohols, sterols and n-C_{35} alkane), others were not (e.g. n-fatty acids and total n-alkanes), and suggested that the OM content of Pakistan margin sediments was determined by OM type, mineral surface area, and mineral type.

Cowie et al. (1999), however, observed minimal organic carbon $\delta^{13}C$ values within the OMZ, and suggested they could be due to the oxygen-related decay state of OM. Thus, the relationship of sedimentary OM quantity and quality with oxygen availability on the Pakistan margin is not straightforward. The evidence suggests that oxygen availability plays some role in controlling the organic geochemistry of the sediments, and that other factors including OM type, sediment texture, and cross-margin transport also exert some influence.

2.1.4 Site Choice and Description

Sites along an offshore transect of the Pakistan margin were chosen to show maximal contrasts in site conditions. Five principal sites were selected at water depths of 140m, 300m, 940m, 1200m and 1850m. In addition, several intermediate sites at depths of 700m, 850m, 1000m and 1100m were selected to provide increased spatial resolution across the OMZ lower boundary (Fig. 2.1).

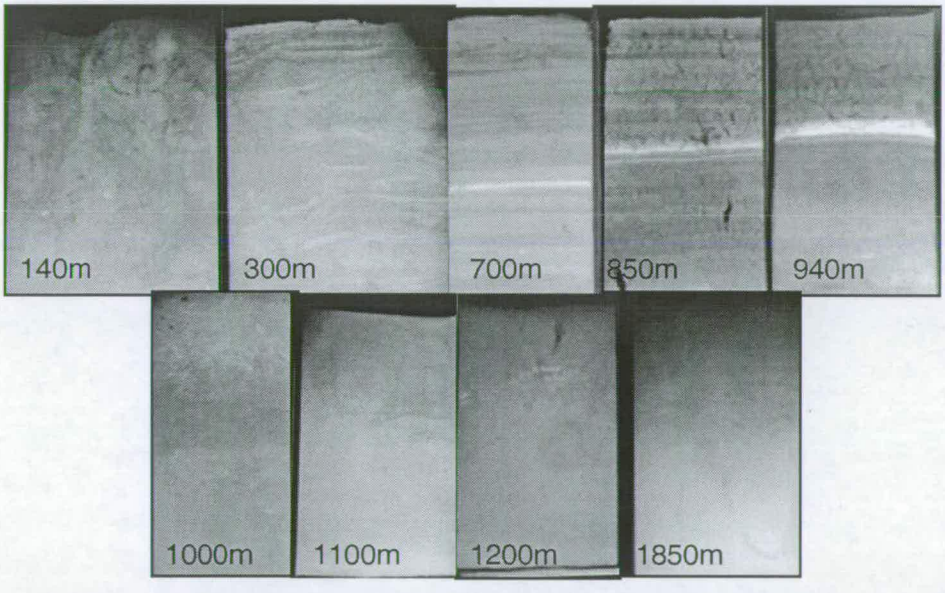


Figure 2.3. X-radiographs of sediments from sites across the Pakistan margin (courtesy of Lisa Levin).

The bottom-water temperatures, DO concentrations, sediment %Corg values and faunal populations found at each of the sites sampled for this study are shown in Table 2.1.

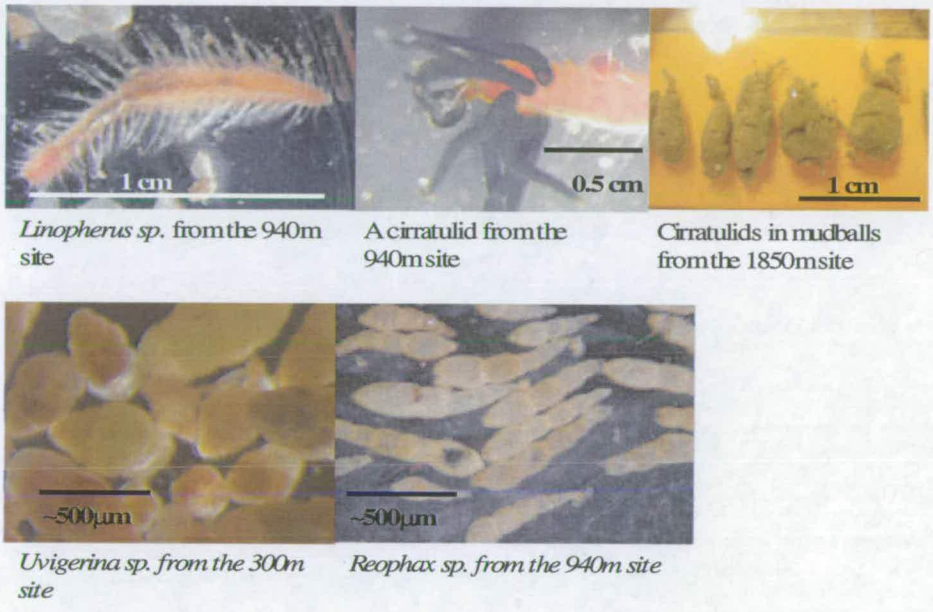
The 140m site was chosen as an oxygenated site above the OMZ (previously visited on SO90). Although the bottom water was far from saturated with DO, the site had

Station (Depth (m)	Temperature (°C)	Dissolved Oxygen ml L^{-1}	Sediment %OC	OM Quality (DI)	Macrofauna Biomass g(wet) m^{-2} / Diversity	Foraminifera Density / Diversity
Pre Monsoon						
140m	22.5	2.05	1.46 ± 0.08		9 / 51 (± 5)	593 / 19
300m	15.5	0.10	2.36 ± 0.09		0.020 (± 0.022) / 2 (± 0.5)	549 / 18
850m	9.7	0.13	3.22 ± 0.06			
940m	9.0	0.13	3.31 ± 0.12		62 (± 45) / 12 (± 1)	80 / 13
1000m	8.7	0.15	3.04 ± 0.01			
1200m	7.2	0.34	3.27 ± 0.26		0.4 (± 45) / 13 (± 2.6)	77 / 16
1850m	3.5	1.78	1.40 ± 0.10		9 (± 15) / 53 (± 6)	24 / 8
Post Monsoon						
140m	18.2	0.11	1.43 ± 0.07	-0.99 ± 0.06	5 (± 2) / 45 (± 3)	1163 / 20
300m	14.8	0.11	2.56 ± 0.29	-0.40 ± 0.12	0.013 (± 0.019) / 1	839 / 14
700m	11.2	0.14	2.59 ± 0.01			
850m	10.1	0.14	3.22 ± 0.06			
940m	9.3	0.17	3.40 ± 0.13	-0.48 ± 0.03	45.7 (± 0.02) / 13 (± 1)	
1100m	8.0	0.24	2.96 ± 0.46			
1200m	7.3	0.27	3.27 ± 0.26	-0.49 ± 0.18	7 (± 11) / 14 (± 0.7)	
1850m	3.7	1.7	1.20 ± 0.25	-1.17 ± 0.14	2 (± 0.9) / 44 (± 4)	

Table 2.1. Site conditions in pre- and post-monsoon seasons. Oxygen concentrations are from CTD casts, %Corg values are for the surface 0-0.5 cm, DI values are averaged over the surface 3 cm. Macrofauna diversity is species number per megacore averaged from 5 cores (Peter Lamont, pers.comm.), foraminifera density data are total number of calcareous individuals in 15 cm^2 of the surface 1 cm of sediment, and diversity data are species number for the same sample volume (Stefanie Schumacher, pers. comm.). For site co-ordinates see appendix A.

abundant macrofaunal and foraminiferal populations, and the sediments were fully bioturbated, with burrows visible in x-radiographs (Figure 2.3). The 300m site was chosen to represent conditions at the heart of the OMZ. Here there was almost no oxygen or macrofauna, and foraminifera dominated the benthic community. X-radiographs showed the sediments to be laminated.

The 940m and 1200m sites were chosen to allow investigation of the way benthic biogeochemical processes varied across the lower OMZ boundary. The 940m site showed OMZ type DO concentrations, while those at the 1200m site were considerably higher. The sediments at both sites were rich in high quality (as measured by the amino acid degradation index, Dauwe and Middelburg, 1998) OM. The 940m site supported the highest macrofaunal biomass among the primary sites, while very little macrofauna was ever recovered from the 1200m site. There may, however, have been large macro or megafauna at the 1200m site that were never recovered in megacores, as sediment x-rays from that site showed very well mixed sediments. At the 940m site, the sediments were laminated with fine burrows (to ~6cm) and a turbidite at ~5-6cm.



Linopherus sp. from the 940m site | A cirratulid from the 940m site | Cirratulids in mudballs from the 1850m site

Uvigerina sp. from the 300m site | *Reophax sp. from the 940m site*

Figure 2.4. Photographs of key fauna from the Pakistan margin (courtesy of Lisa Levin and Kate Larkin).

The 1850m site was chosen to represent abyssal type sediments. At this site oxygen was relatively abundant, and the top 10cm of sediment was well mixed with large burrows. The mixed portion of the sediment was OM-poor, and overlay a grey,

extremely OM poor, low-porosity clay at a fairly distinct boundary (~10cm depth). Both macrofaunal and foraminiferal biomass were relatively reduced at this site.

Surface sediments at all sites were poorly consolidated and highly porous. At the 140m, 940m and 1200m sites they became relatively cohesive by a depth of ~2cm. At the 1850m site porosity decreased more markedly downcore, and at the 300m site lack of cohesion and high porosity persisted to the greatest depth.

Of the intermediate, lower OMZ boundary sites sampled, the 700m site had similar characteristics to the 300m site, and the remaining sites were most similar to the 940m site, with different, but still abundant, macrofaunal communities.

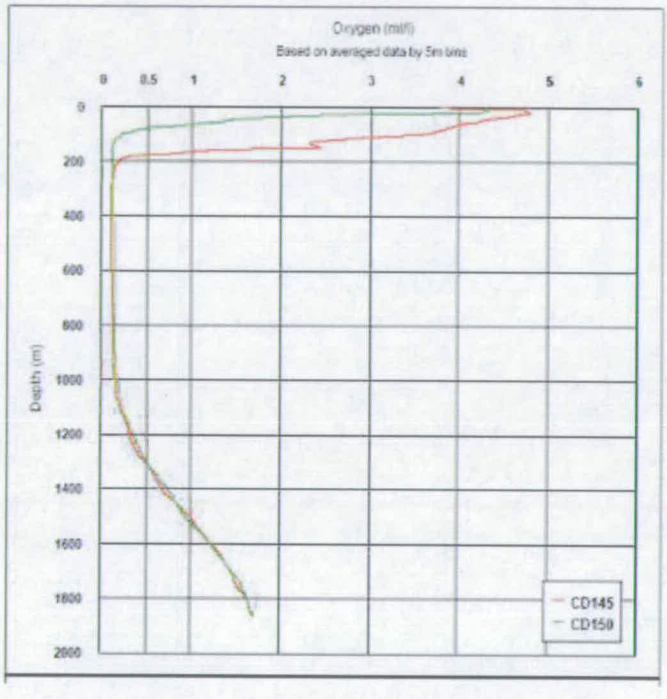


Figure 2.5. The variation in DO concentration with water depth, derived from CTD casts, before (red) and after (green) the summer monsoon of 2003 (from Bett et al.. 2004b).

Where macrofauna were present, the community was dominated by polychaetes. These were relatively diverse at the 140m site, including cirraulids, cossurids, ampharetids, maldanids and spionids. At the 140m and 1850m sites crustacea and molluscs were also found. At the OMZ lower boundary (850m, 940m, 1000m and 1100m) sites, the faunal community was less diverse, and tended to be dominated by large polychaetes, in particular the amphinomid *Linopherus sp.*, and prionospio and machrochaete species. The foraminiferal community was dominated at shallow (140m and 300m) sites by calcareous varieties, particularly *Uvigerina sp.* At deeper sites, agglutinated foraminifera, including reophax, bathysiphon and pelosina dominated the foraminifera. Photographs of key fauna are shown in Figure 2.4.

Studies were conducted both before and after a monsoon, allowing an assessment of the way a seasonal pulse of OM impacted the benthic system. Bottom-water

temperatures after the monsoon were slightly lower than those before, due to upwelling of deep water. The most striking seasonal variation, however, was a reduction in DO concentration at the 140m site, as the OMZ expanded into shallower waters. This was presumably caused by intense productivity and oxygen consumption in the upper-water-column (Fig. 2.5). The concentration of DO at the 140m site decreased from 2.05 to 0.11 ml L^{-1}, thus in the post-monsoon season it was as hypoxic as the 300m site (Bett et al., 2004b).

At other sites, the monsoon-induced OM pulse caused a reduction in sediment oxygen penetration depths (Eric Breuer, pers. comm.), but did not have a measurable impact on the %Corg of surface sediments (Table 2.1).

Thus the selected sites showed the desired cross-margin contrasts in DO, oxygen penetration, %Corg and faunal communities, and variations in response to the SW monsoon.

2.2 Sampling And Experimental

Sampling and experimentation was conducted aboard the RRS Charles Darwin (Bett et al., 2004a, b, Cowie et al., 2005a, b). Two cruises (CD 146 CD 151) occurred immediately before the summer (SW) monsoon of 2003. A third was conducted during the final stages of the same monsoon (CD 150), and a fourth followed, in the immediate post-monsoon season (CD 151).

2.2.1 Sediment Sampling Techniques

Sediment samples were recovered as either multi- (5cm diameter, 12 cores per deployment) or megacores (10.1 cm diameter, 8-12 cores per deployment). Cores intended for organic geochemical sampling were stored at seafloor temperature for no more than 6 hours before sectioning.

Cores were sectioned at 0.5 cm resolution to 2 cm depth, 1 cm to 10 cm depth and 2 cm to either core bottom or 30 cm depth. Sectioning plates, rings and spatulas were rinsed in seawater and distilled water between each section.

Samples for pigment analysis were placed into pre-combusted 20-ml scintillation vials and stored frozen at -20°C.

Samples for carbohydrate analysis were placed in plastic sample bags and frozen at -20°C for later freeze-drying. Most samples analysed for carbohydrates were derived from cores from which porewaters were also extracted, for dissolved organic carbon analysis. These were sectioned in the controlled temperature laboratory under a nitrogen atmosphere. Sections were placed in 30-ml centrifuge tubes. Following porewater extraction by centrifugation (10 mins at 3500 rpm), the residual sediments were freeze-dried and transferred into plastic sample bags.

2.2.2 ^{13}Carbon Tracing Experiments

Carbon isotopic labelling experiments were carried out with the aim of constructing complete, quantitative budgets for the short-term fate of OM in Pakistan margin sediments. Approximately 80% ^{13}C-labelled algal detritus was added to sediment cores (or to the in situ benthic interface), and was traced into the macro, meio and micro fauna, as well as into the bulk sediment, and the dissolved organic and inorganic carbon pools in the porewaters and overlying water. Experiments were conducted on intact sediments containing their whole benthic communities at 7 sites across the margin (the 140m, 300m, 850m, 940m, 1000m, 1200m, and 1850m sites, Table 2.2).

The amount of C added averaged 730 mg m^{-2}, and was the same for each experiment (i.e. the amount was not

	Site	Type	Duration / h	mg C / mg ^{13}C added
Pre-Monsoon	140	Shipboard 2-day	68.1	14.1 / 8.7
	300	Shipboard 2-day	61.4	10.3 / 7.4
	300	Shipboard 5-day	127.3	10.9 / 7.8
	850	Shipboard 2-day	45.8	15.9 / 11.4
	940	Shipboard 5-day	112.0	10.8 / 7.7
	1000	Shipboard 2-day	56.5	10.6 / 7.6
	1200	Shipboard 5-day	114.4	10.4 / 7.4
	1850	Shipboard 2-day	48.1	10.7 / 7.6
	1850	Shipboard 5-day	116.8	15.9 / 11.4
Post-Monsoon	140	Shipboard 2-day	44.2	10.3 / 7.7
	140	Shipboard 5-day	118.3	10.1 / 7.6
	140	In-Situ	56.9	35.8 / 26.8
	300	Shipboard 2-day	58.3	10.3 / 7.7
	300	Shipboard 5-day	154.9	10.4 / 7.8
	300	In-Situ	56.9	35.8 / 26.8
	940	Shipboard 5-day	113.3	10.4 / 7.8
	940	In-Situ	47.9	25.9 / 19.4
	1850	Shipboard 5-day	86.3	29.5 / 9.3

Table 2.2. Details of all the 13C tracer experiments carried out on cruises CD 146 and CD 151. The quantities of C added are totals of the two cores used for each shipboard experiment, and are total amounts for the entire chamber for in -situ experiments. The lander chamber has 5.6 x the surface area of a pair of magacores.

altered in accordance with the natural %Corg of each station). The added C was on average 0.8 ± 0.3 % of the C naturally present in the surface 1 cm of sediment, and this value did not vary systematically among experiments or sites. Uniform C addition was used in order to ensure a constant stimulation of benthic metabolism (e.g. Witte et al., 2003 b), across all experiments.

2.2.2.1 Shipboard Incubations

The majority of tracer experiments were conducted using a shipboard megacore incubation rig under quasi in situ conditions. These were effectively microcosm experiments, but unlike previous microcosm work, they involved intact sediments, and whole, natural, faunal communities.

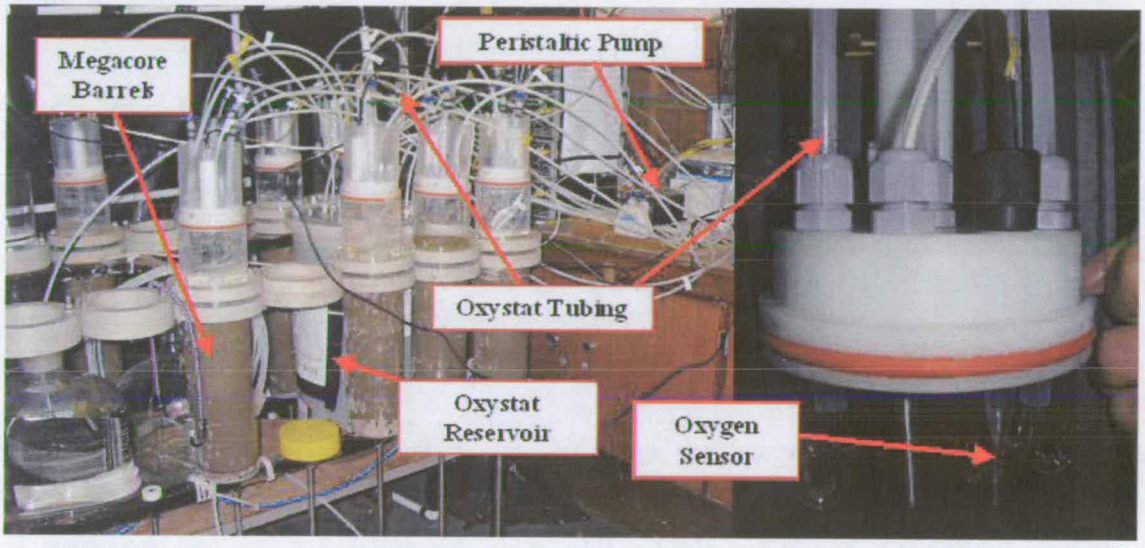


Figure 2.6. Photographs of, on the left, the shipboard incubation rig, and on the right, a core top seal, with key components labelled.

Cores for incubation were sealed top and bottom with all air excluded as soon as they came aboard. This was found to be necessary in order to prevent contamination of hypoxic core top water with atmospheric oxygen. Cores were then quickly transferred to a controlled temperature laboratory (set to on-site seafloor temperature) where they waited not more than 6 hours before experiment initiation. Before incubation, cores were installed on an incubation rig designed for this project (Schwartz et al., in prep.) shown in Figure 2.6. This consisted of a rack capable of holding 14 megacores. The tops and bottoms of each core were fitted with gas-tight caps. The top caps were in contact with water overlying the sediments. Pushing the top seal down expelled air bubbles. The undersides of the core-top caps featured

magnetic stir bars driven by motors on their external top sides. Two ports in each cap allowed the emplacement of oxygen and temperature microsensors, used to monitor conditions in the waters overlying the sediments. Each cap had five further ports, one (central) for bubble expulsion and introduction of tracer, two for the removal of water samples and simultaneous replacement with filtered bottom-water (to maintain constant volume), and two for the connection of each core to an "oxystat" system.

The "oxystat" system was designed to maintain core-top oxygen levels at seafloor conditions despite sediment oxygen consumption, thus keeping fauna alive. These two ports were connected to each other via an ~8m length of gas-permeable tubing (the oxystat gill), submerged in a reservoir of filtered seawater (the oxystat reservoir). This reservoir was sparged with an air and nitrogen mix designed to maintain its DO level slightly above that at the seafloor. A peristaltic pump circulated core top water out of each core barrel, through its dedicated oxystat gill, and back. Diffusion across the tubing in the reservoir replenished core top water with dissolved oxygen which may have been consumed by benthic organisms. Sub-aerial parts of the oxystat system were made of gas impermeable tubing. Thus the core top water of each core remained isolated from all other simultaneous experiments.

Experiments commenced when algal tracer, freeze-dried onto silica or kaolin ballast and made into a slurry with Milli-Q water, was introduced through the central core-top port by syringe. Oxystat pumping was switched off during tracer introduction (to avoid filling the oxystat tubing with algae), but slow stirring was maintained to ensure even coverage of settling tracer over the core surface. Stirring and oxystat pumping were then left off for 30-60 minutes to allow complete settling of tracer, whereupon they were resumed.

Incubations proceeded at seafloor temperature in the dark (cores were wrapped in black plastic sleeves) for either 2 or 5 days. Each experiment was conducted on two cores simultaneously for the purpose of replication.

Core-top water was sampled at 0, 12, 24, 36, 48, 96 and 120 hours after stirring commenced (fewer sampling points for shorter experiments). Samples were preserved, poisoned with $HgCl_2$, in headspace vials for DIC and $\delta^{13}C$ DIC analysis,

and filtered (with 0.22 μm milipore filters) and frozen in screw-top tubes for nutrient analysis. Samples for DOC/TDN analysis were GFF filtered, acidified with concentrated H_3PO_4, and stored in flame sealed ampoules.

Experiments were terminated by removing cores from the rig and extruding them. Sectioning intervals of 0.5cm thickness to 2cm depth, 1cm thickness to 10cm depth and 2cm thickness to 20cm depth were used. Each section was divided in half. One half was placed in a petri dish for extraction of fauna, and the other half was placed in a centrifuge tube for porewater extraction.

After centrifugation (3500 rpm for 10 mins), the porewater samples were preserved in headspace vials for DIC and $\delta^{13}C$ analysis as described above. The sediment remaining in the centrifuge tubes was placed into plastic sample bags and stored frozen prior to freeze-drying.

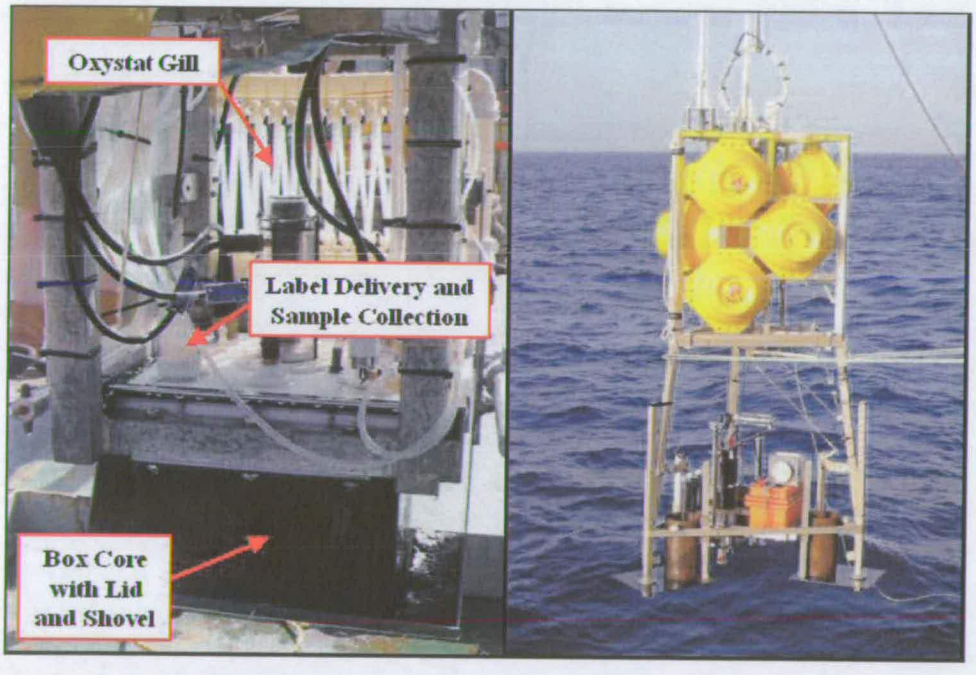


Figure 2.7. The benthic lander (on the right), and its chamber (on the left), with key components labelled.

All fauna were extracted under 12x-20x magnification following wet sieving (with filtered seawater) and retention of residues from 300μm, 150μm and 63μm sieves. All 300μm residues down to 10cm depth were sorted for metazoan macrofauna. All 300μm and some 150μm residues down to 1cm depth were sorted for foraminifera. All unsorted residues were frozen.

Extracted fauna were identified to the lowest taxonomic level possible, rinsed in Milli-Q, placed in pre-weighed tin capsules or glass vials and stored frozen at -20°C.

2.2.2.2 In-Situ Experiments

Isotope labelling experiments were also conducted on the seafloor using a benthic chamber lander (Black et al., 2001, Fig. 2.7) (KC Denmark). The lander was an autonomous research platform that descended unattached to the sea floor. In one of its functioning modes (ELINOR), it inserted a Teflon-lined box core (30x30cm) into the sediment. After a settling period (~ 2 hours) the lid was closed, thus isolating a portion of sediment and overlying water and forming an incubation system analogous to the shipboard system. The water was similarly homogenised by stirring (avoiding resuspension), and pumped through an oxystat gill located outside the chamber in contact with ambient seawater (thus similarly permitting near-ambient DO conditions to be maintained within the incubation chambers). Sensors positioned in the lid allowed monitoring of chamber oxygen levels and pH. Isotope labelling experiments were conducted in situ following the same principles as on board ship. After lid closure, algal slurry was introduced by a pre-programmed syringe, after which it was allowed to settle before stirring and oxystat pumping commenced. Further syringes withdrew water samples at pre-determined times. At the end of the incubation, a hydraulically-driven shovel closed off the bottom of the chamber (thus recovering sediments) and ballast was released, permitting the attached buoyancy spheres to bring the lander to the sea surface. Once the lander was recovered, the water samples were preserved as described for shipboard incubation samples. The recovered sediment was sub-sampled using two short megacore tubes. These cores were sectioned, and samples were treated as described for shipboard incubations.

2.3. Analytical

2.3.1 Bulk Carbon Elemental and Stable Isotopic Analysis

2.3.1.1 Sediments

Aliquots of freeze-dried sediment between 10 and 30 mg mass (corresponding to 200-600μg of C) were weighed into silver boats. Sediments were de-carbonated by addition of 2-3 drops of double-distilled 6N HCl, followed by drying at 60°C.

Samples were analysed for C and N content and $\delta^{13}C$ on a dedicated Europa Scientific (Crew, UK) Tracermass isotope ratio mass spectrometer with a Roboprep Dumas combustion sample converter, calibrated with acetanilide standards. Carbon and N weight percentages were determined from IRMS peak areas. The r^2 values of calibration curves were usually higher than 0.99. Average relative standard deviations for replicate analyses were 4.6% and 5.0% for %Corg and $\delta^{13}C$ respectively (n = 27), and acid treated blanks did not contain detectable C.

2.3.1.2 Fauna

Faunal samples were air dried at 45°C, and those in tin boats were re-weighed to obtain sample dry weights. Fauna in vials were transferred wet to pre-weighed tin boats, dried and re-weighed. Soft-bodied fauna were de-carbonated by placing tin boats inside silver boats and adding 1-2 drops of 1N HCl, followed by drying at 45°C. Mollusc and foraminifera samples were de-carbonated by placing tin boats inside silver boats followed by addition of 2-3 drops of double-distilled 6N HCl (Hidetaka Nomaki, pers. comm.) to ensure complete de-carbonation. Faunal samples were analysed by combustion IRMS as detailed above, with acetanilide standards tailored to suit very low C samples.

2.3.2 Carbohydrates

Carbohydrates were analysed as alditol acetates. For discussion of the method see Fox et al. (1989).

2.3.2.1 Reagent Preparation

A Mixed aldose external standard, and an internal myo-innositol standard were prepared by dissolving aldoses in 1:1 pyridine:water (Milli-Q) to give concentrations of ~0.025M.

A KBH_4 solution was freshly prepared for each sample batch by dissolving 0.71g of KBH_4 in 10ml of Milli-Q water using sonication. This solution could not be capped/shaken, and was always used immediately.

2.3.2.2 Sample Preparation

1) 0.2-0.3g sub-samples of freeze-dried sediment were weighed into plastic tubes.

2) Samples were wetted with Milli-Q and treated with double distilled 6N HCl until effervescence ceased.

3) Samples were dried in a centrifugal evaporator for 2 hours.

2.3.2.3 Hydrolysis

4) A clean Teflon stir bar and 1ml of 12M H_2SO_4 were added to each sample, which was then mixed to a paste using a glass stirring rod.

5) Samples were sealed with drilled caps, and thoroughly mixed using a vortex mixer and sonication.

6) Extraction commenced with a preliminary period of 30 minutes at room temperature (with magnetic stirring).

7) After 30 minutes, 8ml of Milli-Q water was added to each tube, and samples were placed in a boiling water bath (on a heated magnetic stirrer). Hydrolysis proceeded for 3 hours.

8) Hydrolysis was quenched in an ice bath, and samples finished cooling in a freezer.

9) 25µl of a 0.025M solution of myo-inositol (as an internal standard) was added to cooled samples followed by vortex mixing.

10) Samples were neutralised by the gradual addition (with vortex mixing) of the stoichiometric quantity of $Ba(OH)_2$ powder (ground) required to bring the mixture to a pH of 5.5-6.5 (usually ~3.8g for 1ml of H_2SO_4).

11) Eight ml of Milli-Q water was added and mixed into each tube, and samples were centrifuged for 10mins at 3000 rpm. The supernatant was transferred to pear-shaped flasks, and the residue was washed with 5ml of Milli-Q water. After vortex mixing and re-centrifugation, this rinse was added to the previous aliquot.

12) Sample volumes were reduced using rotary evaporators (with a water bath temperature of 40°C) and samples were transferred to 10-ml glass culture tubes.

13) A standard of mixed sugar solution and myo-inositol internal standard solution was prepared and dried in a centrifugal evaporator. This was treated hereafter the same as the samples.

2.3.2.4 Derivatisation

14) Freshly prepared KBH_4 solution (0.5 ml) was added to each sample, and each tube was sealed. Reduction proceeded at room temperature overnight (16 hours).

15) Reduction was halted by dropwise addition of glacial acetic acid with shaking until effervescence stopped, the precipitate changed colour, and pH reached 5.5.

16) Samples were passed through 5ml of cation exchange resin followed by 2ml of anion exchange resin and rinsed out with 7ml of Milli-Q into pear shaped flasks

17) Sample volumes were reduced by rotary evaporation. Samples were transferred into Pyrex culture tubes and dried in a centrifugal evaporator for 3-3.5 h, with assistance from a heating lamp for part of the time (temperature never exceeded 50°C).

18) Dried samples were washed with 1ml followed by 2x 0.5ml of methanol, drying under nitrogen each time.

19) 0.25ml of methyl immadazole and 2ml of acetic anhydride (both stored in dessicators) were added, and acylation proceeded at room temperature for 10 minutes.

20) Acylation was quenched by addition of 4 ml of Milli-Q water, and samples were then allowed to cool.

21) 1.5 ml of dichloromethane (DCM) was added to each sample with mixing, followed by sonication to separate layers.

22) The lower, DCM, layers were transferred to injection vials using a Pasteur pipette. These were dried under N_2, and the solvent extractions were repeated with 0.5ml of DCM.

23) Dried samples were taken in 200µl of pyridine and transferred to GC injection vials with inserts.

2.3.2.5 Chromatography

Samples were analysed by gas chromatography with flame ionisation detection (FID), using a Supelco SP2330 60m x 0.32mm column with a 25µm film thickness.

The oven program started at 90°C, and ramped to 190°C at 20°C / min. The

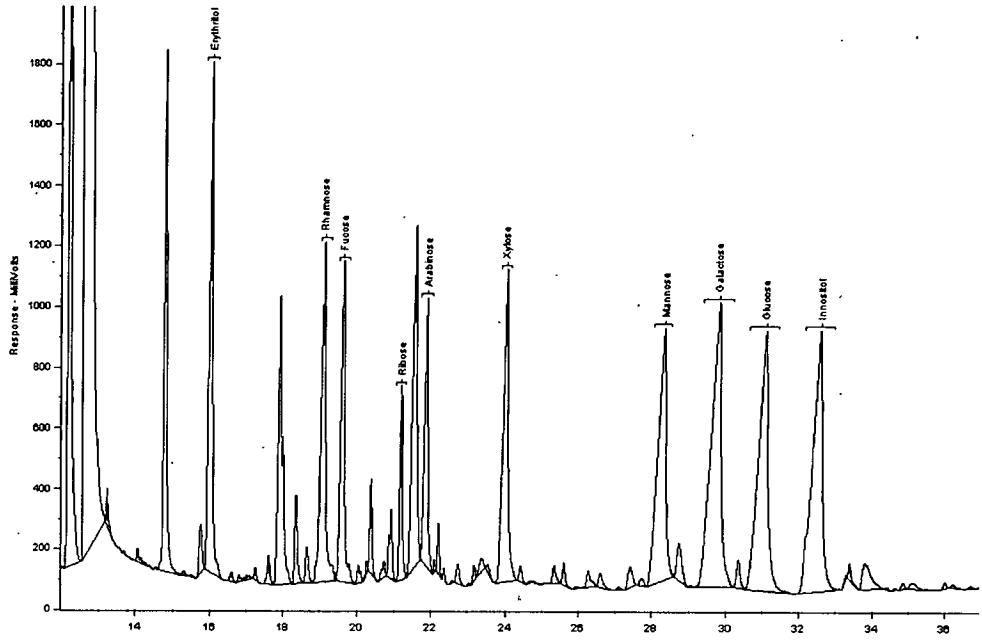


Figure 2.8. A typical carbohydrate chromatogram from a sediment sample (pre-monsoon, 140m).

temperature then increased to 250°C at 4 °C / min and was held for 11 minutes. A final ramp took the oven temperature to 270°C at 20°C / min, with a hold for 7 minutes to prevent sample carry over. Splitless injection (injector temperature was 280°C) and a flow rate of 2.5-3 ml/min were used. The initial purge time was 1.5 mins, and the flow rate of He carrier gas was 2 ml / min. The FID temperature was 250°C. Good separation and clean baselines were achieved, and a typical sample chromatogram is shown in figure 2.8.

Sugars were quantified using a single-point calibration of analyte:standard amount ratio against analyte:standard response (peak area) ratio. Linearity of FID response was demonstrated using standards containing varying amounts of analyte and a constant amount of internal standard, and also by serial dilutions of a single standard. R^2 values from these tests were always greater then 0.9, and usually greater than 0.98. From each core at least one sample was run in duplicate or triplicate. The average relative standard deviation of all sugars from 10 sets of replicates was 12 %.

2.3.3 Pigments

Pigments were analysed by high performance liquid chromatography (HPLC) as described below.

2.3.3.1 Extraction

1) Frozen sediment samples were thawed refrigerated, and kept in the dark overnight.
2) Sub-samples (2-3g) were weighed into 100-ml glass bottles to which ~20ml of glass beads had been added.
3) 20ml of 90% acetone (10% Milli-Q water by volume) was added and samples were shaken to mix.
4) Extraction was performed by shaking for 20 seconds in a cell homogeniser, while cooling with CO_2.
5) Acetone/sediment slurry extracts were decanted into glass test tubes and centrifuged (1300 rpm for 5 mins) to remove the sediment.
6) Acetone extracts were transferred into 5-ml vials with septum caps for HPLC analysis.

2.3.3.2 HPLC Analysis

Samples were analysed on a Waters instrument using a Nova-pak C18, 4μm x 15cm reverse phase column with a Nova-pak C18 5μm guard column. The detectors used were a Waters diode array detector 996 and a Waters fluorescence detector 474. The eluents and gradients used are shown in Table 2.3. The flow rate was 0.8 ml / min and the sample injection volume was 60μl. A typical chromatogram is shown in Figure 2.9.

Time / min	%A	%B	%C
0	100	0	0
4	0	100	0
18	0	20	80
23	0	100	0
25	100	0	0

Table 2.3. The eluants and gradients used in HPLC analysis of pigments. All gradients are linear. Solvent A = 0.5M ammonium acetate in 85:15 methanol:Milli-Q water, Solvent B = Acetonitrile: Milli-Q water Milli-Q90:10, Solvent C = Ethylacetate

A photodiode array was used for peak identification (by comparing spectra with library data) and for quantitation. The chromatogram of absorbance at 665nm was used to quantify chlorophyll-a, pheophytin and pheophorbide, and that at 450nm was used to quantify β-carotene, alloxanthin, diatoxanthin, and zeaxanthin.

Quantitation was performed using a single-point external calibration using standards sold by Sigma, Fisher, Roth and VKI, and stored in the freezer at -80°C.

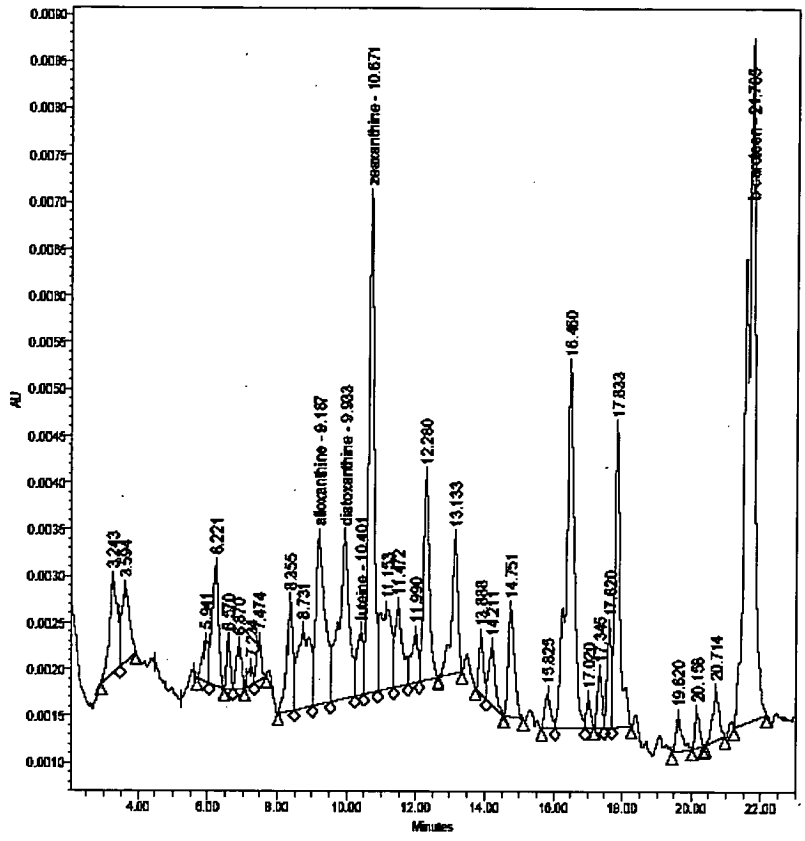


Figure 2.9. A typical pigment absorbance chromatogram at 450nm from the photodiode array detector.

Two samples from each core were run in either duplicate or triplicate. The average relative standard deviation across all pigments from 15 sets of replicates was 7%.

2.4 Data Processing

2.4.1 Porosity Corrections

Pigment data was collected from wet samples, and required correcting to give values in units of μg / g dry sediment. This was achieved using porosity data (courtesy of G. Law), derived from measuring the wet and dry weights of samples. The porosity data, together with bottom water salinity values derived from CTD casts, was also used to correct the dry weights of carbohydrate samples for their salt contents, before carbohydrate data was normalised to sample weight.

2.4.2 Correlation Analysis

Parameters were tested for their covariance using the correlation tool in the Microsoft Excel data analysis add in. This generated a correlation coefficient ρ for each pair of data sets tested, which was equal to the covariance of the data sets divided by the product of their standard deviations. Tables were used to determine the significant value of ρ at the 5% level. For the data sets in this study, which usually had n = 10-14, ρ was usually ~ 0.5 (exact values given in each chapter).

2.4.3 Multivariate Analysis

Principal component analysis was used to detect subtle compositional changes within biochemical classes between sites and samples. This is a method of producing a single parameter from a large and complex data set. Principle component analyses were performed in Minitab, using either mole or weight percentage data. This type of analysis generated scores for each sample on the first and second principle axes, which were based on the suites of biochemicals in question. It also generated factor coefficients for each biochemical in the suite, which indicated in what sense and with what magnitude they affected the sample scores.

2.4.4 T-Tests

Two tailed Students' t-tests were used to determine whether apparent differences between data sets were statistically significant. These were performed using the Excel function. T-tests generated values for p. A significant difference at the 5% probability level (i.e. 95% probability that the data sets are different) was signified by values of t smaller than 0.05. The 10% probability level can also be used, in which case a value of t below 0.1 indicates a statistically significant difference.

CHAPTER 3

A Method for The Quantitative Detection of ^{13}C-Labelled Amino Acids In Marine Sediments and Fauna

3.1 Introduction

The uptake and metabolism of several compound classes by marine fauna and bacteria has been studied using stable isotopic labels. These biochemical classes include the lipids (Sun et al., 1999, Sun, 2000, Sun et al., 2002, Bradshaw et al., 1991), amino acids (Thomas and Blair, 2002, Berthold et al., 1991), and bacterial biomarkers, including D-Alanine and bacterial phospholipid fatty acids (PLFAs) (by gas chromatography-combustion-isotope ratio mass spectrometry, GC-C-IRMS, Boschker and Middelburg, 2002, Boschker et al., 2001, Veuger et al., 2005). Such studies are essential to improving our understanding of the cycling of OM in seafloor sediments, and to the discovery of the biochemical alterations that occur in faunal guts and during decay in the sediment, which eventually produce uncharacterisable, refractory OM.

Gas chromatography-mass spectrometry (GC-MS) techniques (as opposed to GC-C-IRMS techniques) for the tracing of labelled biochemicals face quantitation difficulties, as standards with the correct level of isotopic labelling are either prohibitively expensive or unobtainable. For this reason, many previous studies have only traced labelled biochemicals in a qualitative, or relatively quantitative (i.e. mole percentage) sense (Thomas and Blair, 2002, Berthold et al., 1991). This problem is however surmountable, using a calibration technique devised for the tracing of lipids by Sun (2000).

Amino acids are the building blocks of proteins, and so are major constituents of all biomass, particularly in the marine environment. They also constitute significant portions of both reactive and refractory sedimentary OM. In the water column, and during early sediment diagenesis, amino acids have been shown to undergo selective degradation, and thus their abundances and suites have been used as indices of OM degradation state (Dauwe and Middelburg, 1998, Cowie and Hedges, 1994). The ability to study the fate of amino acids during faunal digestion and sedimentary OM decay would therefore add greatly to our knowledge of both the role of fauna in OM cycling, and the processes and factors which combine to determine OM burial efficiency.

The aim of this study was to develop the tracing of ^{13}C-labelled amino acids into a fully quantitative technique by applying the calibration method developed by Sun, 2000) to the derivatisation procedure used by Thomas and Blair (2002). The method was applied to fauna and sediment samples taken from shipboard and in situ ^{13}C tracing experiments (described in chapter 2), thus producing quantitative labelled biochemical data for samples from whole-community feeding experiments.

3.2 Method Theory

3.2.1 Tracing ^{13}C-Labelled Molecules Using Mass Spectrometry

When organic molecules are subjected to ionisation in preparation for mass spectrometry they undergo fragmentation. This produces mass spectra consisting of fragments, the masses and relative abundances of which are constant for, and therefore diagnostic of, a given molecule.

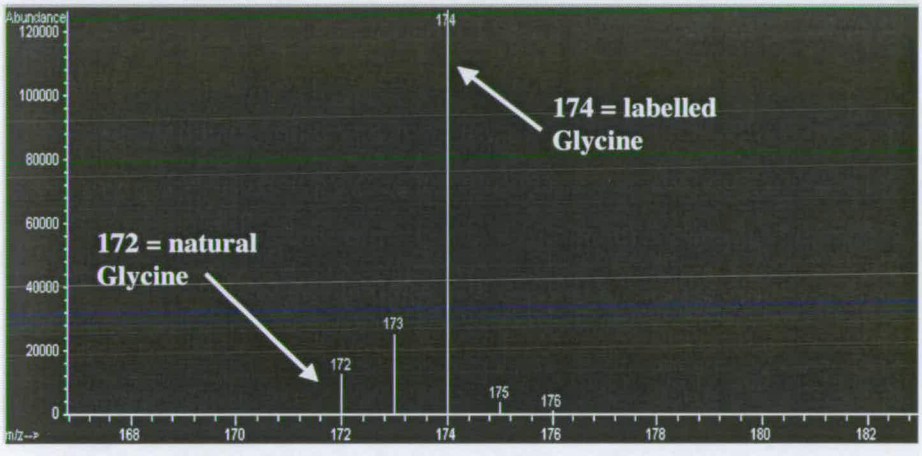


Figure 3.1. An illustration of the different ions (both of the same fragment) produced by 100% ^{12}C and 100% ^{13}C glycine (a 2 C amino acid).

During GC-MS analysis therefore, compounds are identified on the basis of both their retention times in the GC column, and on their mass spectra. From the mass spectrum of each compound a particular (abundant) fragment is chosen, the abundance (peak area) of which is used for quantitation.

The quantifier ion for a given compound typically contains only ^{12}C atoms, and has a mass M. Should that ion contain a single ^{13}C atom, it would have mass M+1. If the original molecule was derived from 100% ^{13}C-labelled algae, every carbon atom in the quantifier ion would be the ^{13}C isotope, and it would have mass M+n, where n is the number of carbon atoms (from the original molecule) the ion contains. If the

quantifier ion is the molecular (or quasi-molecular) ion, then n is equal to the number of carbon atoms in the original molecule. Thus by detection of the ions with masses M and M+n, the abundance of both naturally occurring and 100% ^{13}C-labelled molecules can be quantified simultaneously (Fig. 3.1).

The protein amino acids all contain between 1 and 9 C atoms. Thus for the majority of them (all those containing 3 C atoms or more), the natural occurrence of a 100% ^{13}C-labelled version of the molecular ion is extremely unlikely. Therefore, whenever a fully-labelled molecular ion is detected, it can always be regarded as being derived from the added, labelled OM. For amino acids containing only 1 or 2 C atoms, the amount of M+1 or M+2 response that would naturally occur (based on the abundance of the natural version) must be subtracted from the M+1 or M+2 peak areas before they are used to quantify the amount of artificially labelled compound present.

If the ^{13}C source algae is not 100% ^{13}C-labelled, then not every molecule will produce the M+n ion, and all ions in the series M+1, M+2...M+n will be produced. Alterations in faunal samples of the relative abundances of these partially labelled ions (away from those in the source algae) indicates metabolic alteration of molecules, including the cleavage of highly labelled C chains, and the addition of new unlabelled functional groups.

3.2.2 Choice of Derivative

Trifluoroacetyl iso-propyl (TFA) esters were chosen as suitable amino acid derivatives for this work (Darbre and Islam, 1968). Although these derivatives are less capable of resolving some basic amino acids (Thomas and Blair, 2002, Mabbott, 1990) than other methods (Persson and Näsholm 2001, Woo and Lee 1995), they have other significant advantages. Firstly, TFA esters do not add elements with significantly abundant M+1 and M+2 isotopes to the amino acid molecules. Such isotopes cause each fragment ion to 'run on' in a series of small peaks, reducing the absolute response of the fragment used for quantitation, and introducing error (Persson and Näsholm 2001). The correction of M+1 peak areas for naturally occurring ^{13}C, and the identification of new, partially labelled compounds would also be confounded by the use of a derivative containing an element with abundant M+1 or M+2 isotopes, unless a fragment without that element could be used for quantitation (Sun et al, 2000). Thus, TFA ester derivatives were chosen in favour of

tert-Butyldimethylsilyl derivatives (Persson and Näsholm 2001, Woo and Lee 1995), which involve adding an atom of Si that has abundant M+1 and M+2 isotopes. Ttifluoroacetyl iso-propyl esters have future potential for use in chiral separations, and have been found to be compatible with GC-C-IRMS (Silfer et al., 1991).

3.3 Method Practice

3.3.1 Reagent Preparation

A charge-matched (Cowie and Hedges, 1992) mixed internal standard solution containing ~2.5mM α-aminoadipic acid, norleucine and hydroxylysine in 1N HCl was prepared. Acidified 2-propanol was prepared by standing 2-propanol in a ice bath, and adding, dropwise, 250μl of acetyl chloride for every 1ml of alcohol. The reagent was usually prepared freshly for each batch, and was always used within 48 hours.

3.3.2 Sample Preparation

1) 100mg aliquots of freeze-dried sediments were weighed into pre-combusted 5ml ampoules. Fauna samples were placed, complete with their tin boats, into ampoules. Steps 2 and 3 only applied to samples of sediment and foraminifera.
2) Samples were wetted with Milli-Q and treated with double distilled 6N HCl with vortex mixing until effervescence ceased.
3) Samples were dried in a centrifugal evaporator for 2-3 hours.

3.3.3 Hydrolysis

4) Degassed, double distilled 6N HCl was prepared by bubbling N_2 through the acid for 10 mins while sonicating.
5) Sample ampoules were flushed with N_2.
6) 2ml of degassed double distilled 6N HCl was added to each sample, with vortex mixing.
7) Ampoules were flushed again with N_2, and flame sealed.
8) Hydrolysis was conducted at 150°C for 70minutes.
9) The reaction was quenched in an ice bath.

10) The amount of N in each sample was estimated, and 16 µl of 2.5mM mixed internal standard solution per 10µg of expected N were added.

11) Samples were vortex mixed and dried in a centrifugal evaporator for 6 hours at room temperature, or for 3 hours with a heating lamp.

3.3.4 Cation Exchange

12) Dried hydrolysates were vortex mixed with 2ml of 0.1M HCl.

13) Samples were centrifuged, and 0.7ml of each extract was transferred to a clean ampoule, dried and frozen.

14) 20ml cation exchange columns were prepared with 2ml of Dowex 50Wx8 resin, which was then washed with 10ml of Milli-Q.

15) Samples were loaded onto the cation exchange columns with a Pasteur pipette.

16) Organic acids were washed out with 10ml of Milli-Q.

17) Amino acids were eluted with 3.5ml of 4N NH_4OH, followed by 4ml of Milli-Q. The eluent was collected in 10ml Pyrex culture tubes.

18) Samples were dried overnight (8 hours) in a centrifugal evaporator at room temperature.

3.3.5 Derivatisation

19) A standard, containing amino acid standard solution (2.5mM, Sigma), and an equivalent amount of mixed internal standard was prepared and dried, and was treated hereafter as a sample.

20) Dry extracts were dissolved in 0.5ml of acidified 2-propanol.

21) Esterification was conducted at 110°C for 70 minutes.

22) The reaction was quenched in an ice bath, and the samples finished cooling in the freezer.

23) Esters were dried under a stream of N_2 at 40°C.

24) Esters were twice washed with 0.3 ml of dichloromethane (DCM) and dried under a stream of N_2.

25) Dry esters were dissolved in 0.5 ml of trifluoroacetic anhydride and 0.5 ml of DCM.

26) Acylation was conducted at 110°C for 10 minutes.

27) The reaction was quenched in an ice bath.

28) Derivatives were dried and then twice washed and dried with 0.3ml of DCM at 0°C (tubes stood in an ice bath during this step).

29) Samples were dissolved in 200µl of DCM and transferred to GC injection vials with inserts. Standards were taken up in 600µl of DCM and transferred to GC injection vials.

3.3.6 Chromatography

Samples were analysed by GC-MS using a 30m, 0.32mm diameter, 0.25µm film thickness Equity 5 column (Supelco). The oven program began at 35°C, for an initial time of 1.5 minutes. The oven temperature was then ramped at 5.5°C min^{-1} to 100°C, then at 4°C min^{-1} to 190°C, by which time all peaks had eluted. A final ramp at 70°C min^{-1} to 280°C and a 5 minute hold was used to bake the column. This was followed by a downward ramp at 30°C min^{-1} to 35°C, and a 5 min hold, which ensured the MS source cooled from its maximum temperature (~154°C) to its set point (136°C) before the next run. The injector temperature was 290°C, the MS interface was at 280°C, and the flow rate was 1.5ml min^{-1}. Good peak separation was achieved, and all amino acids eluted within 35 minutes (Fig. 3.2).

3.3.7 Ionisation

Chemical ionisation was used because, with TFA esters, it gives pseudo-molecular ions and more diagnostic spectra than electron ionisation (Dallakian and Budzikiewicz, 1997). Positive ion chemical ionisation was used, with methane (at 20%) as the reagent gas.

3.3.8 Detection

Amino acids were detected by using a HP 5973 mass selective detector. The ions used to quantify natural and ^{13}C-labelled amino acids are shown in Table 3.1. The

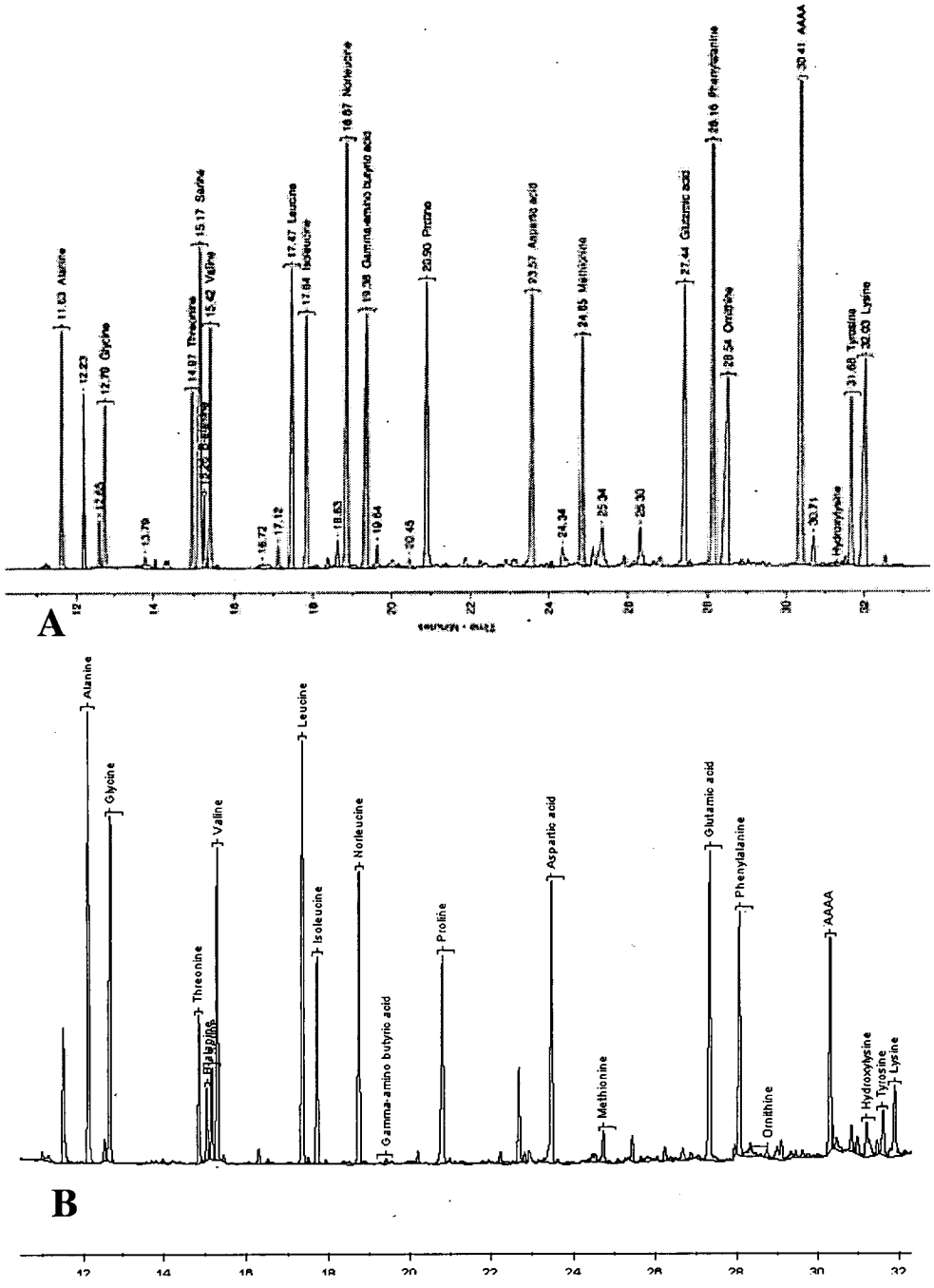


Figure 3.2. The chromatographic separation of amino acids in A) a standard and B) a sample of algae. These traces are from a flame ionising detector.

quadrapole temperature was 106°C. The source temperature (136°C) was relatively cool compared to normal operating conditions (230°C), but was hotter than during previous uses of this method (Dallakian and Budzikiewicz, 1997, Thomas and Blair, 2002).

A cool ion source was required to preserve the heavy and characteristic quasi-molecular ions. These are the most useful ions for identification and quantification of the amino acids, especially in cases of co-elution. Previous studies have set the MS source temperature at 0°C (Dallakian and Budzikiewicz, 1997), but have noted that the actual temperature during operation was 60-90°C due to unavoidable heating by the filament. In this study however, it was not possible to set extremely low source temperatures.

The source temperature was selected such that it would not change dramatically during MS operation, and so as to ensure that the heating of the source during each sample run would be reproducible. Therefore steps were taken to ensure the source cooled to its set point between runs (see above), and thus started each run at the same temperature, avoiding progressive heating with consecutive samples. A reproducible source temperature was necessary, as higher temperatures were observed to cause more intense fragmentation, and thus to alter the proportion of the total response represented by the quantifier ion. It is worth noting that operating the MS with a low source temperature makes it necessary to clean the source more frequently than usual to avoid a reduction in sensitivity.

Amino Acid	Natural Mass Fragment	^{13}C Mass Fragment
Alanine	186	189
Glycine	172	174
Threonine	312	316
β-Alanine	186	189
Serine	298	301
Valine	214	219
Leucine	228	234
Isoleucine	228	234
Norleucine	228	-
γ-Aminobutyric Acid	200	204
Proline	212	217
Aspartic Acid	272	276
Methionine	246	251
Phenylalanine	262	271
Glutamic Acid	286	291
Ornithine	325	330
α-Aminoadipic Acid	300	-
Hydroxylysine	337	-
Tyrosine	374	383
Lysine	339	345

Table 3.1. The ions used for quantification of the natural and ^{13}C labelled version of each amino acid. All ^{13}C fragments are the 100% labelled versions. All fragments are (M+H)-C_3H_6, where M is the molecular ion (amino acid + derivative additions), and C_3H_6 is a propanol moiety added to carboxylic and hydroxyl groups during esterification (Dallakian and Budzikiewicz, 1997).

Peak separation and retention time replication were sufficiently good to allow the use of selective ion monitoring (SIM).

Compounds that eluted close together were grouped, and for the retention time window occupied by each group, the MS selectively monitored the natural and ^{13}C-labelled quantifier ions for only those compounds. Sections of flat baseline longer than 2 minutes were suitable for changing from one group to the next. This was found to significantly increase sensitivity, especially for small samples (~0.3mg of faunal tissue), or where ^{13}C-labelled amino acids only represented a small percentage of the total.

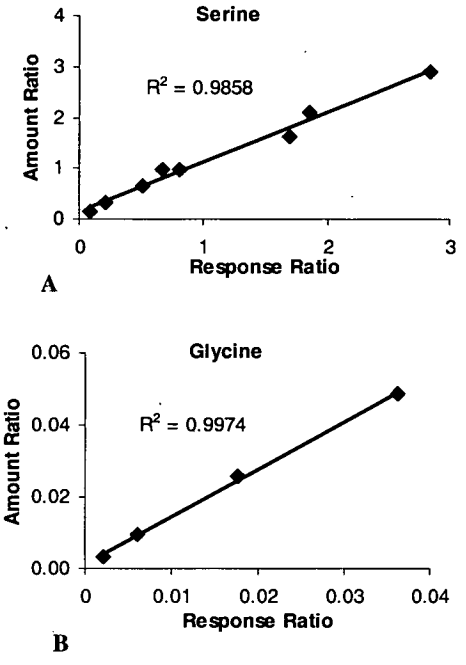


Figure 3.3. Examples of multi point calibration curve linearity for analyte to internal standard amount ratios of A) ~1 and B) ~0.01.

All samples were also run in scan mode, in which the MS monitored all of the ions present. This allowed quantitation of partially labelled molecules, which was necessary for the detection of newly produced, partially labelled amino acids. When scan data files were processed the peak areas of all ions from M (^{12}C only), through M+1, M+2 to M+n were recorded.

3.3.9 Calibrations and Corrections

3.3.9.1 Linearity of Multi Point Calibrations

Amino acids were quantified by the comparison of sample analyte:internal standard response ratios with those of a single standard. Multi point calibration tests supported the use of single point calibrations by showing that response ratios varied linearly with amount ratios ranging from ~1 to ~0.01 (the ratios in question were analyte:internal standard). Average r^2 values for plots of amount ratio versus response ratio were 0.9234 and 0.9253 for the two amount ratio ranges (Fig. 3.3).

3.3.9.2 MS Response Thresholds

During standard tests peak areas were 7-8 orders of magnitude. Samples, however, produced peak areas for natural and ^{13}C-labelled amino acids that ranged between 4 and 8 orders of magnitude.

A test in which serial dilutions of a standard were run revealed that for a constant amount ratio, and at small (4-6 orders of magnitude) analyte peak areas (responses), the analyte to internal standard response ratio varied dramatically depending on the magnitude of response (fig. 3.4).

Figure 3.4 shows that for glycine (as an example of all the amino acids), three of the dilutions produced similar response ratios, and the fourth and weakest dilution produced a significantly different (lower) ratio. This suggests there is a threshold quantity of compound entering the MS, below which its response is dramatically reduced. Since there was generally more internal standard present than analyte, the analytes tended to drop below this threshold while their internal standards remained above it, the result being the observed drop in analyte:internal standard response ratio for a constant amount ratio. This issue was further complicated by the fact that the threshold seemed to occur at different absolute quantities of analyte for the different amino acids.

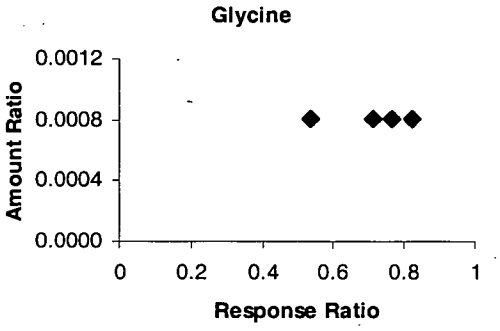


Figure 3.4. An example of the way the analyte:internal response ratio varies at low analyte responses for a constant amount ratio. (Note, response ration did not simply fall progressively as absolute analyte quantity fell).

The solution to this problem was to run three standards (with different analyte:internal standard amount ratios), each at two different dilutions, with each batch of samples. Thus single point calibrations were generated for each analyte for the full range of magnitudes of analyte response, and conditional formulae in the data processing worksheet ensured that for each analyte in each sample the relevant calibration was used.

3.3.9.3 Quantitation of ^{13}C-Labelled Amino Acids

The standards used contained natural abundances of ^{13}C, and were thus suitable for quantifying the natural amino acid contents of the samples. They could not however be used to quantify ^{13}C-labelled amino acids as, due to the fact that the ^{13}C-labelled algae was not 100% labelled, the 100% labelled fragments used to quantify each labelled compound represented unknown fractions of the total amount of each amino acid from the labelled algae that was present (the measured ~80% ^{13}C in the algae was probably not evenly distributed among all the biochemicals, and will have produced unknown relative quantities of partially and fully labelled versions of each compound).

The absolute quantitation of ^{13}C labelled amino acids followed a procedure developed by Sun (2000). Calibration curves were constructed which related the ^{13}C to ^{12}C response ratio of each amino acid to its ^{13}C to ^{12}C amount ratio. In order to quantify the ^{13}C amino acids in a sample, the ^{12}C amino acids were first quantified using the internal standards and single point calibrations described earlier. The ^{13}C to ^{12}C response ratios of each amino acid, and the Sun et al. (2002) type calibration curves were then used to generate ^{13}C to ^{12}C amount ratios, which were converted to ^{13}C amounts using the previously determined amounts of ^{12}C amino acids.

The calibration curves were constructed by mixing ^{13}C-labelled algae and natural flying fish derivative solutions in 1:1, 10:1, 50:1 and 100:1 volumetric ratios (^{13}C:^{12}C). The amino acid concentrations in each of these derivatives (before they were mixed) were quantified independently by GC with flame ionising detection (GC-FID). In the case of the ^{13}C algae solution these concentrations were multiplied by ~80% (the level of ^{13}C-labelling, thus assuming uniform labelling of all amino acids). Thus the ^{13}C to ^{12}C amount ratio in each mix was calculated.

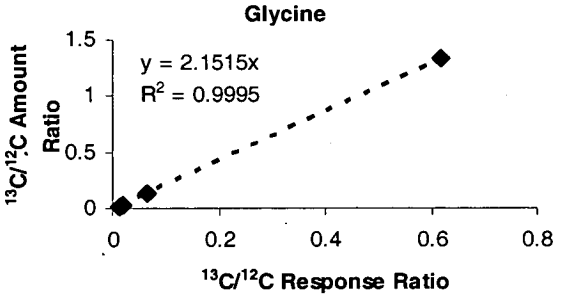


Figure 3.5. An example Sun et al. (2000) calibration curve relating $^{13}C/^{12}C$ response ratio to $^{13}C/^{12}C$ amount ratio.

The mixes were then analysed by GC-MS, and the ratio of peak areas for the ^{13}C and ^{12}C ions (^{13}C to ^{12}C response ratios) of each amino acid were plotted against the

calculated ^{13}C to ^{12}C amount ratios. The Sun (2000) type calibration curves showed good linearity (Fig.3.5), with an average r^2 of 0.9988. The actual responses (peak areas) of ^{12}C and ^{13}C amino acids in the derivative mixes used to construct these calibration curves ranged from 5-8 orders of magnitude, thus they are applicable to samples showing a wide range of responses.

This (2000) type calibration relies on the assumption that the fragmentation pattern of each amino acid is constant, regardless of the level of isotope labelling. This same assumption is made by Sun (2000), and could be tested in the future using a limited number of 100% labelled amino acid standards.

3.3.9.4 Correction of M, M+1 and M+2 Responses

The fact that the ^{13}C-labelled algae was not 100% labelled introduced a further complication, as it meant that the labelled algae contained small but significant amounts of unlabelled amino acids, which produced the natural ions with mass M. The abundance of the naturally present M ion of each amino acid was used in the quantitation of added, labelled amino acids (see above). If algae-derived M ions were part of that abundance the calibration would become slightly circular.

Amino Acid	Average RSD (%)
Alanine	7.8
Glycine	7.9
Threonine	10.8
β-Alanine	15.0
Serine	12.5
Valine	5.5
Leucine	8.1
Isoleucine	10.2
Proline	22.9
Methionine	28.2
Phenylalanine	32.8
Tyrosine	38.2
Aspartic Acid	47.1
Ornithine	65.8

Table 3.2. The average relative standard deviations of each amino acid, derived from 5 sets of replicates.

In order to avoid this problem the portions of the M ion responses that were due to M ions from the added algae were subtracted out, based on the abundances of the equivalent M+n ions. The ($^{13}C/^{12}C$) or (M+n/M) response ratios for each amino acid in the source algae were used to calculate the amounts of M response produced by the algae for a given M+n response (Sun et al., 2000). These amounts were subtracted out from the sample M responses, and the $^{13}C/^{12}C$ response ratios were recalculated for use in quantitation (see above). The correction of M responses and recalculation of $^{13}C/^{12}C$ response ratios was done using equation 1 (Sun, 2000):

$$^{13}C/^{12}C \text{ response ratio} = M+n \,/\, [M-(M+n \,/\, \text{source ratio})] \qquad (1)$$

where $^{13}C/^{12}C$ response ratio is the corrected (M+n/M) response ratio in the sample, M+n is the response of the fully labelled ion in the sample, M is the response of the unlabelled ion in the sample, and source ratio is the $^{13}C/^{12}C$ response ratio in the ^{13}C-labelled algae (Sun, 2000). The corrected M responses were taken from the corrected $^{13}C/^{12}C$ response ratios using the M+n responses.

The responses of the M+1 and M+2 partially labelled fragments were corrected for the natural occurrence of such ions in fragments containing 2 or more and 5 or more carbon atoms respectively. This was done on the basis of the probability of such ions occurring naturally, given the natural abundance of ^{13}C (1.1% of all C). Corrected M+1 ion responses were generated using equation 2, and corrected M+2 ion responses were generated using equation 3:

$$(Corrected\ M+1) = M+1 - (n \times 0.011 \times corrected\ M) \quad (2)$$

$$(Corrected\ M+2) = M+2 - (\{([n \times 1.1]^2 / 200) / 100\} \times corrected\ M) \quad (3)$$

where M+1 and M+2 are the original responses for those ions, corrected M is the M ion response after correction, as detailed above, and n is the number of C atoms in the fragment ion (including both the original amino acid and C added during derivatisation).

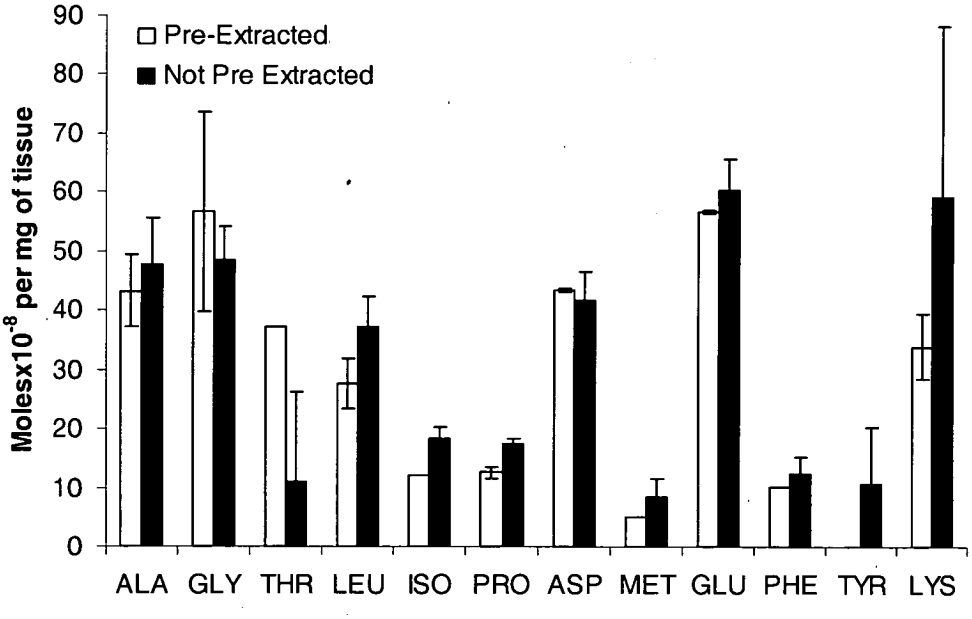


Figure 3.6. The amino acid yields of flying fish tissues with and without prior lipid extraction. Error bars are 1 standard deviation.

3.4 Assessment

3.4.1 Precision

Six of the macrofauna samples analysed using this method were split into 2 or 3 parts before analysis, allowing an assessment to be made of the precision of the technique. The average relative standard deviations of ^{13}C-labelled amino acid mole percentages thus generated are shown in Table 3.2. They represent relatively good precision, especially considering that the samples were too small to be homogenised before they were split, thus some of the variability can be attributed to real variations in composition among sub-samples. It is unclear why the compounds towards the bottom of Table 3.2 show relatively high RSD's. It may be because these amino acids tended to be slightly less abundant, or it may be related to the chromatography, as they all eluted late.

3.4.2 Sample Pre-Treatment

Tests were carried out using un-labelled flying fish tissue to determine whether samples could be analysed for both ^{13}C-labelled lipids and amino acids. Lipids were extracted from several samples of flying fish tissue by sonication in 9:1 DCM:methanol (v/v) for 30 minutes at room temperature. These were analysed for their amino acid contents (using GC-FID), along with several flying fish samples that had not been pre-extracted.

The amino acid contents of the two sets of samples were not different beyond analytical error, thus the extraction of lipids before amino acid analysis had not caused any measurable amino acid loss (Fig. 3.6).

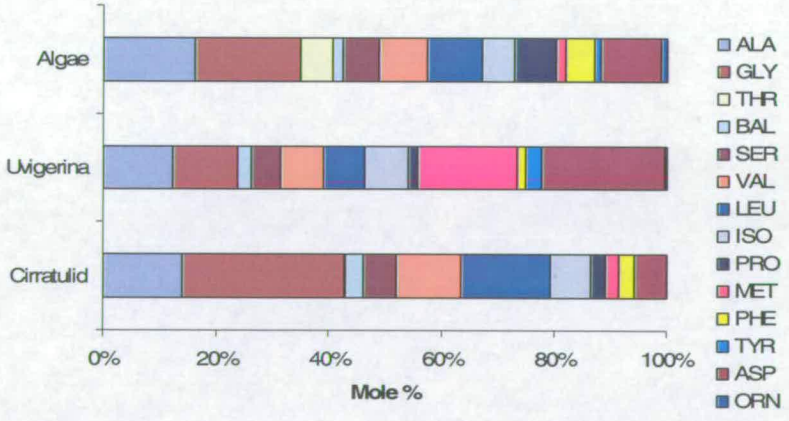


Figure 3.7. The ^{13}C-labelled amino acid suite of ^{13}C-labelled algae, and the altered suites found in foraminifera (*Uvigerina sp.*) and in a cirratulid polychaete.

The success of these tests allowed the collection of twice as much biochemical data as would have been possible if separate samples had had to be used for the separate analyses.

3.4.3 Application to Fauna and Sediment Samples

The analytical technique described in this study was used for the analysis of macrofauna, foraminifera and sediment samples derived from ^{13}C-tracing experiments (described in chapter 2). It was successful in highlighting selective assimilation patterns among the amino acids, which were taxon specific (Fig. 3.7, see chapter 5).

3.5 Conclusions

- The quantitative tracing of ^{13}C-labelled amino acids in sediment and fauna samples has been demonstrated.
- Replicate analyses showed good precision.
- It was found that samples could be pre-extracted for lipids before amino acid analysis, without measurable amino acid loss.
- The ability to quantitatively trace ^{13}C-labelled amino acids will allow quantitative amino acid budgets to be constructed from future experiments.

CHAPTER 4

Short term processing of organic carbon by benthic organisms across the Pakistan Margin: Spatial and seasonal variations

4.1 Introduction

Continental margin sediments are important as sites for the accumulation and burial of organic matter (OM) in the marine environment (DeMaison and Moore, 1980).

The multiple, often interrelated factors that influence carbon burial efficiency (the proportion of OM delivered to the sediment which is eventually buried), include OM supply (generally linked to surface ocean productivity), OM quality or lability, oxygen availability and exposure time, organic and inorganic sedimentation rates, and sediment texture (e.g. Canfield, 1994, and references therein).

The availability of high food-quality OM and the availability of oxygen are also the dominant factors controlling the size, composition and behaviour of benthic communities (e.g. Levin et al, 2000, Pinet, 1992, Flach et al., 1998, Nilsson and Rosenberg, 1994). At low dissolved oxygen concentrations in particular, benthic communities are observed to exhibit a wide range of structures and behaviours (Rosenberg, 2001, Levin 2003, and chapter 1). In turn, benthic fauna, with varied digestion, burrowing and irrigation activities, have varied effects on sediment biogeochemistry, mixing, microbial activity and redox conditions, influencing both OM cycling and oxygen exposure time (e.g. Aller, 1982, Sun et al, 2002, Kristensen, 2000). Thus, faunal communities, sediment organic geochemistry and oxygen exposure times are intimately and mechanistically linked.

While oxygen exposure time is generally found to have the dominant control on OM burial efficiency in the marine environment, coastal and continental margin environments, with low oxygen exposure times due to short water columns, rapid sediment accumulation rates and limited sedimentary oxygen penetration, show a wide variation in OM burial efficiency (Hartnett et al., 1998). A hypothesis of the Arabian Sea project was that spatial variation in benthic communities and their activities is responsible for this variation in burial efficiencies among low oxygen exposure time environments.

Abundant evidence exists to show that the activities of macro and meiofaunal communities have significant effects on benthic OM cycling. Many studies have aimed to characterise and quantify the physical movement of sediment and OM brought about by burrowing and feeding activities (Lauerman et al., 1997, Miller et

al., 1998). For example, tracers have been observed to penetrate to depth in a non-diffusive manner, indicating active non-local transport of fresh OM down burrows, the depth of which is highly dependent on the dominant species present (Thomas and Blair, 2002, Sun et al., 1999, Levin et al., 1997, DeMaster et al., 1994). This vertical transport of OM may reduce oxygen exposure time and increase the rate at which it is buried, or may unearth partially buried OM and expose it once more to oxygen, and thus increase decay (Hartnett et al., 1998). The polarity of the bioturbation effect therefore depends on the direction of transport of OM, and whether the OM in question decays differently under oxic and anoxic conditions. Sediment irrigation through burrow ventilation, whether constant or intermittent, has been observed to significantly increase the oxygenated volume of sediment and the occurrence of fluctuating redox conditions, and thus overall OM decay rates (Sun et al., 2002, Sun et al., 1999, Aller and Aller, 1998, Kristensen, 2000, Aller 1994). Macrofauna cause further microbial stimulation by the introduction of reactive OM at depth in the sediment, either through bioturbation, excretion, or secretion of burrow linings (Aller, 1982, Levin et al., 1997, Sun et al., 1999). The presence of such high-quality OM initiates bacterial respiration (Cook et al., 2000), and the resultant increased abundance of extracellular enzymes allows co-metabolism of refractory OM, which may otherwise have become buried. Finally, benthic macro- and meiofauna consume, and are thus directly responsible for respiration, and chemical alteration of sedimentary OM, (Lauerman et al., 1997).

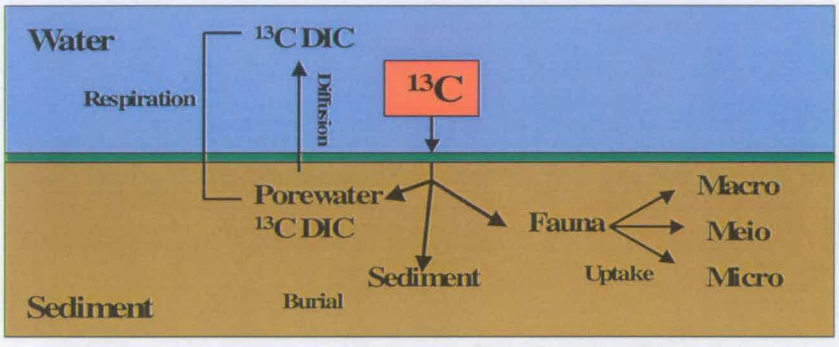


Figure 4.1. A schematic representation of ^{13}C tracing experiments, showing how a quantitative C budget is constructed.

In summary, the effects of faunal activity are the least well characterised and quantified aspects of OM cycling and burial in marine sediments. Consequently, the inclusion of faunal processes in the modelling of seafloor C-cycling is currently restricted to a bioturbation term, which is usually diffusive, and thus may not reflect the actual geometry or dynamics of biological mixing, and an exponential decay

constant for bulk OM, which is most representative of microbial processes (Heip et al., 2001). This general lack of information is due to the inaccessibility of the seafloor, together with the localised and patchy nature of faunal processes (burrows, faecal pellets etc.), which are missed by normal geochemical sampling, and the extensive potential for variation introduced by the possibilities of species-specific effects. Previous studies of the effects of fauna on OM cycling and burial have often concentrated on a single process, such as bioturbation, and often are performed in microcosm, using sub-sets of the benthic community (e.g. Sun et al., 1999, 2002, Thomas and Blair, 2002), thus an holistic view of faunal processes is lacking. In the relatively rare cases where whole and intact faunal communities have been considered, studies have usually been limited to a single coastal or deep-sea site, or an offshore transect with co-varying oxygen, organic matter and temperature availability (e.g. Witte et al., 2003 a, b). Together, these limitations have prevented an assessment of how the key variables affect the role of fauna in OM uptake and cycling.

Previous studies involving the tracing of isotopically labelled OM into the benthic community have found that foraminifera and bacteria take up significant proportions of added label, and that these groups of organisms are probably responsible for the majority of OM remineralisation in sediments (Moodley et al., 2000, 2002). Other studies however, have found that the macrofauna not only transport fresh OM to considerable depths (e.g. Levin et al., 1997, DeMaster et al., 1994), but they also carry out the majority of faunal OM uptake over short timescales (Witte et al., 2003 a, b). In addition, in some continental margin environments, the macrofauna have been shown to be responsible for up to 50 % of sediment community oxygen consumption, and thus OM remineralisation (Heip et al., 2001). For a fuller description of this previous work, see chapter 1, and the discussion section below.

Some of the key remaining questions regarding the role of fauna in sedimentary OM cycling are as follows:

1) How does the role of fauna in OM processing vary with varying benthic community, and with varying oxygen and OM availability?
2) Which group of fauna are most influential in the sediment OM cycle?
3) How important are species-specific effects?

4) How does the role of fauna in OM processing change in response to a pulsed input of OM to the seafloor, such as is typical of many deep-sea environments?

This study aimed to investigate the role of the benthic community in the short term processing of OM using ^{13}C-labelled algal detritus as a tracer. Whole, undisturbed sediment communities were investigated under seafloor conditions, and, where possible, in situ. The tracing of isotopic label into fauna was part of a broader tracking of the label into bacterial biomass, sediment and dissolved inorganic and organic carbon (DIC and DOC respectively) pools, thus allowing the construction of some of the most complete and detailed sediment carbon budgets to date, at sites with contrasting conditions and benthic communities (Fig. 4.1).

The study was carried out at sites spanning the oxygen minimum zone (OMZ) on the Pakistan margin of the Arabian Sea. This margin displays steep gradients in oxygen availability and sediment OM quantity and quality, and consequently benthic communities vary dramatically between sites. This setting allows direct assessments of the way the role of fauna in benthic C-cycling varies in response to all these factors, and gives a unique picture of faunal OM processing in a wide range of environments.

4.2 Methods

4.2.1 Study Area

The study was conducted at sites along an offshore transect of the Pakistan Margin of the Arabian Sea (in an area roughly defined by, 23°17'N and 22°52'N, and 65°59'E and 66°43'E, chapter 2). Experiments and sampling were conducted before (April-May) and after (September-October) the summer monsoon of 2003 during RRS Charles Darwin cruises CD146 and CD151 respectively.

Twice a year, monsoon winds cause upwelling and intense productivity in the Arabian Sea. In July the winds blow from the SW (the SW monsoon), and in December they blow from the NE (the NE monsoon). This results in twice yearly, pulsed inputs of OM to the sediment-water interface (maximum particle flux of 180-190 mg m^{-2} day^{-1}, compared to 0.1-60 mg m^{-2} day^{-1} between monsoons, particles are

3.8-10 % organic carbon, Haake et al., 1993a), where it forms the base of the benthic food chain.

The Arabian Sea also exhibits a permanent and pronounced midwater oxygen minimum zone (OMZ), where oxygen levels fall below 0.2 ml L^{-1} between depths of roughly 150m and 1000m (Table 4.1). The OMZ is the result of a combination of the intense biannual plankton blooms, and lack of midwater ventilation (Sarma, 2002, Cowie, 2005, and references therein). Where the OMZ impinges on the continental margin, low oxygen conditions are enforced on the sediment. The weight percentage organic carbon contents (%Corg) of Pakistan margin sediments varies broadly according to oxygen availability, with maximal values found within and slightly below the OMZ (Cowie et al., 1999). The availability of oxygen across the margin also influences benthic community structure. Above and below the OMZ, the sediments host diverse macrofaunal communities, which produce well-mixed sediment structures. Within the core of the OMZ (ca. 250-700m), macrofauna are unable to survive, foraminifera dominate the community, and annual laminations are preserved in the sediment (Schulz et al., 1996). At the lower boundary of the OMZ, as oxygen levels rise, macrofaunal biomass shows a marked maximum. Full details of site conditions are given in Table 4.1.

Study sites were chosen at depths of 140m, 300m, 850m, 940m, 1000m, 1200m and 1850m, to fall above, within and below the OMZ and thus span a range of redox conditions and contrasting benthic communities. The 140m, 850m and 940m sites were partially oxygenated OMZ boundary sites. The 300m site was at the hypoxic core of the OMZ, the 1200m site was relatively well oxygenated and near the sedimentary %Corg maximum, and the 1850m site represented a fully oxygenated, OM-poor, abyssal end-member.

All sites were visited in April-May 2003, before the summer monsoon. In order to study the effect on the benthic community of a pulsed input of OM, experiments were also carried out at a sub-set of sites (the 140m, 300m, 940m and 1850m sites) after the summer (SW) monsoon, in September-October of 2003.

4.2.2 Experimental

Pulse-chase tracer experiments with a ^{13}C-labelled OM substrate were carried out on intact sediments with whole benthic communities present. Two approaches were used; shipboard incubation of recovered sediment cores (Schwartz et al., in prep.), and in-situ incubation, using a benthic chamber lander (Black et al., 2001).

4.2.2.1 Shipboard Experiments

Sediment megacores were recovered, and then installed onto a core incubation rig (Schwartz et al, in prep), situated in a controlled temperature laboratory maintained at seafloor temperature. During incubation, dissolved oxygen concentrations were maintained, despite sediment community oxygen consumption, by circulation of core-top water through dedicated "oxystat" gills (gas-permeable tubing situated in a reservoir maintained at ambient seafloor oxygen concentrations). Time-series samples of core-top water were taken using sampling ports protruding through the core top-seals. Incubations were carried out in the dark.

Tracer experiments were initiated by the introduction of a slurry of ~80% ^{13}C-labelled diatom detritus, freeze dried onto an inert ballast of either silica (pre-monsoon studies) or kaolinite (post-monsoon studies). The dose used was 100-150 mg of algal detritus, equal to a carbon delivery of 5-8 mg of C, including 3-6 mg of ^{13}C per megacore, or 600-1000 mg C m^{-2}. After a settling period of 30-60 minutes, gentle water column stirring was initiated (sufficient to ensure homogenisation without disturbing sediments). During each experiment, overlying waters were sampled for DIC, $\delta^{13}C$ of DIC, DOC, $\delta^{13}C$ of DOC, and nutrients, at intervals of 0, 12,24,36 and 48 hours after experiment start for 2-day incubations, and 0, 24, 48, 96 and 120 hours after experiment start for 5-day incubations. This data is not presented here.

Each experiment was conducted in duplicate simultaneously on megacores from the same corer deployment and thus the same exact coordinates.

Incubations of 2- and 5-days duration were carried out. Experiments of both durations performed at the same site (140m post-monsoon, 300m pre and post-monsoon, and 1850m pre-monsoon) allowed an assessment of the progression of label through the sediment system over time.

When incubation periods were finished, the megacores were removed from the incubation rig, and sectioned at intervals of 0.5cm thickness to 2cm, then 1cm thickness to 10cm, followed by 2cm thickness to 20cm. Each core section was divided in half. One half was placed in a Petri dish to be sorted for fauna, and the other half was placed into a 35 ml centrifuge tube for extraction of porewaters.

Porewaters were extracted by centrifugation (~ 3500 rpm, for 10 mins). The sediment residues were transferred to bags and freeze dried.

Sediment for fauna extraction was immediately wet sieved using filtered seawater, and residues from 300μm, 150 μm and 63 μm sieves were retained and refrigerated. Residues from the 300μm sieve were examined under x12-x20 magnification and all live macrofauna (from all depth horizons to 10 cm) and foraminifera (from the surface 1 cm only) were removed. Foraminifera were also extracted from some 150 μm sieve residues. Due to time constraints foraminifera were not collected from pre-monsoon experiments at the 850m and 100m sites. Fauna samples were sorted into the lowest taxonomic groups possible, and preserved frozen in glass vials and pre-weighed tin boats.

Time-zero control experiments were conducted as described above, except that core sectioning took place immediately after the labelled algae slurry had been allowed to settle.

4.2.2.2 In Situ Experiments

Benthic lander experiments followed the same principles as shipboard incubations (see chapter 2 for a full description). After the lander arrived at the seafloor, disturbed sediment was allowed to settle, and the chamber lid was closed. Tracer was delivered by automated syringe, and settling was allowed before gentle stirring of overlying water in the chamber commenced. The amount of tracer introduced was scaled up according to the difference in surface area of the chamber box-core compared to that of a megacore (resulting in addition of 25-35 mg of C, including 20-27 mg of ^{13}C). Pumping of chamber water through an oxystat gill in contact with bottom water prevented any significant decline in chamber dissolved oxygen concentration. After incubation for ~48h, the chamber shovel was activated, thus isolating the experimental sediment, and the release of ballast allowed the lander to ascended to the surface. The recovered box-core was sub-sampled with two

megacore tubes, and these sub-cores were processed as described above for shipboard incubated megacores.

In this study, bulk isotopic data from fauna and solid sediments will be presented and discussed.

A summary of all shipboard and in-situ experiments carried out at each site in each season, together with exact durations and quantities of label delivered can be found in chapter 2.

4.2.3 Bulk Isotopic Analysis

4.2.3.1 Sediment

Samples of freeze-dried sediment (10-30 mg, corresponding to 200-600μg of C) were weighed into silver capsules. Sediments were de-carbonated by addition of 2-3 drops of double distilled 6N HCl, followed by drying at 60°C. Samples were analysed for C and N content and $\delta^{13}C$ using a Europa Scientific (Crew, UK) Tracermass isotope ratio mass spectrometer (IRMS) with a Roboprep Dumas combustion sample converter. Carbon and N quantities were determined from IRMS peak areas and calibrated against standards of acetanilide. Calibration curves typically had r^2 values greater than 0.99 and intercepts were no more than 10μg away from the origin. Replicate analyses produced average relative standard deviations of 4.6% and 5.0% for %Corg and $\delta^{13}C$ respectively (n = 27), and acidified blanks did not contain measurable amounts of C.

4.2.3.2 Fauna

Fauna samples were air dried at 45°C and those in tin boats were re-weighed to obtain sample dry weights. Samples in vials were transferred into tin boats and weighed. Soft-bodied fauna were de-carbonated by placing tin boats inside silver boats and adding 1-2 drops of 1N HCl, followed by drying at 45°C. Molluscs and foraminifera were de-carbonated by placing tin boats inside silver boats followed by addition of 2-3 drops of double distilled 6N HCl (Hidetaka Nomaki, pers. comm.) to ensure complete de-carbonation. Fauna samples were analysed by combustion IRMS as detailed above, with standards tailored to suit very low C samples.

Station (Depth (m)	Temperature (°C)	Dissolved Oxygen ml L^{-1}	Sediment %Corg	OM Quality (DI)	Macrofauna Biomass g(wet) m^{-2} / Diversity	Foraminifera Density / Diversity
Pre Monsoon						
140m	22.5	2.05	1.46 ± 0.08		9 / 51 (± 5)	593 / 19
300m	15.5	0.10	2.36 ± 0.09		0.020 (± 0.022) / 2 (± 0.5)	549 / 18
850m	9.7	0.13	3.22 ± 0.06			
940m	9.0	0.13	3.31 ± 0.12		62 (± 45) / 12 (± 1)	80 / 13
1000m	8.7	0.15	3.04 ± 0.01			
1200m	7.2	0.34	3.27 ± 0.26	-0.49 ± 0.18	0.4 (± 45) / 13 (± 2.6)	77 / 16
1850m	3.5	1.78	1.40 ± 0.10		9 (± 15) / 53 (± 6)	24 / 8
Post Monsoon						
140m	18.2	0.11	1.43 ± 0.07	-0.99 ± 0.06	5 (± 2) / 45 (± 3)	1163 / 20
300m	14.8	0.11	2.56 ± 0.29	-0.40 ± 0.12	0.013 (± 0.019) / 1	839 / 14
940m	9.3	0.17	3.40 ± 0.13	-0.48 ± 0.03	45.7 (± 0.02) / 13 (± 1)	
1850m	3.7	1.7	1.20 ± 0.25	-1.17 ± 0.14	2 (± 0.9) / 44 (± 4)	

Table 4.1. Summary of experiments conducted at all sites in both seasons, with exact durations and quantities of ^{13}C added and site conditions. Oxygen concentrations are from CTD casts, %Corg values are for the surface 0-0.5 cm, DI values are averaged over the surface 3 cm (Sandra Vandewiele, pers. comm.). Macrofauna diversity is species number per megacore averaged from 5 cores (Peter Lamont, pers.comm.), foraminifera density data are total number of calcareous individuals in 15 cm^2 of the surface 1 cm of sediment, and diversity data are species number for the same sample volume (Stefanie Schumacher, pers. comm.).

4.2.4 Data Analysis

Correlation analyses were conducted in Excel. They generated correlation coefficients (ρ) for pairs of data sets, equal to their co-variance divided by the product of their standard deviations. For data sets containing 10-12 data points, a value of 0.5 or more indicates a significant correlation at the 5% level.

4.3 Results

4.3.1 Site Conditions

Site conditions were as described earlier, with minimal oxygen concentrations between ~150m and 1000m, roughly coincident with a %Corg and OM quality maximum that occured at or slightly below the lower OMZ boundary (at the 1200m site). There was no measurable change in sediment %Corg between seasons, but carbohydrate and lipid analyses did show a slight increase in concentrations, attributable to a seasonal OM pulse (Rachel Jeffreys, pers. comm., and chapter 7). The most significant seasonal signal was a decrease in bottom water dissolved oxygen concentrations at the 140m site, from 2.05 ml L^{-1} to 0.11 ml L^{-1} (Table 4.1). Macrofaunal communities were present above and below the OMZ, but were absent from the 300m site, which was populated only by foraminifera. The 140m site had the highest macrofaunal abundance, followed by the 940m, 1200m and 1850m sites. The OMZ lower boundary sites (the 940m, 850m and 1000m sites), due to large individual animal sizes, displayed the highest macrofaunal biomass, followed by the 140m, then 1200m and 1850m sites. Sediment X-ray data suggested that these last two sites were also populated by large, deep burrowing fauna, however these were never recovered in megacores, and so were not present in isotope tracing experiments (Peter Lamont and Lisa Levin, pers. comm.). Foraminifera were present at all sites. Calcareous foraminifera dominated at the shallower 140m and 300m sites, and were replaced by agglutinated taxa as depth increased. At the OMZ lower boundary, where the macrofauna were particularly abundant, foraminiferal populations were reduced. (Stefanie Schumacher and Kate Larkin, pers. comm.). Thus foraminifera dominated the benthic community under the very lowest oxygen conditions, and macrofauna showed their greatest dominance where high quality OM was most

abundant, and oxygen levels were just sufficient.

Season	Site	Experiment Duration	% Label Recovered From Macrofauna	% Label Recovered From Foraminifera	% Label Recovered From Total Fauna
Pre-Monsoon	140	2 day	5.6 ± 0.5	1.2 ± 0.4	6.8 ± 0.1
Pre-Monsoon	300	2 day	0.0	0.8 ± 0.2	0.8 ± 0.2
Pre-Monsoon	300	5 day	0.0	0.93 ± 0.09	0.93 ± 0.09
Pre-Monsoon	850	2 day	3.8 ± 0.1	N/A	3.8 ± 0.1
Pre-Monsoon	1000	2 day	12.0 ± 5.5	N/A	12.0 ± 5.5
Pre-Monsoon	940	5 day	7.3 ± 2.6	0.2 ± 0.1	7.5 ± 2.8
Pre-Monsoon	1200	5 day	0.04 ± 0.03	0.11 ± 0.07	0.15 ± 0.1
Pre-Monsoon	1850	2 day	0.07 ± 0.02	1.18 ± 0.09	1.25 ± 0.07
Pre-Monsoon	1850	5 day	0.11 ± 0.05	0.6 ± 0.2	0.7 ± 0.2
Post-Monsoon	140	2 day	1.4 ± 1.0	2.7 ± 0.9	4.14 ± 0.06
Post-Monsoon	140	5 day	1.5 ± 0.7	4.9 ± 0.2	6.4 ± 0.4
Post-Monsoon	140	2 day *in situ*	1.6 ± 0.4	2.1 ± 0.6	3.7 ± 0.9
Post-Monsoon	300	2 day	0.0	0.79 ± 0.09	0.79 ± 0.09
Post-Monsoon	300	5 day	0.0	2.8 ± 0.1	2.8 ± 0.1
Post-Monsoon	300	2 day *in stiu*	0.0	2.8 ± 0.2	2.75 ± 0.22
Post-Monsoon	940	5 day	10.6 ± 1.7	0.16 ± 0.0	10.7 ± 1.7
Post-Monsoon	940	2 day *in situ*	13.6 ± 0.1	0.18 ± 0.03	13.78 ± 0.08
Post-Monsoon	1850	5 day	0.20 ± 0.09	0.46 ± 0.02	0.66 ± 0.07
Time Zero Controls					
Post-Monsoon	140	T0 Control	0.4	0.2	0.6
Post-Monsoon	940	T0 Control	5.6	0.0	5.6

Table 4.2. Percentage of total label added recovered from the macrofauna and foraminifera in each experiment. High percentage uptake in the 940m T0 control was due entirely to rapid uptake by specimens of the polychaete *Linopherus sp.*, which are not representative of the rest of the faunal community. Errors are 1 standard deviation. Exact experiment durations are given in appendix B.

4.3.2 Data Quality

Two time-zero (T0) control experiments were conducted, one at the 140m site, and one at the 940m site, both in the post-monsoon season. Labelled algae and time constraints prevented further control experiments. Apparent label uptake and/or faunal ^{13}C contamination in the 140m site control experiment was only ~ 10 % of that after 2 or 5 days (Table 4.2), and was not significant. Apparent uptake in the 940m control experiment was considerably greater; however, this result was due to rapid uptake of labelled OM by a few individual polychaetes (*Linopherus sp.*), rather than to contamination (visual observation of rinsed fauna). The 940m site time-zero control experiment could, in fact, be viewed as a 2-hour incubation experiment (roughly the time taken to remove fauna from contact with labelled algae), instead of as a T0 control, and such rapid uptake is not representative of other sites with less active macrofauna. It is therefore thought that time-zero controls showed there was no significant contamination of fauna with ^{13}C label, and that even uptake values below that of the 140m T0 control (i.e. at the 1200m and 1850m sites) are significant above experimental artefact.

Each experiment was run using a pair of megacores for the purpose of replication. It became clear, however, that no half-megacore section provided sufficient faunal samples either to be representative of the faunal community, or to allow all the planned analyses to proceed. For this reason, data from the two replicate cores of each experiment was pooled, and reported as one experiment. As the exact source of each sample was recorded however, replicate cores could be considered separately in order to produce standard deviations on uptake values (but for all other purposes, data was pooled). Relative standard deviations of total faunal uptake values had a range of 0.9-153 %, with an average value of 42 %. These are considered reasonable, given the small numbers of faunal samples and the limited degree of replication that was possible. They reflect the local patchiness of benthic faunal communities. Further confidence in the data stems from the facts that trends between sites were outside error and were maintained between seasons, and that shipboard and in-situ methods returned similar results (Table 4.2). The general correspondence (difference in uptake between shipboard and lander experiments was not as great as that between sites), and lack of systematic difference, between

shipboard and lander-derived data indicated that any artefact associated with sediment core recovery and incubation at atmospheric pressure was small for the depths considered (maximal depth of comparison was 940m).

4.3.3 Artefacts Of Variable ^{13}C Dosing

Previous studies have shown that even slight additions (on the order of those used here) of fresh organic matter to deep-sea sediments can produce significant increases in the rate of sediment community oxygen consumption (e.g. Witte et al., 2003 b). This is an unavoidable artefact when using algal detritus as a tracer, and thus all uptake and respiration rates produced by studies such as this must be regarded as maximal.

This raises the concern that the similarities and differences in faunal response to OM addition among sites may be forced by differences in absolute or relative C dosing (Moodley et al., 2005). In this study, similar absolute amounts of algal carbon were added to all experimental cores. Given the differences in Corg content among the study sites (Table 4.1) this could have led to different doses relative to natural OM abundance. Consequently, it should be noted that differences in faunal stimulation might have resulted, and this factor needs to be taken into account in the

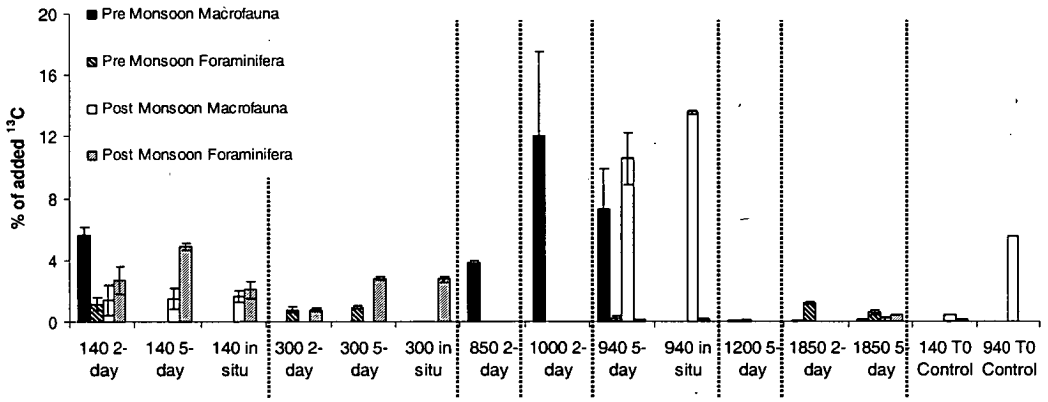


Figure 4.2. The percentage of added label recovered from the macrofauna and foraminifera in each experiment. The amount of label added per m^2 did not vary significantly between experiments. Error bars are 1 standard deviation.

interpretation of comparative data. In principle, the stimulus would be more pronounced at OM-depleted sites. In fact, the effect does not appear to have been

significant. Calculation of dosing shows that all sites received an algal carbon input equivalent to 0.8 ± 0.3 % of the organic carbon naturally present in the surface 1 cm of sediment, and there was no systematic (or %Corg related) trend between sites. The only exceptions were the post-monsoon experiments at the 1850m site, which received quantities of algal carbon equivalent to 4.7 % of the carbon naturally present in the top 1 cm of sediment. This difference did not create an obvious artefact, however, as this experiment produced the lowest faunal response to added carbon of all (Table 4.2 see discussion below).

4.3.4 Variation in Total ^{13}C Uptake Across The OMZ

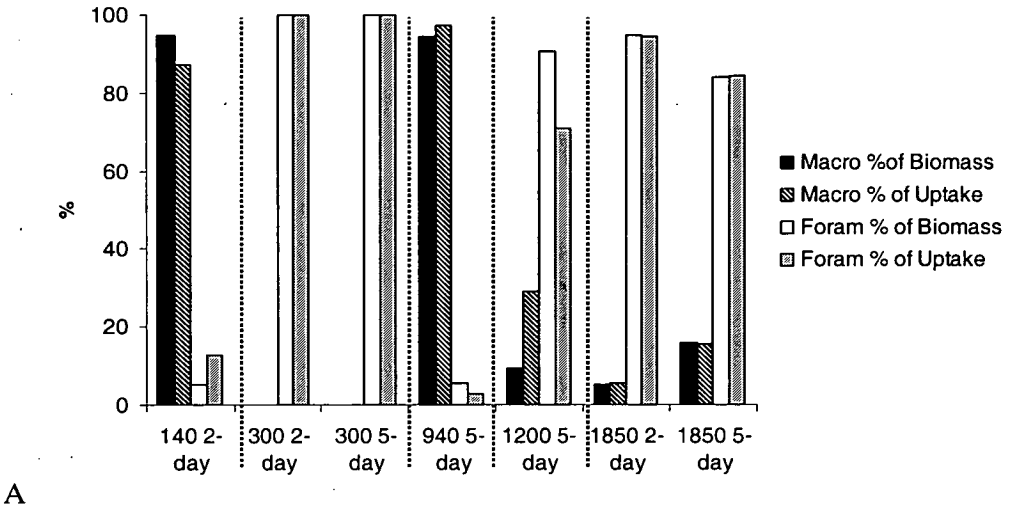
Uptake of label by the benthic community is presented as the percentage of the total label added to any experiment recovered in faunal samples. It should be noted that the surface-area-normalised quantity of label added to each experiment varied very little, and the data therefore show the same site and season relationships and trends whether plotted as "% of label added" or as "absolute amount of label recovered".

Total label uptake by the benthic community ranged from 0.2-13.8% of the total label added. The highest values by a considerable margin were found at the 940m site, followed by the nearby 850m and 1000m sites. The 140m site exhibited intermediate levels of faunal uptake of OM, followed by the 300m, then 1200m and 1850m sites, at which uptake was extremely low (Fig. 4.2, Table 4.2).

4.3.5 Variation in Uptake Between 2 And 5-Day Experiments

Two- and five-day experiments performed at the same site during the same season allow an assessment of the way fauna uptake and processing of OM varies over time after its introduction. This comparison is possible at 300m site in both seasons, at the 1850m site in the pre-monsoon season only, and at the 140m site in the post-monsoon season only. In both seasons, 5-day experiments at the 300m site showed increases in total label uptake, although in the pre-monsoon season this increase was almost within error. At the 140m and 1850m sites (in post and pre-monsoon seasons respectively) increase in uptake after 5-days as opposed to 2-days was exhibited by the foraminifera (although, again, at the 1850m site, this was almost within error), and the amount of label present in the macrofauna was constant (Fig. 4.2, Table 4.2).

The difference in uptake between 2 and 5-day experiments was always subtle, and on



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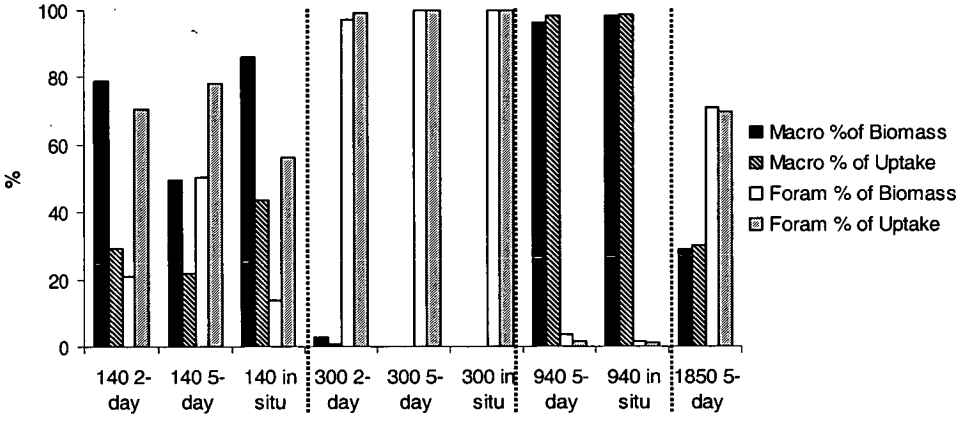


B

Figure 4.3. The percentage of biomass and uptake represented by the macrofauna and foraminifera in each experiment for A) the pre-monsoon season and B) the post-monsoon season.

a much smaller scale than variation in uptake between sites.

4.3.6 Faunal Class Dominance

At the 940m site, and at the 140m site before the monsoon, the macrofauna dominated OM uptake, being responsible for 87% at the 140m site and 98% at the 940m site of total faunal uptake. In contrast, at the deeper 1200m and 1850m sites,

and at the hypoxic 300m site, the foraminifera were responsible for 70-100% of OM uptake (Fig. 4.3).

The 140m site showed a seasonal switch in faunal class dominance of OM uptake. Accompanying the decrease in oxygen concentration, the percentage of total uptake accounted for by the macrofauna decreased (from 83 % to 43 %), and that accounted for by the foraminifera increased accordingly. Associated with this was a change in the balance of macrofaunal and foraminiferal biomass, but the macrofauna remained dominant in that sense (Fig. 4.3).

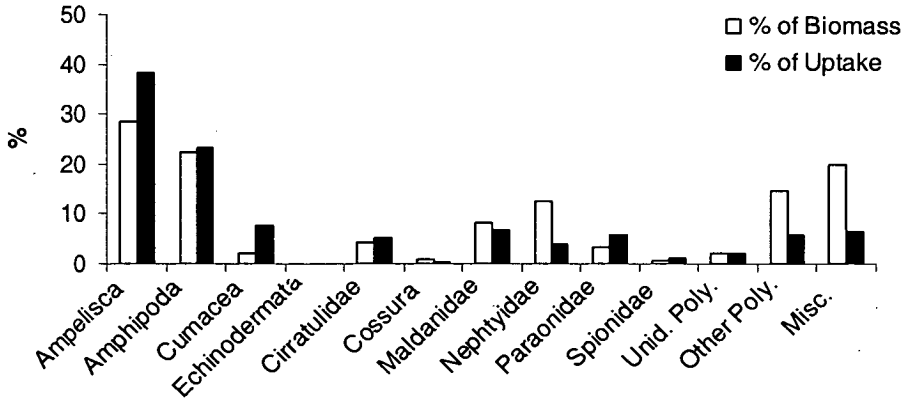
Macrofaunal data showed a positive correlation between sample total C content (equivalent to animal size) and label content ($\rho = 0.65$), suggesting that taxa might be expected to take up OM in proportion to their biomass. Comparison of the percentage of biomass and of total label uptake represented by each faunal class provides a community structure independent means of assessing the efficiency with which the two main faunal groups processed OM (Fig. 4.3). Generally, there was broad correspondence between the contribution to total biomass and to total OM uptake by the faunal groups, however the macrofauna were more efficient (i.e. were responsible for a greater percentage of OM uptake than the percentage of the biomass they constituted) at the 1200m and 940m sites, and the foraminifera were more efficient at the 140m site. At the 300m and 1850m sites there was no significant difference between the percentages of OM uptake and biomass contributed by each group (Fig. 4.3).

4.3.7 Species Effects

As for the macrofauna and foraminifera, the percentages of uptake and biomass (within each faunal class) represented by each family / genus / species (depending on level of sorting) can be used to asses which taxa dominated absolute OM uptake, and which were relatively more or less efficient at OM uptake (Fig. 4.4).

Across several experiments, some taxa consistently consumed more than their 'fair share' of label; that is they consumed a percentage of total OM uptake that was greater than their contribution to the total biomass.

Taxa that consumed more than their 'fair share' of label included the crustaceans Ampelisca, Amphipoda and Cumacea, the polychaetes Cirratulidae, Paraonidae,



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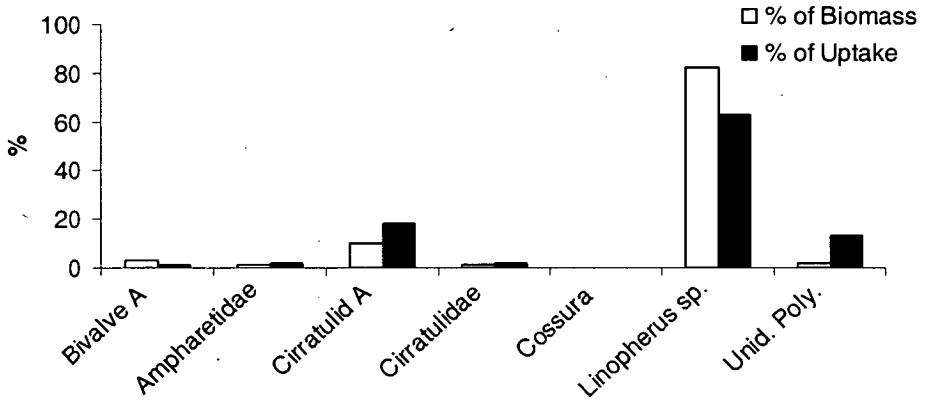

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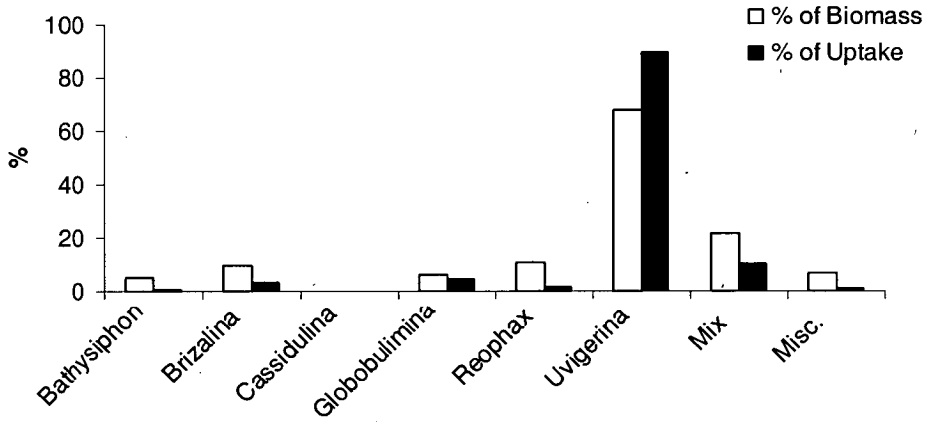


C

Figure 4.4. Example plots comparing the percentages of the biomass and label uptake represented by the various faunal groups for; A) the 140m site 2-day experiment macrofauna, B) the 940m site 5-day experiment macrofauna, C) the 300m site 2-day experiment foraminifera. All data displayed is from pre-monsoon season experiments. Plots for all other experiments are given in appendix B.

Ampharetidae and Prionospio, and the foraminiferan *Uvigerina sp.*. In addition, the broader groups of polychaetes and molluscs at the 1200m and 1850m sites, respectively, were particularly efficient at OM uptake.

The amphinomid polychaete *Linopherus sp*, found at the 940m and 850m sites, consumed slightly less than its fair share of OM; however, it was so dominant in terms of macrofaunal biomass at the 940m site that it also dominated OM processing, despite its relative inefficiency at feeding (Fig. 4.4, and appendix B).

4.3.8 Seasonal Effects

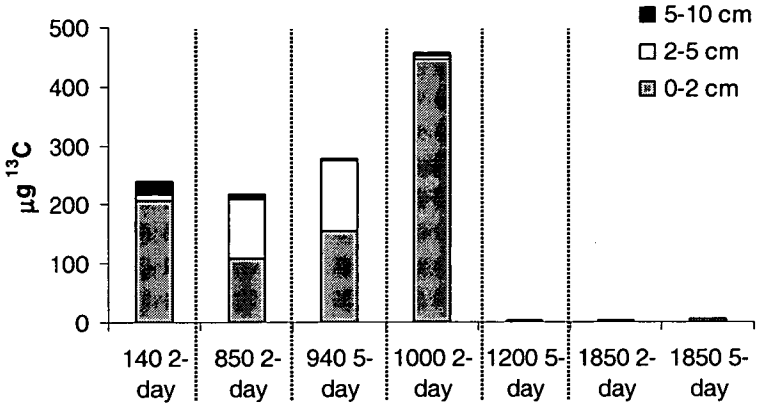
In addition to the seasonal switch from macrofaunal to foraminiferal dominated uptake at the 140m site, a further seasonal effect was evident in the 300m and 940m 5-day experiments. In these experiments, the percentage of label recovered from the fauna was greater (in excess of error, Fig. 4.2, Table 4.2) after the monsoon. The increases were 0.9% to 2.8% and 7.5% to 10.7% for the 300m and 940m experiments respectively (Fig. 4.2, Table 4.2). Total uptake did not however increase after the monsoon in 2-day experiments at the 140m and 300m sites, nor in 5-day experiments at the 1850m site, where uptake was constant (or even decreased slightly) between seasons (Table 4.2).

4.3.9 Vertical Distributions of Labelled Fauna

In all experiments, the majority of labelled organisms were recovered from the, 0-2cm horizon. Labelled organisms were only found below 2cm at the 140m and 940m sites (Fig. 4.5).

The 140m and 940m sites displayed different patterns of macrofauna vertical movement. At the 940m site, a large proportion (35-40%) of label in macrofauna was recovered from the 2-5cm horizon, and none was found any deeper. In contrast, at the 140m site, only 13-30% of label was generally found between 2cm and 5cm;

however, some of that (8-16%) was found between 5 and 10cm (Fig. 4.5).



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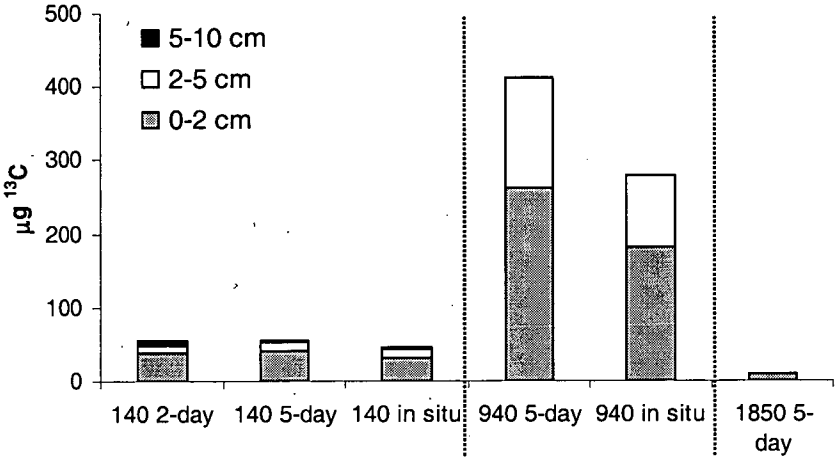


B

Figure 4.5. Depth distribution of label recovered in the macrofauna, in experiments at stations where significant macrofauna was present. Data shown as absolute amounts of ^{13}C recovered in macrofauna in the depth horizons 0-2 cm, 2-5 cm, and 5-10 cm. A) pre-monsoon, B) post-monsoon.

4.3.10 Vertical Distribution of Label and Bioturbation

Isotopic analyses of bulk sediments revealed very little ^{13}C enrichment of sub-surface sediments above time zero controls, and only rare sub-surface peaks (Fig. 4.6). Downcore sediments were enriched in ^{13}C above natural levels (~ -19 to-23 ‰, Cowie et al., 1999); however, visual inspection of the time zero control data (Fig. 4.6) showed that subsurface $\delta^{13}C$ values up to –15 ‰ were to be considered as

experimental artefacts of smearing during sectioning. Considering this value for the background, and sediment x-radiographs (Fig. 4.7), the mixing at each site during each season is summarised below.

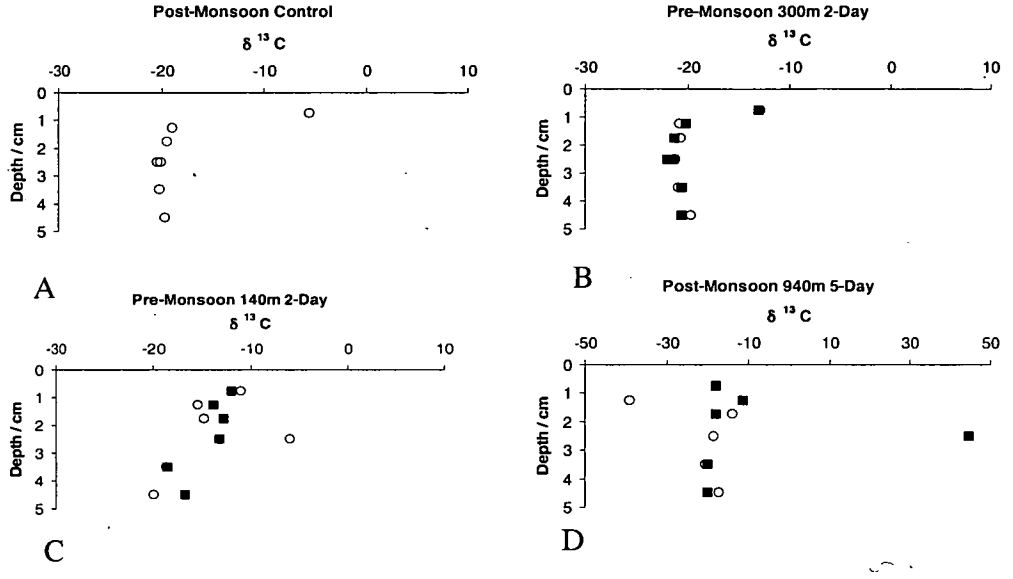


Figure 4.6. Representative examples of downcore $\delta^{13}C$ profiles of bulk sediments following labelling experiments. A) Time zero control from the 140m site, B) a typical 300m site experiment profile, C) a 140m site profile showing a sub-surface ^{13}C peak at 2.5 cm, D) a 940m site experiment showing the most significant sub-surface peak of all experiments conducted, again at 2.5 cm. Note that data for the surface (0-0.5cm) horizon is not shown on the scales used. These values were typically 500-1000 per mil. Where two sets of symbols are used they represent the two replicate cores of the experiment in question.

- 140m. Mixing and occasional sub-surface peaks were present to a depth of 2.5 cm, with deeper mixing in 5-day and *in situ* experiments. Burrowing was visible in sediment x-rays.
- 300m. No suggestion of mixing. X-rays showed laminated sediment.
- 850m. Sediment x-rays showed strong laminae present, but discrete burrows could also be seen.
- 940m. Some high enrichment to 0.75 cm (~ 400 ‰) and 2.5 cm (45 ‰) in the post-monsoon season, and sub-surface peaks of more moderate size present at 2.5 and 4.5 cm in the pre-monsoon season. X-rays showed burrows, but also preservation of laminae.

- 1000m. Sediment x-rays showed discrete burrows in homogenous sediment.
- 1200m. No suggestion of mixing in sediment ^{13}C profiles, but x-rays showed the sediment was well mixed.
- 1850m. Generally sediment ^{13}C profiles showed no evidence of mixing, but large and deep burrows were present in sediment x-rays. The animals associated with these burrows were never recovered, in megacores, thus their bioturbation signal would not be expected in incubation experiments.

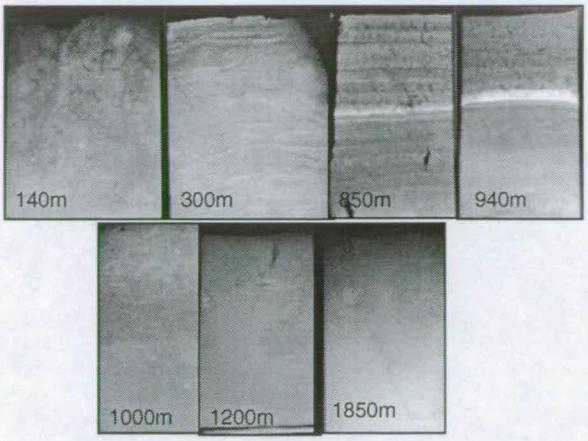


Figure 4.7. X-radiographs of sediments from the sites investigated (courtesy of L. A. Levin), each representing the sediment surface and down to ~15cm depth.

4.4 Discussion

4.4.1 Summary of Results

In summary, the added label was taken up most strongly at the macrofauna-dominated and relatively OM rich 140m, 850m, 940m and 1000m sites, all of which had lower oxygen availability than the colder but oxygenated 1200m and 1850m sites (Fig. 4.2, Table 4.2).

Foraminifera dominated uptake at the extremely low oxygen (300m) and cold, deep, but oxygenated (1850m) sites, leaving macrofauna dominant only at the OM rich sites at OMZ boundaries (the 140m (pre-monsoon), 940m, and most probably 850m and 1000m sites). Thus, the macrofauna were able to adapt to low oxygen conditions to take advantage of abundant high-quality OM, but below a threshold oxygen concentration were unable to survive.

Summer monsoon-driven upwelling and productivity caused reduced oxygen concentration and an associated switch from macrofaunal to foraminiferal dominance

of OM uptake at the 140m site. At the 300m and 940m sites, the seasonal input of OM to the seafloor had a priming effect, causing greater benthic community response to the artificial OM input after the monsoon.

Comparison of the percentage of biomass contributed by faunal groups and individual species with the percentage of uptake that they performed revealed that these generally co-varied. In some environments, however, either the macrofauna or foraminifera were relatively more efficient at OM uptake, and this was chiefly related to oxygen availability. In addition, individual species were identified as being particularly efficient and / or significant in total OM uptake and processing at certain sites.

Thus, oxygen availability, OM availability and benthic community composition combine to determine the way in which the benthic community responds to OM input.

Label tracing was also used to assess the vertical movement behaviour of the macrofauna at the 140m and 940m sites. The macrofaunal community at the 140m site exhibited less frequent vertical movement, but to a greater maximum depth than those at the 940m site. Deep vertical mixing of the sediment was, however, virtually undetectable during the time frame of these experiments.

4.4.2 Faunal Uptake in Total C Processing Budgets

At first glance, the percentages of added label recovered from the fauna seem relatively inconsequential fractions. It should be noted however that in this study, on average 16.5 % of added label was recovered from processed pools (macrofauna, foraminifera, bacteria and respiration) at experiment termination, and this is comparable with other similar experiments (e.g. Moodley et al., 2000, Witte et al., 2003 b). The highest percentages of added label to be processed occurred at the macrofauna populated 140m and 940m sites, and the lowest values occurred at the foraminifera dominated 300m and 1850m sites (Johan H. Andersson, pers. comm.). Across all experiments, faunal uptake constituted a very significant ~ 5-83 % of the total processed label. The majority of un-processed label remained near the surface of the sediment. Some may have been transformed into dissolved prganic carbon, and this may be quantified in the future.

The division of processed label between macrofaunal uptake, foraminiferal uptake, bacterial uptake and total respiration varied systematically among sites.

At the 140m site respiration always accounted for 70-75 % of processed label. Macrofaunal, foraminiferal and bacterial uptake represented roughly equal percentages. The macrofauna dominated before the monsoon, but foraminifera and bacteria dominated afterwards.

At the 300m site, respiration accounted for roughly 55-75 % of total processed label, and there was no macrofaunal uptake. Foraminiferal uptake was greater than bacterial uptake in the pre-monsoon season, and this dominance switched after the monsoon.

At the 940m site, macrofaunal uptake roughly equalled, or even (in the in situ case) totally dominated over respiration (constituting 45-83 % of total processed label). Bacterial uptake was the next most significant fate of label, followed by foraminiferal uptake.

At the 1850m site respiration displayed its greatest dominance (~ 85-95 %) of the fate of processed OM. Foraminiferal uptake accounted for the greatest part of the remaining label, and macrofaunal and bacterial uptake were roughly equal in significance (Andersson pers. comm).

Comparison of faunal uptake data with bacterial uptake and respiration data highlights that faunal processing is of comparable importance in sediment C-cycling as bacterial processing and respiration, which are typically thought to be the most significant processes (e.g. Moodley et al., 2002, Witter et al., 2003b). The observed variation in the relative significance of different processed OM fates means that the Pakistan margin exhibited sites comparable to those featured in most other studies. The respiration dominated deep-sea 1850m site was similar to Mediterranean and NE Atlantic sites (~ 1500-200m depth), where respiration was the fate of the vast majority of added OM, with only small (5-10 %) percentages taken up by bacteria and fauna (Moodley et al, 2005). The 140m site was similar to an estuarine, a North Sea, a deep Norwegian fjordic, a Goban Spur abyssal plain, and a Porcupine Abyssal Plain site, where respiration accounted for ~ 75 % of processed label, and macrofaunal uptake for ~ 10-15 % (Moodley et al., 2005, Witte et al., 2003 a, b, Heip et al., 2001). The 940m site was similar to a Goban Spur continental shelf site where

the macrofauna were found to account for over 50 % of sediment community oxygen consumption (Heip et al., 2001). The 300m site was similar to a 2170m deep site off NW Spain, where respiration accounted for ~ 50 % of recovered label, and the rest was split relatively evenly between foraminiferal and bacterial uptake (Moodley et al., 2002). There is no simple relationship, in the previous studies cited, between site depth and the distribution of processed OM among respiration and bacterial, foraminiferal and macrofaunal uptake. The results of this study suggest that variations in faunal communities, OM supply and oxygen availability such as are seen across the Pakistan margin could account for the variation in results between previous single site studies.

4.4.3 Controls On Total Faunal Response

4.4.3.1 Oxygen

Patterns of uptake at the 140m and 300m sites provide evidence to suggest that the availability of oxygen exerts a strong control over the way OM is processed by benthic communities.

Uptake patterns at the 140m site between pre and post-monsoon seasons showed that under conditions of low oxygen availability (in the post monsoon season) total uptake was reduced, and the foraminifera became more efficient at OM uptake than the macrofauna (Figs. 4.2 and 4.3). In pre-monsoon, relatively oxygenated conditions, the macrofauna at the 140m site dominated both the biomass and OM uptake, despite the fact that the foraminifera were more efficient at OM uptake (per unit biomass). In response to a monsoon-induced reduction in oxygen availability (from 2.05-0.11 ml L^{-1}), however, a shift in the balance of biomass in favour of the foraminifera, together with an increase in their relative uptake efficiency, switched the system at the 140m site to one where the foraminifera dominated OM uptake and processing (Figs. 4.2 and 4.3).

This shift in OM uptake pattern at the 140m site in response to a reduction in oxygen concentration indicates that oxygen availability controls the faunal response to an OM pulse in a threshold manner. Below this threshold (which appears to lie between 0.11 ml L^{-1}, the value at the 140m site, post-monsoon, where foraminifera dominated, and 0.13 ml L^{-1}, the value at the 940m site, post-monsoon, where

macrofauna dominated. The threshold is however probably taxon specific (Levin et al., 2000)), the macrofauna become unable to function effectively, and so are out-competed by the foraminifera, which then dominate OM processing. This is also shown by the oxygen driven absence of macrofauna from, and dominance of foraminifera at the 300m site, at which faunal uptake of OM is, as a result, reduced (see community structure section).

In addition, low oxygen conditions seemed to generally inhibit the functioning of foraminifera. In the post-monsoon season, there was less foraminiferal OM uptake at the intensely hypoxic 300m site than at the 140m site. This was despite the facts that these sites had similar foraminiferal communities (Stefanie Schumacher, pers. comm.), and the temperature, and availability of high-quality OM (see below) at the 300m site would otherwise provide ideal conditions for rapid processing of OM.

4.4.3.2 Availability of High-Quality OM

Faunal communities from sites where higher-quality OM (as indicated by the amino acid degradation index, DI, Dauwe and Middelburg, 1998, Table 4.1, and pigment concentration, chapter 6) was naturally abundant were better able to respond to an artificial pulse of fresh algal detritus. Label uptake was highest at the 140m, 300m, 850m 940m and 1000m sites, where OM was more abundant and of higher quality than at the 1850m site (Fig. 4.2). This was the case despite the fact that macrofauna were present at the 1850m site, and absent from the 300m site. Thus, at sites where high quality OM was naturally abundant, the faunal community was better adapted and primed for responding to an artificial OM pulse (Table 4.1, Fig. 4.2).

The effect of the natural availability of high quality OM on the ability of faunal communities to process labelled algae is further illustrated by comparing the macrofauna dominated uptake at the 940m and 140m (pre-monsoon only) sites. The 940m site exhibited higher quality and more abundant OM than the 140m site, and had a lower bottom water dissolved oxygen concentration (Table 4.1), but total OM uptake was, on average, a factor of 2 greater than at the 140m site.

This suggests that of the two main controls on faunal abundance and activity, food supply and oxygen supply, the former is dominant in controlling faunal OM uptake, so long as the latter is above a threshold level. The macrofauna at the 940m site appeared to have adapted to low oxygen conditions, in order to take advantage of the

abundant, high quality OM. This is consistent with previous work, which has found that OM supply often exerts a stronger control on macrofaunal abundance and activity than oxygen availability (e.g. DeMaster et al., 1994, Duniveld et al., 2001, Smith et al., 2000, Levin et al., 2000, Cook et al., 2000).

The 1200m site was an exception to this trend, as it exhibited maximal OM quality and availability, but minimal faunal OM uptake. The reason for this was probably the absence from experimental megacores of the large burrowers thought to populate this site (Levin, pers.comm.).

4.4.3.3 Temperature

With increasing water depth, seafloor temperatures fell from ~23°C at the shallow 140m site to ~3-4°C at the deep, 1850m site (Table 4.1.). A previous study has shown (by incubating shallow marine samples from the Mediterranean (~16°C) at deep-sea temperatures) that low temperatures inhibit C processing by the benthic community (Moodley et al., 2005), and also slow the response of the benthos to an input of OM. The 1200m and 1850m sites showed this effect, as despite high natural availability of OM at the former, and the presence of macrofauna and relatively high oxygen availability at both, they exhibited minimal OM uptake (Fig. 4.2, Table 4.2). Furthermore, time-series water sampling of these experiments for the release of ^{13}C-labelled DIC revealed relatively slow C remineralisation rates, and, at the 1850m site, a time lag before respiration commenced (Johan H. Andersson, pers. comm.).

4.4.3.4 Community Structure

Correlation analysis revealed that among the macrofauna, the amount of ^{13}C label recovered from each specimen was positively related to the overall size of that specimen as measured by its total C content ($\rho = 0.65$). This correlation could partially result from the presence of large quantities of labelled algal matter in macrofaunal guts, making heavily labelled individuals also seem relatively large. None the less, the correlation implies that the larger an individual was, and the more abundant a species was, the greater was its role in OM processing. Thus community structure exerts some control over the scale and pattern of the processing of OM by fauna. Middelburg et al. (2000) have previously observed this control of commuity

structure on OM processing, when consumption of ^{13}C-labelled algae in an estuarine environment was seen to occur in proportion to consumer biomass.

Of all the sites studied, the 140m, 850m, 940m and 1000m sites showed significantly greater (t-test, $P = 4x10^{-5}$) total label uptake than the 300m, 1200m and 1850m sites (Fig. 4.2). The difference between these two groups of sites is that, at those in the first group, macrofauna dominated the biomass (Fig. 4.3). The 940m, 1000m and 850m sites in particular exhibited especially high macrofaunal biomass, consistent with their above-threshold oxygen levels (see section 4.4.3.1) and abundant high-quality OM (Levin, 2000), and hence showed the highest label uptake values by a considerable margin (Fig. 4.2).

Very small macrofaunal communities were recovered from megacores at the 1200m and 1850m sites (Table 4.1), and faunal OM uptake (macrofauna and total) at these sites was correspondingly low (Fig. 4.2, Table 4.2). Sediment x-radiographs (Fig. 4.7), however, suggested that large and deep–burrowing (5-10 cm) organisms (thought to be shrimp) were present at these sites but were not recovered (Lisa Levin pers. comm.). Had such organisms been present in experimental megacores, the measured faunal OM uptake at these sites may have been significantly larger.

Thus, it seems that a relatively high macrofaunal biomass results in maximal rapid faunal processing of freshly deposited OM. This highlights the overall importance of macrofauna in OM cycling, as when they are present and dominant, a large proportion of fresh OM will be subject to internal digestion before it is made available to the rest of the faunal community (Witte et al., 2003a, b, Lauerman et al., 1997, Miller et al., 2000). Although downcore transport of OM was not measurable in this study, the presence of macrofauna will generally not only increase total OM uptake, but in many settings will lead to the rapid transport of OM to depth in the sediment (e.g., Blair et al., 1996, Levin et al., 1997), and this has further implications for OM burial versus decay.

Macrofauna possess features and abilities that make their importance in OM uptake compared to the foraminifera unsurprising. They are larger, and therefore able to process greater volumes of sediment. They are also more able to move horizontally and vertically through the sediment in search of food, and many species are thought to be able to ingest selectively on the basis of particle size, age, or food quality (e.g.

Miller et al, 2000, Smith et al., 1993). Thus, they are more likely to process freshly introduced OM than the foraminifera, which exhibit limited motility, even though they may be selective feeders (Heinz et al., 2002).

4.4.3.5 Summary of Controls

In summary, low oxygen availability, low OM quantity and quality, low temperatures, and the absence of macrofauna all tend to reduce the amount of early processing of fresh OM by the benthic community. In addition to this, oxygen availability exerts a threshold-type control on the pattern of OM uptake among the faunal classes, prompting domination by foraminifera at low concentrations. In addition, rapid macrofaunal community structure changes through the OMZ lower boundary (Lisa Levin, pers. comm.) suggest that where oxygen availability is near critical for macrofauna (e.g. 850-1200m), dramatic changes in benthic community structure occur, as specific taxa adapt and dominate communities to take advantage of bioavailable OM (i.e. the oxygen threshold is taxon specific). Thus, community structure, oxygen availability and bioavailable OM interact (and co-vary) in a complex fashion to control the rate and pathways of early OM uptake and cycling by fauna, with temperature having an additional imprint on the magnitude and timing of community response.

4.4.4 Seasonal Variation

Monsoon-driven upwelling and intense primary productivity in the Arabian Sea has been shown to result in twice-yearly discrete inputs of OM to the sediment (Haake et al., 1993a), constituting the kind of food pulse characteristic of many deep-sea environments. By conducting labelling experiments both before and after the summer monsoon, we were able to assess whether, having received such an OM pulse, the faunal communities would respond differently to an artificial OM addition. Sediment %Corg data did not show a consistent measurable increase in surficial sediments between pre-and post-monsoon seasons at any site, although there were slight increases in the 0-0.5cm horizons at the 140m, 300m and 1850m sites (Greg Cowie, pers. comm.). The pigment contents of the surface sediments displayed a similar general lack of seasonal increase (chapter 6), and the same was found for amino acids (Sandra Vandewiele pers. comm.), but carbohydrate abundance

generally showed slight increases in surficial sediment concentrations after the monsoon (chapter 7). Other evidence for a monsoon-driven OM flux included shallower post-monsoon sediment oxygen penetration depths at all sites, and corresponding shifts in porewater redox boundaries, as evident from trace-metal and nutrient concentration profiles (pers. comms.: Breuer, Law, Brand, Bett et al., 2004a, b, Cowie et al., 2005a, b). Thus, it appears that a pulse of fresh OM was delivered to the sediments of the Pakistan margin as a result of the summer monsoon, but, perhaps due to rapid degradation, this pulse had only a relatively subtle effect on sediment OM content by the time of sampling.

Five-day experiments at the 300m and 940m sites showed measurable increases in total faunal uptake of label after the monsoon (Fig. 4.2). This suggests that the seasonal OM pulse could have had a priming effect on these benthic communities, which responded by shifting into a more active and efficient feeding mode.

This seasonal priming effect was not seen in 2-day experiments at the 140m or 300m sites, or in 5-day experiments at the 1850m site (Fig. 4.2). The lack of seasonal signal at the 140m site is likely to be the result of any OM pulse related priming effect being cancelled out by the inhibitory effect of the observed reduction in oxygen availability.

At the 300m site, a seasonal increase in uptake occurred in 5-day experiments, but not in the 2-day experiments. A series of long-term isotope tracing experiments carried out by Witte et al. (2003b) revealed a significant time lag (on the order of a week) between the immediate location and uptake of label by macrofauna and the subsequent rise to domination of OM processing by the foraminifera and bacteria. Therefore, it is possible that a seasonal increase in foraminiferal label uptake would only ever be evident in longer (i.e. 5-day) experiments. It is also possible that any seasonal increase in total uptake in the 300m 2-day experiments was obscured by within-site variation or experimental error.

The lack of seasonal increase in OM uptake at the 1850m site may have been because the seasonal OM pulse that occurred there was not sufficiently large or reactive to have a measurable priming effect on the benthic community. This is a distinct possibility, as even the experimental addition of fresh algal detritus (such as would never naturally reach these sediments) failed to produce a dramatic response

by the benthos. Alternately, the absence of the largest and most active fauna of this site from experimental megacores may have reduced the tendency of the community to show a seasonal priming effect, or the observed time-lag in faunal response to label delivery (Johan H. Andersson, pers. comm.) may have masked such an effect. The latter suggestion is supported by the fact that total label remineralisation at the 1850m site increased (roughly twofold) between pre and post-monsoon seasons, thus there was some evidence of a seasonal priming effect.

In general, although evidence for priming of the benthic community by a seasonal OM pulse was seen at the 300, 940 and 1850m sites, the effect was relatively subtle compared to variation in uptake observed between sites (Table 4.2, Fig 2). This may be due to the relatively low impact of the seasonal OM pulse on sediment biogeochemistry.

4.4.5 Uptake Over Time

A measurable increase in the amount of label in fauna between 2 and 5-day experiments (at the same site, and in the same season) was only ever observed for the foraminifera. Indeed, at sites where macrofauna were present, they showed constant label content between the two experiment durations, while the foraminifera in the same experiments increased their label contents between the 2- and 5-day time points (see the post-monsoon 140m site and pre-monsoon 1850m site experiments, Fig. 4.2).

The lack of increase in macrofaunal ^{13}C content between 2- and 5-day experiments may have been a function of the fact that a large proportion of the label recovered from macrofauna is likely to have been present as gut contents. Once the macrofauna fill their guts with labelled algae, their OM holding capacity is saturated. It is likely that the macrofauna continued to process label between the 2- and 5-day time points, but aside from the relatively small proportion of ingested OM that is assimilated (0.2-8 %, Miller et al., 2000, Lauerman et al., 1997), they at no one time contained any more labelled OM than could be housed in their guts. Thus, it seems that the amount of label present in the macrofauna probably reached its maximum very rapidly (potentially long before the 2-day point), and plateaued, despite

continued OM processing (i.e. actual assimilation may have been relatively minor over the 2-5 day period).

By contrast, the foraminifera at the 140m, 300m and 1850m sites seemed to respond more slowly the input of labelled algae, and therefore showed increased label contents after 5-days. A slower response by foraminifera than macrofauna to an artificial OM pulse has been observed previously (Witte et al., 2003b), and it has been suggested that foraminifera typically consume more degraded OM than the macrofauna. Other studies of recently deposited marine snow on the deep-sea floor have, however, shown a relatively rapid colonisation of fresh OM by foraminifera (Gooday, 1988). In fact, the food preferences and feeding behaviour of foraminifera are likely to vary considerably among taxa and environments (Nomaki et al., 2005a).

4.4.6 Faunal Class Dominance

In terms of absolute quantity and percentage of total OM uptake, the macrofauna were observed to dominate at the 940m site, in the pre-moonsoon season at the 140m site, and most likely (by analogy with the 940m site) at the 850m and 1000m sites, and this was largely due to their dominance of the biomass. The foraminifera dominated OM uptake at the 300m, 1200m and1850m sites and, in the post-monsoon season at the 140m site. In the first three cases this was due to foraminifera dominating the biomass, illustrating the control that community structure exerts over faunal OM processing. In the last case, however, the dominance of OM processing by the foraminifera was in spite of domination of the biomass by the macrofauna, and was instead attributable to low oxygen availability.

In order to de-convolve the activity of groups of fauna in OM processing from their dominance of the biomass, the percentage of the total biomass constituted by each class is compared to the percentage of total faunal uptake that class performed (Fig. 4.3). There was general agreement between these values; thus benthic community composition dictates both the magnitude of total faunal OM uptake, and the distribution of OM uptake among faunal groups, and thus the pathways of early OM cycling. Slight deviations from this correspondence showed that the macrofauna accounted for a slightly greater percentage of uptake than of biomass at the 940m and 1200m sites, and this scenario was reversed at the 1850m site.

Studies of gut contents and feeding rates have suggested that the macrofauna are able to process 30-40% of the OM flux to the sediment (Miller et al, 1998). In an investigation of holothurian gut ^{234}Th and ^{210}Pb contents, Lauerman et al (1997) calculated that only 0.2-4% of OM passed through megafaunal guts. However, Miller et al (2000) re-examined this data and produced a revised figures of 1.2-24%.

Isotope labelling studies have produced a range of estimates as to which faunal groups are responsible for most OM uptake. In shallow estuarine and coastal environments the 'fauna' (macrofauna plus foraminifera) have been observed to take up a similar proportion of added label to the bacteria (Moodley et al., 2005). Deep-sea studies have concluded that the bacteria and foraminifera are responsible for similar proportions of OM processing (Moodley et al., 2000), and a considerably greater percentage of OM uptake (~20% and 29% respectively) than the macrofauna (3.5%) (Moodley et al, 2002). In this study, the macrofauna were observed to dominate OM uptake in the 940m site, pre-monsoon 140m site, and probably 850m site and 1000m site experiments (Fig. 4.3). The high incidence of macrofauna dominance of OM uptake in this study is likely to be due to the unusual macrofaunal communities that were present on the lower boundary of the OMZ, a feature of OMZs worldwide (Levin, 2003). Dominance of macrofauna over foraminifer and bacteria in short-term OM uptake has, however, also been observed in non-OMZ continental shelf and deep-sea settings (Witte et al., 2003 a, b). The present study supports the suggestion by the latter authors, that the macrofauna, despite their relatively small contribution towards total sediment biomass, are surprisingly important in immediate uptake and processing of freshly deposited OM, and also the suggestion that the macrofauna can, given appropriate conditions, equal or (in the case of the 940m site) dominate over the foraminifera (Moodley et al 2002) in OM uptake and processing. Those appropriate conditions seem to be near-critical oxygen levels, under which selected macrofauna adapt to take advantage of abundant, high-quality, bioavailable OM (Rosenberg, 2001).

4.4.6.1 Controls On Macrofaunal versus Foraminiferal Dominance Of OM Processing

Consideration of several particular examples shows how the environmental factors OM and oxygen availability interact to influence the relative efficiencies of the different faunal groups in OM processing.

In the pre-monsoon experiment at the 1200m site, the macrofauna were responsible for a considerably greater percentage of uptake than could be explained by their biomass contribution (Fig. 4.3). Thus at this relatively oxygenated, OM rich site, the macrofauna were more efficient than the foraminifera at locating and consuming fresh OM (the foraminifera still dominated overall OM processing due to their overwhelming dominance of the biomass). In addition, the degree of dominance of the macrofauna over the foraminifera in OM processing was greater at the relatively oxygen-poor but OM-rich 940m site, than at the (pre-monsoon) 140m site (where, in fact, the foraminifera always consumed more OM per unit biomass than the macrofauna).

These examples suggest that under conditions of sufficient oxygen and high OM availability, the macrofauna are more efficient processors of OM, while under conditions of low oxygen or low OM availability, the foraminifera are better adapted, and thus more efficient.

Label uptake dominance patterns also suggest a link between water depth and the dominant faunal group. Excepting examples where low oxygen conditions played a role, the macrofauna dominated OM uptake at the shallower (140m, and 940m) sites, and the foraminifera dominated at the deeper (1200m and 1850m) sites. The data thus support the common observation that with increasing water depth, the average size of the dominant fauna decreases (Rowe et al., 1991, Pinet, 1992, Levin et al., 2000). This study shows that this is true not only of the dominance of biomass, but also of the dominance of role in OM processing (Mahaut et al., 1995). The increasing influence of smaller organisms with increasing depth is usually linked to the normal downslope reduction in naturally available OM and OM quality, and is thus consistent with the suggestions above that macrofauna are favoured over foraminifera in the presence of abundant high quality OM. Consistent with the decrease in macrofaunal influence downslope, respiration had its maximum share of

total OM processing at the 1850m site. As faunal uptake at this site was the lowest on the margin, it can be assumed that the majority of that respiration was performed by bacteria (although it should be noted that the large fauna thought to be present at the 1850m site were not present in experiments).

Previous work has lead to suggestions that interactions between different fractions of the benthic community also influence faunal class dominance. Moodley et al. (1998) found that where macrofauna were particularly abundant (due to high OM availability), they suppressed foraminiferal activity, a suggestion which is consistent with the label uptake pattern observed at the 940m site.

4.4.6.2 Contrasting Macrofaunal and Foraminiferal OM Processing

Dominance of OM uptake by macrofauna as opposed to foraminifera has profound implications for its short-term fate. Metazoan macrofauna are relatively large organisms, capable of active location and selective uptake of food (Fauchald and Jumars, 1979), and of significant vertical movement through the sediment. Thus macrofaunal OM processing has the potential to rapidly redistribute OM through the sediment column (over 10 cm depth or more, e.g. Levin et al., 1997), and either make it available to sub-surface fauna and bacteria, bury it below the redox front, and so enhance preservation, or re-expose it to oxygen, depending on feeding mode.

Foraminifera, by contrast, display limited vertical movement, and may only mix the top 0.5 cm of sediment (Nomaki et al., 2005 a), a suggestion borne out by the preservation of laminae in foraminifera-dominated sediments at the 300m site. It has also been suggested, based on the observation that foraminiferal OM uptake can be delayed by days to weeks compared to that of the macrofauna, that the foraminifera consume more degraded OM than the macrofauna (Witte et al., 2003 b, Moodley et al., 2002). This suggestion is partly supported in this study by the observed relatively high OM uptake efficiency of foraminifera at the 140m site, where the naturally present OM was of relatively low quality. However, while some foraminifera are thought to be deposit feeders (Gooday et al., 2002), significant foraminiferal label uptake was observed after 2-5 days, supporting other observations made after natural and stimulated OM pulses that foraminifera are also capable of selective feeding (Heinz et al., 2002), and of rapidly colonising and utilising freshly deposited OM (Gooday, 1988, Kitazato et al., 2000). Nomaki et al. (2005 b) used

their observations of species-specific response to an OM pulse during in-situ isotope labelling experiments to suggest that this apparent contradiction arises from the presence of different species, with different food preferences and habits. Deep-sea, agglutinated foraminifera may have a preference for consuming degraded OM, and therefore there may be a 'time consuming activation of metabolism' required before response to a pulse of fresh OM is observed (Nomaki et al, 2005 b). Gooday et al. (1992) noted that foraminifera consume different forms of OM, but do so at a low trophic level, and thus constitute an important link with metazoans in the seafloor C-cycle through predation and colonisation of burrows.

Macrofauna and foraminifera also likely differ in their digestive and metabolic chemistry, as shown by tracing of labelled amino acids (chapter 5), and processing of OM by these different faunal groups may impart different biochemical imprints on the sediment.

Thus, the observed variation among sites between macrofauna- and foraminifera-dominated OM uptake, in response to community structure, oxygen concentration and OM availability, have profound implications for the short-term fate of freshly deposited OM.

4.4.6.3 OM Remineralisation

The dominant carbon sink in most benthic isotopic labelling studies is respired CO_2 (e.g. Moodley et al., 2002, Moodley et al., 2005), and the question of which organisms are responsible for the respiration has been the subject of considerable study. The traditional view is that, in spite of rapid and significant uptake of OM by the macrofauna, microbial biomass in the sediment is so large (generally ~95% of the total) that bacterial re-mineralisation must eventually exceed that carried out by other fauna (Witte et al., 2003 a). This is supported by experiments performed by Moodley et al. (2005), in which de-faunated sediment showed similar sediment community oxygen consumption (SCOC) rates as sediment with a natural community present. This view has led to suggestions that larger animals are likely to affect OM cycling principally through microbial stimulation, mixing, irrigation and biodeposition, rather than through direct uptake and respiration (Heip et al., 2001, Kristensen, 2000).

Heip et al. (2001), however, divided responsibility for measured SCOC between faunal groups using respiration rates calculated for each species, based on the body size-respiration rate equation published by Mahaut et al. (1995), and biological survey data of mega, macro and meiofauna. Remaining SCOC was attributed to the microbial community. While it should be noted that the application of theoretical individual animal respiration rates is problematic, their study revealed that although the bacteria vastly dominated the biomass (at 75-90%, the macrofauna coming second with 10%), a portion of that biomass seemed to be dormant, and that, on the continental shelf and upper slope, the macrofauna were responsible for up to 50% of total respiration. Thus, observations of rapid and significant macrofaunal OM uptake in this study may be accompanied at the 140m and lower OMZ boundary sites by significant contributions of the macrofauna to OM re-mineralisation.

This is a relatively novel finding, and one that should be investigated on the Pakistan margin through similar methods as employed by Heip et al. (2001). In order to transcend the problems related to the microcosm-based and theoretical respiration rates however, future studies should also attempt to directly isolate and measure the respiration of different faunal groups from a real sediment community. This approach would have the advantage of including the effects of species-specific variations and inhibitory or stimulatory effects among the faunal classes that will have been missed by previous approaches. It may also reveal similarities among different sites and communities, which could form the basis of regional scale modelling.

4.4.6.4 Uptake versus Throughput

It is important to note that 'uptake' values in this study do not represent total label throughput of the fauna during the entire experiment duration, but are simply snapshots of the amount of label in faunal guts and tissues at the time of experiment termination. There was sufficient time in all experiments for all taxa to have ingested and subsequently egested significant percentages of the labelled OM.

In order to assess what the total label throughput of any faunal group was during any experiment, typical feeding rates and gut turnover times must be considered. Small animals have been observed to have shorter gut turnover times, and faster metabolic rates than larger fauna (Mahaut et al., 1995); thus, an estimation of the label

throughputs of the macrofauna and foraminifera in all Pakistan margin experiments would most likely enhance the role of the foraminifera in OM processing relative to the macrofauna.

Mahaut et al. (1995) proposed the relationship between body size and metabolic rate to be $R=aW^b$, where R is respiration rate, W is the organism weight, and a and b are constants that vary with water depth, such that respiration is slower in deeper waters. The percentage of the biomass represented by smaller faunal classes increases with increasing depth, and the rate at which respiration slows with increasing depth is greater for larger organisms. Thus, the shift in relative dominance of OM uptake, turnover and remineralisation in favour of the foraminifera brought about by estimating total OM processing by the faunal groups during each experiment will increase with increasing water depth.

An actual attempt to calculate the total label throughput of faunal classes during labelling experiments is hampered by a lack of knowledge as to the gut turnover times and feeding rates of the organisms present on the Pakistan margin. Studies of different taxa (from holothurians to polychaetes) suggest a range of residence times for OM in the gut ranging from ~ 3-24 hours (Ahrens et al., 2001, and references therein). Published foraminiferal vacuole turnover times have not been found.

If we assume continuous feeding and the shortest published gut turnover time for polychaetes (~3 hours, Ahrens et al., 2001), macrofauna at the 140m and 940m sites would have processed all of the added label several times over during a 2-day experiment. As a possible alternative extreme, if we use a conservative gut turnover time of 18 h (Ahrens et al., 2001 recorded times of 12-36 hours for polychaetes that were generally larger than those in this study), and an assumption that polychaetes only feed for 50% of the time (Masson et al., 1995), the result is that an estimated 20-30% of the added label was processed by the macrofauna in 2-5 day experiments at the 940m site. The total throughput estimates therefore are clearly very sensitive to the gut turnover times and feeding habits used to calculate them.

Thus, without gut and vacuole turnover times directly related to the macrofaunal and foraminiferal species present on the Pakistan margin, reliable estimates of total label throughput are not attainable. It is unlikely that all of the added label was processed by the macrofauna several times during each experiment, as considerable proportions

of the added label were observed to remain in an undisturbed layer at the sediment surface when experiments were terminated. Thus the second scenario, or something like it, is likely to be closer to actuality. Considering that scenario to be a conservative estimate of total faunal OM throughput during experiments, this exercise serves to highlight that, although they only contain relatively small proportions of the added OM at any one time, faunal processing is a very significant path in the early diagenesis of OM.

4.4.7 Faunal Group Dominance At The Family Level And Keystone Taxa

The percentages of total macrofaunal or foraminiferal label uptake performed by individual taxa compared to the percentage of the biomass constituted by those taxa allowed assessment both of which taxa were most significant in the cycling of OM at each site, and of which taxa were most efficient at OM uptake (or consumed more than their 'fair share') (Fig. 4.6 and appendix B).

Taxa that consumed more than their fair shares include the Ampelisca, Amphipoda and Cumacean crustaceans (at the 140m site), the Cirratulidae, Paraonidae, Ampharetidae (at the 140m and 940m sites) and Prionospio (at the 850m sites) polychaetes, and the foraminiferan *Uvigerina sp.* (at the 140m and 300m sites) (Fig. 4.6). The amphinomid *Linopherus sp.*, of the 940m and 850m sites, notably, usually consumed less than its fair share.

A keystone taxon was originally defined as a species that plays a critical role in an ecosystem, such that should its abundance or behaviour alter, the rest of the ecosystem would also be altered (Paine, 1969). This original definition was based on consideration of the impacts of changing predator/prey relationships in an ecosystem. However, the reciprocal controls of the benthic community on and by its geochemical environment discussed above suggest that a keystone taxon thus defined would also play a role in determining and maintaining its chemical environment.

Species have previously been posited as keystone taxa on the basis that they ingested a large proportion of the available fresh OM at the sediment surface and egested it at depth, introducing a patchy, high-quality food source to deeper-dwelling fauna, which re-locate to populate those areas, and by doing do concentrated oxygen

demand and hypoxia into discrete pockets (Miller et al., 2000, Levin et al., 1997). These studies both identified large species, a holothurian and a maldanid polychaete, respectively, as keystone taxa.

On this basis, the polychaete *Linopherus sp.* found at the Pakistan margin OMZ lower boundary, qualifies as a keystone taxon in the environment it inhabits. At the 940m site, this species accounted for 82-97% of macrofaunal biomass and for 63-85% of macrofaunal OM uptake. At the termination of experiments at the 940m site, between 4.5% and 11.4% of added label was found in the gut contents and tissues of *Linopherus sp.* (Table 4.2), and it can be assumed that during the course of the experiments, a considerably greater percentage of the total label added passed through its gut. Thus, in spite of always consuming less than its fair share, *Linopherus sp.* dominated faunal OM processing at the 940m site, due to its size and abundance. No definite evidence was found for *Linopherus sp.* egesting at depth (a sub-surface ^{13}C peak in the sediment was only found in one core out of 6), however visual observation showed that it did create sizeable burrows, and will therefore have had a significant impact on sediment redox conditions. In addition, gut passage and digestion causes substantial biochemical alteration of OM (e.g. Thomas and Blair, 2002; Sun et al., 1999), and this has profound implications for the quality of OM as a food source to the rest of the benthic community. In this way, *Linopherus sp.* has a large potential effect on its chemical and biological surroundings and the decay and preservation of OM.

In contrast, at the 850m site, *Linopherus sp* was less of an important constituent of the benthic community. Here *Linopherus sp* still dominated the biomass, but the presence of a prionospio polychaete that was extremely efficient at fresh OM uptake, reduced the percentage of label uptake accounted for by *Linopherus sp.* to only 36%. Thus, at the 850m site, the prionospio polychaete could also be called a keystone taxon.

The foraminiferan *Uvigerina sp.* was a keystone taxon at the 300m site, and at the 140m site in the post-monsoon season. In both of these cases, the foraminifera dominated uptake. *Uvigerina sp.* constituteed 40-55% of the biomass and 65-80% of label uptake in these experiments, and thus effectively out-competed other species of foraminifera for food, and possibly suppressed their populations. At the 140m site

Uvigerina sp. were responsible for the majority of foraminiferal label uptake (Fig. 4.6 and appendix B), and were thus key organisms involved in the observed switch from macrofaunal to foraminiferal domination of OM uptake under low oxygen conditions. Previous work is consistent with the positing of *Uvigerina sp.* as a keystone taxon, as calcareous foraminifera in general, and *Uvigerina* in particular, have been shown to be capable of feeding selectively on fresh OM (Kitazato et al., 2000 Nomaki et al., 2005 b).

The keystone taxa identified in this study were either large and abundant, and / or capable of efficient location and uptake of fresh OM (Miller et al., 2000, Levin et al., 1997). Abundance and feeding preferences may therefore be used to predict which species will play key roles in other environments. Making such predictions would be far from straightforward, however, as the functioning of a species as a keystone taxon depends not only on the species itself, but also upon its interactions with, and effects upon, the rest of the benthic community.

A prediction that arises from this study is that where cirratulids dominate the macrofaunal community they may be a keystone taxon. They were observed here to be efficient at OM uptake (they consumed more than their fair share), but were only ever a minor component of the benthic biomass, and thus did not play a particularly significant role in OM processing. In other environments they may however dominate the biomass, and would then be expected to play key roles in sediment C-cycling.

In summary, the significance of a particular species in OM cycling at a particular site is dependent not only upon its predisposition and ability to actively seek and consume fresh OM, but also on its abundance relative to other species. On the Pakistan margin, several crustacean and polychaete taxa were found to be efficient consumers of fresh OM. The amphinomid *Linopherus sp* was found not to be an efficient consumer of OM, as the foraminiferan *Uvigerina sp.* was, but both of these taxa were shown to be remarkably dominant in terms of biomass and OM uptake at sites where they were present.

4.4.8 Feeding Guilds

Several taxa, including the Cirratulidae, Ampharetidae and Paraonidae were found to be relatively efficient at the location and uptake of fresh OM (Fig. 4.6, appendix B). This is consistent with previous findings about the feeding behaviour and selectivity of these families. Although there have been few studies, covering few (and rarely deep-sea) species of each family (e.g. Maurer and Leathem, 1981, Carrasco and Carbajal, 1998), it is highly likely that cirratulids, ampharetids and paraonids are all deposit feeders, which, despite showing some burrowing activities, feed predominantly at the surface. They are also all thought to be capable of selective ingestion, favouring fresh algal detritus, although this is rather less certain for the paranoids than for the other families (Fauchald and Jumars, 1979, and references therein).

The amphinomid *Linopherus sp*, found at the 850m and 940m sites, was observed to consume less than its fair share of labelled OM. Amphinomids living on hard substrates are often carnivorous. This feeding strategy is however unlikely to be adopted in the soft sediments of the deep sea, where a carrion-feeding or scavenging strategy is more likely (Fauchald and Jumars, 1979, and references therein). This, once again is to some extent consistent with the relatively low efficiency with which *Linopherus sp* ingested labelled algae. Visual observations of some *Linopherus sp* guts full of algae suggest that it feeds in an opportunistic way, and this, together with its natural stable isotopic composition, suggest it is better described as an omnivore (Rachel Jeffreys and Lisa Levin, pers. comm.).

The Cossura and Nephtyidae families were observed to consume less than their 'fair share' of algal OM, and this also ties into their previously established feeding guilds. The first of these families feeds from the sub-surface and therefore would have had severely reduced and delayed access to the added label. The second is often carnivorous, but this strategy is likely to be replaced in the deep sea by carrion-feeding, scavenging or omnivory (Fauchald and Jumars, 1979, and references therein), suggesting it may only have access to the labelled algae through consumption of the tissues of primary consumers.

The foraminiferan *Uvigerina sp.* was observed to dominate foraminiferal OM uptake, especially at the 300m site and (post-monsoon) at the 140m site. The particular

propensity of Uvigerina to consume freshly-deposited OM has been previously observed in in-situ and microcosm feeding experiments, and has been attributed to its relatively shallow living depth (within the surface 1cm) and food preferences (Nomaki et al., 2005 a, b). Other studies have also found that calcareous foraminifera, the group to which *Uvigerina sp.* belongs, are more capable of selective feeding than the agglutinated foraminifera (such as bathysiphon, observed to consume less than its fair share in this study) (Suhr et al., 2003).

Thus, the results of isotope tracing in this study are consistent with previous findings on the feeding biology of the main taxa present, and serve as important direct support for those findings.

4.4.9 Vertical Distribution of Fauna

The vertical distributions of labelled fauna at experiment terminations showed that at the 940m site, a relatively large proportion of the benthic community travelled to between 2 and 5 cm depth (compared to the 140m and 1850m site macrofauna), but did not go any deeper. In contrast, at the 140m site, a smaller percentage of labelled fauna were found below 2 cm, but some of these burrowing organisms penetrated below 5 cm depth (Fig. 4.5). At the 1200m and 1850m sites labelled fauna were rarely found below 2 cm depth. The labelled fauna found between 2 and 5 cm depth at the 940m site were predominantly the amphinomid *Linopherus sp*, while the fauna found deep (2-10 cm) in the sediment at the 140m site were cirratulids, ampharetids and maldanids, thus the different burrowing patterns were likely due to variations in benthic community composition. Labelled fauna vertical distribution patterns at these two sites were consistent with sediment X-radiographs (Fig. 4.7), which showed well-mixed sediment to at least 10 cm at the 140m site, and discrete burrows which terminated at a dense, turbidite layer (5-6 cm) at the 940m site.

Fauna in experimental cores may not have showed their natural depth distributions, as the introduction of OM at the surface may have prompted upwards migration. This stimulus was however equally applied in all experiments.

The shallower maximum depth of vertical movement at the 940m site may have been due to the lower bottom water oxygen concentrations there, compared to the 140m site in the pre-monsoon season. Burrowing fauna have been observed to migrate

upwards and even emerge from the sediment in response to falling oxygen levels (Nilsson and Rosenberg, 1994, and personal observations in experiment cores that went anoxic). This leads to the expectation that the depth of vertical movement at the 140m site in the post-monsoon season should have been reduced, in response to the observed seasonal reduction in oxygen concentration. However, similar proportions of label were found at depth at the 140m site in both seasons (Fig. 4.5), thus, the primary effect of reduced oxygen availability appears to have been a suppression of macrofaunal activity, rather than a reduction in burrowing depth.

4.4.10 Bioturbation

The vertical distribution of labelled fauna can only reveal details of the movement of the fauna, and not the depth or magnitude of biomixing that they carry out. The observed general lack of sub-surface $\delta^{13}C$ enrichment of bulk sediments, and of sub-surface $\delta^{13}C$ peaks (Fig. 4.6) suggests that most fauna fed and egested close to the surface, and thus caused minimal vertical OM movement within the time frame of the experiments.

Sediment x-radiographs from the Pakistan margin showed that, at the 140m, 1200m and 1850m sites, the sediments were well mixed (Fig. 4.7). The rate at which this bioturbation occurs must however be relatively slow, as, due partly to dilution and the obscuring effect of smearing, it was rarely possible to resolve it above experimental artefact in isotopic tracer experiments lasting 2-5 days.

Sediment x-radiographs of the 1200m and 1850m sites (Fig. 4.7) displayed such a high degree of biomixing that individual biological structures could not be counted. The lack of measurable bioturbation at these sites is thus particularly incongruous. The relatively large animals thought to cause such intense and deep mixing (ca. 1cm-wide burrows were observed to > 15cm at the 1850m site) were never recovered in megacores, however, and therefore were probably not present in labelling experiments. This may account for the lack of vertical label movement (and possibly for low overall label uptake) observed at these sites, but does not account for the lack of bioturbation signal at the 140m and 940m sites.

The lack of measurable biomixing puts the Pakistan margin in stark contrast with the sites of other isotope labelling experiments, where considerable and deep (~10cm)

sub-surface peaks have been observed to form over similar experiment durations (Blair et al., 1996, Thomas and Blair, 2002, Levin et al., 1997, Moens et al., 2002, Sun et al., 1999). The majority of these studies (excluding Moens et al., 2002 and Sun et al., 1999) were carried out on the oxygenated continental slope of North Carolina, in open, in-situ experiments, and in comparable water depths to those in this study. Thus, the amount of bioturbation observed here is comparatively low. This is likely to be partly due to the different faunal communities found on the Pakistan margin compared to the Carolina margin, where most vertical label transport was carried out by abundant large maldanids, which were not present in this study. A further possible explanation for the general lack of measurable penetration of label to depth in and around the OMZ on the Pakistan margin may be the focussing of hypoxia-resistant fauna close to the sediment-water interface by low oxygen levels and shallow sediment oxygen penetration depths. This is consistent with the rather less abundant faunal community found on this margin compared to that on the Oman margin (Levin et al., 2000), where bottom waters are better ventilated and sufficient deep bioturbation occurs, even within the OMZ, to eliminate sediment laminations. A further possible explanation for the lack of observed vertical label transport is that the availability of introduced OM at the surface may have resulted in fauna altering their behaviour to remain close to the source of food.

4.4.11 Further Work

This study, together with previous work, has furthered our understanding of the role of fauna in early OM processing, however the experiments performed to date have their shortcomings, and these lead to gaps in our knowledge.

Future studies should be conducted in situ where possible, and should be of extended duration, so that early faunal OM processing can be linked to the long-term fate of OM in terms of burial and decay. These extended durations may require the use of laboratory or shipboard incubation methods. Further experiments should be conducted in an ever-wider range of environments in a way that allows direct comparisons, with the eventual goal of producing sufficient information to make accurate generalisations about the whole seafloor. In addition, the effects of the least well described faunal process, digestion, must be further investigated, and properly

linked to sediment organic geochemistry. Finally, a detailed understanding of the eventual fate of the OM processed by fauna would be aided by the devising of a way of directly observing which fauna are responsible for measured respiration, as this would make the current reliance on theoretical respiration rates unnecessary.

This work, and the improved process modelling which may arise from it, would benefit from an improved knowledge of the biology of benthic communities, including feeding rates and gut turnover times. This information is only available for a restricted selection of taxa, most of which are megafauna from shallow water environments.

4.5 Conclusions

- The uptake of fresh OM by macrofauna and foraminifera are significant processes in the short-term processing of OM even when compared with bacterial uptake and total respiration.
- Oxygen availability controls the faunal uptake of OM in a threshold manner. Above the threshold macrofauna dominate, and below the threshold foraminifera dominate.
- The natural availability of high-quality OM, where oxygen levels are sufficient, allows macrofaunal communities to flourish, and dominate OM processing.
- The exact composition of the benthic community influences the role that fauna play in early OM uptake and cycling. Where macrofauna are present, total faunal uptake tends to be enhanced
- Greater natural availability of high-quality OM produces benthic communities that are more prone to rapid and efficient uptake of OM.
- The predisposition of the various taxa studied to ingest fresh OM was consistent with their previously established feeding modes.
- A number of keystone taxa have been identified, marked out by their predisposition to consume fresh OM, and/or by their size and abundance.

CHAPTER 5

The Effect Of Macrofaunal Gut Passage On Sediment Geochemistry; Results Of An Amino Acid Tracing Study

5.1 Introduction

In many seafloor settings it is thought that as much as 10-30% of freshly deposited organic matter (OM) may pass through the guts of key macro- and megafauna before it becomes available to other members of the benthic community (Miller et al., 2000), and eventually all residual OM may be processed in this way (Lauerman et al., 1997). Internal digestion, involving specific acids and enzymes, and assimilation processes, is designed to bring about biochemical change of gut contents for the benefit of the organism (i.e. hydrolytic breakdown of polymers into compounds amenable to assimilation, Michael and DeVillez, 1978). Enzyme concentrations in polychaete guts can be as much as five orders of magnitude greater than in the surrounding sediment, and this is possible as they are retained efficiently within the gut and not lost to the environment like bacterial extracellular enzymes (Mayer et al., 1995). Such organisms therefore are likely to strongly influence the composition and food quality of the OM made available to the wider benthic community, and are the agents of one of the earliest stages of diagenetic alteration. The implications of this for other fauna and for OM preservation will depend on the composition change occurring during gut passage.

Polychaetes and other widespread sediment dwellers have been observed to utilise a wide range of food sources with varying selectivity, and at varying depths in the sediment (e.g. Fauchald and Jumars, 1979). Different fauna have been found to have different metabolic process and gut chemistries (Mayer et al., 1997), and to require different suites of biochemicals from their diets (Meister, 1965). Gut architecture has also been observed to vary among taxa, and this leads to variation in particle mixing during gut passage, and thus in digestion and assimilation efficiency (Penry, 1989). Together, these species-specific factors suggest that the influence of faunal OM processing on sediment geochemistry could potentially vary widely among locations and taxa, and the implications of this for bulk OM preservation are unclear.

Efforts to gain a full understanding of sediment OM cycling, and the factors influencing OM burial and the make-up of sedimentary records, have long been hampered by the largely un-characterisable nature of refractory sedimentary OM, and lack a of molecular-level information on key diagenetic transformation processes.

An approach to deducing the composition or biochemical provenance of uncharacterisable humic matter would be to follow fresh OM through early diagenesis, to study those compounds that are preferentially lost, as well as those that are recalcitrant and thus likely to be incorporated into geopolymers. By tracking OM through early diagenetic transformation processes, the appearance of the products of partial metabolic alteration or *de novo* synthesis may also be monitored. Such tracing requires a means of distinguishing between naturally present OM and that which is added in an experiment. Stable isotopic labelling provides for this need, and newly- developed analytical techniques now allow detection of labelled OM at the biochemical level.

A limited number of studies have used isotopically labelled algal detritus and biochemical analysis to trace the fate of fresh carbon on a detailed level. Sun et al. (1999) were able to identify new lipid compounds generated in the presence of macrofauna, and used the loss of labelled lipids to show that the presence of macrofauna tended to enhance overall rates of OM decay. Thomas and Blair (2002) used feeding studies to show how the suite of amino acids present in fresh algae was altered in animal tissues and faecal pellets. These studies were however conducted in microcosm and on a limited number of species.

Some of the remaining questions are:

1. What are the bulk and molecular-level compositional changes associated with OM transformation by benthic faunal digestive processes?
2. Can biochemical changes observed in labelling experiments be shown to lead to compositions characteristic of decay typically found in natural seafloor sediments?
3. Is the biochemical change that occurs during gut passage species-specific, and therefore is the composition of OM made available to the wider benthic community dependent on the keystone taxa present?
4. Apart from species, what governs the amount and nature of biochemical change occurring during gut passage, and can this be predicted?

During OM sinking and deposition (i.e. early diagenesis), organic nitrogen in general, and amino acids in particular, have been observed to represent high food quality OM components, and to be comparatively labile (Cowie et al., 1992, Cowie and Hedges, 1996). Notably, this degradation appears to occur in a systematic pattern, with loss of intracellular amino acids, and preservation of those associated with cell walls. Principal component analysis of the amino acid suites of sediments has been used to produce an index of OM degradation state (Dauwe and Middelburg, 1998, Dauwe et al.,1999). This index was made possible by the diverse structural and chemical natures of the 20 protein amino acids (and several non-protein ones), and has proved widely applicable due to the abundance and ubiquity of amino acids in marine fauna and sediments. In addition, although proteins are observed to be one of the more labile fractions of sedimentary OM, NMR studies of un-characterisable humic matter have shown that a large proportion of un-characterisable organic nitrogen may be present in the form of amine groups (Zang et al., 2000), and experiments have shown that amino acids readily form melanoidins, possible pre-cursors to humic matter (Hedges, 1978). All of these features of the amino acids and their geochemistry make them a logical target for the study of biochemical alteration during digestion.

By analogy with documented amino acid compositional changes during OM decay, it may be expected that proteins and component amino acids will be subject to significant alteration during faunal digestion. Conversely, since proteins are major and fundamental components of most organisms, it might be predicted that amino acid requirements will be relatively invariant among different taxa. Thus, one might expect amino acids to be assimilated in the same proportions in which they are present in the food source. However, the nature and degree of protein (or other biochemical) alteration during benthic invertebrate gut passage remains a largely open question.

Previous studies on this topic have been rare and, due to analytical difficulties, almost never fully quantitative. The complex nature of the experimental work has resulted in most experiments being performed in artificial microcosms and on individual species (e.g. Thomas and Blair, 2002, Sun, 2000). The consequence is that the overall effects of benthic fauna on sedimentary OM alteration, including

collective digestive effects, as well as possible interspecies enhancement or inhibition, remain poorly characterised or quantified. Even the effects of individual fauna remain uncertain due to possible artefacts caused by removal of animals from their natural environment.

In summary, we have relatively little knowledge on the impact of faunal digestion on sediment geochemistry. Studies of sediments and holothurian gut contents from the Procupine Abyssal Plain, and the Arabian Sea have suggested that assimilation of certain sterols by holothurians was the reason for their low abundance in the sediment (Horsfall and Wolff, 1997, Ginger et al., 2001). Microcosm studies of individual taxa fed with ^{13}C labelled algae have shown accelerated decay rates and production of new compounds of lipids, and alteration of the suite of amino acids present in digested OM (Sun, 2000, Thomas and Blair, 2002). While the impact of faunal activities such as bioturbation / irrigation and digestion have repeatedly been shown to accelerate the decay of various classes of biochemicals (e.g. Sun et al., 2002, Sun 2000, Bianchi et al., 2000), the impact of such processes on the biochemistry of ingested and subsequently egested OM is not clear. Where biochemical alteration during digestion has been investigated, microcosm work with single taxa has inhibited the making of links between observed alterations and ambient sediment biogeochemistry, and a holistic view of the impact of digestion on sediment composition has so far been hampered by the fact that usually only one biochemical class is studied at once.

This aim of the present study was to quantitatively investigate the effects of benthic invertebrate gut passage on sedimentary OM amino acid composition. Previous limitations were addressed by conducting tracer experiments, on whole, undisturbed, faunal communities. In addition, this study paralleled a study tracing lipids, and was supported by analyses of natural and labelled sediment, so that for the first time, a wide range of biochemicals were examined, and a direct link with sediment biogeochemistry was made.

5.2 Methods

5.2.1 Field Area

Experiments were conducted at depths of 140m, 300m, 850m and 940m along an offshore transect of the Pakistan Margin of the Arabian Sea, before and after the summer monsoon of 2003. This area is subject to monsoon-driven upwelling and intense productivity, which, coupled with limited mid-water ventilation, produces a permanent oxygen minimum zone (OMZ) between depths of ~150m and 1000m (Sarma, 2002). Where low-oxygen waters impinge on the continental margin the sediments exhibit impoverished faunal communities and organic matter enrichment (Cowie, 2005). The sites used in this study were at the upper (140m) and lower (850m, 940m) margins of the OMZ, where macrofauna were present and abundant, and at the core of the OMZ (300m), where macrofauna were absent and only foraminifera were found. Bottom-water oxygen levels were 0.13-0.16 mlL^{-1} at the deeper two sites (850m and 940m), and 0.11 mlL^{-1} in the core of the OMZ (300m). The bottom-water dissolved oxygen concentration at the 140m site before the monsoon was 2.01 mlL^{-1}, but following the summer monsoon this fell to 0.11mlL^{-1}, due to a monsoon-induced shoaling of the OMZ upper boundary (Table 5.1A).

5.2.2 Experimental

^{13}C enrichment experiments were carried out both aboard ship using incubated megacores, and *in situ* using a benthic chamber lander. In all cases, ~80% ^{13}C-labelled algae, freeze-dried onto silica or kaolinite ballast, was added to intact sediment (megacores or *in situ*), giving a dose of 200-700mg ^{13}C per m^2 (constant within related experiments and between replicates). Sediment was incubated in the dark, at seafloor temperature, and with ambient oxygen levels maintained for 2-5 days. Experiments were terminated when cores (megacores, or core sub-samples of the lander chamber) were extruded and sectioned. Fauna were extracted by sieving sediment through a 300μm mesh using filtered seawater, followed by picking under 10-20x magnification. Sediment samples were frozen at -20°C.

Fauna samples were washed in distilled water, and placed in pre-weighed tin capsules or pre-combusted glass vials. No attempt was made to let the fauna void

Site	Experiment Type	Duration (h)	Label Added (mg ^{13}C m^2)	Site Temperature (°C)	Dissolved Oxygen Concentration (ml L^{-1})	Sediment %Corg	OM Quality (DI)	Macrofauna Biomass g wet m^{-2} / Diversity	Foraminifera Density / Diversity
Pre-Monsoon									
140	2 day	68	535	22.5	2.05	1.46 ± 0.08		9.2 / 51 (± 5)	593 / 19
850	2 day	46	695	9.7	0.13	3.22 ± 0.06			
940	5 day	112	471	9.0	0.13	3.31 ± 0.12		62.2 (± 45.3) / 12 (± 1)	80 / 13
Post-Monsoon									
140	2 day	44	473	18.2	0.11	1.43 ± 0.07	-0.99 ± 0.06	4.6 (± 2.2) / 45 (± 3)	1163 / 20
140	5 day	118	465	18.2	0.11	1.43 ± 0.07	-0.99 ± 0.06	4.6 (± 2.2) / 45 (± 3)	1163 / 20
300	5 day	155	470	14.8	0.11	2.56 ± 0.29	-0.40 ± 0.12	0.013 (± 0.019) / 1	839 / 14
940	5 day	113	477	9.3	0.17	3.40 ± 0.13	-0.48 ± 0.03	45.7 (± 0.019) / 13 (± 1)	
940	2 day *in situ*	48	216	9.3	0.17	3.40 ± 0.13	-0.48 ± 0.03	45.7 (± 0.019) / 13 (± 1)	

Table 5.1A. Summary of experiments conducted at all sites in both seasons, with exact durations and quantities of ^{13}C added and site conditions. Oxygen concentrations are from CTD casts, %Corg values are for the surface 0-0.5 cm, DI values are averaged over the surface 3 cm. Macrofauna diversity is species number per megacore averaged from 5 cores (Peter Lamont, pers.comm.), foraminifera density data are total number of calcareous individuals in 15 cm^2 of the surface 1 cm of sediment, and diversity data are species number for the same sample volume (Stefanie Schumacher, pers. comm.).

Site	Experiment Type	List of Specimens
140	2 day	Amphuipod (0.25), Ampelisca (0.25, 1.75), Polychaete unknown x 2 (0.25, 0.75), Gastropod (0.25), Maldanid (6.0), Cossura (2.5), Nephtyidae (0.75), Sea Urchin (0.25)
850	2 day	Ampelisca (0.75), *Linopherus sp.* x 4 (0.13, 1.75, 2.5 in 2 splits, 3.5 in 2 splits), Prionospio (0.75, 2.5)
940	5 day	Bivalve (0.25), *Linopherus sp.* x 6 (1.25, 1.25 in 2 splits, 1.75, 2.5 in 3 splits, 3.5, 3.5)
140	2 day	Gastropod (0.25), 3 x Cirratulidae (0.75, 1.75, 4.5), Polychaete unknown (0.75), Uvigerina 8 samples of 40-80 individuals (6 from 0.5, 2 from 0.25)
140	5 day	Gastropod (0.25), Cirratulidae x 6 (0.75, 1.25, 2.5, 3.5, 4.5, 8.5), Polychaete unknown (1.5)
300	5 day	Uvigerina, 1 sample of 55 individuals (from 0.5)
940	5 day	Cirratulidae (0.25, 3 splits), *Linopherus sp.* x 3 (2.5 in 2 splits, 3.5, 3.5)
940	2 day *in situ*	*Linopherus sp.* x 3 (1.25, 1.75, 2.5), Macrochaetae (0.75, 1.25, 2.5)

Table 5.1B. List of specimens analysed from each experiment (depth of specimen in cm).

their guts, as most fauna were too small to make collection of separate gut contents feasible.

On return to the laboratory, fauna samples were air dried at 45°C and re-weighed. Sediments were freeze-dried.

Time and equipment constraints dictated that we were unable to perform the full suite of experiments (2- and 5-day shipboard, and a 2-day in-situ experiments) at all sites in both seasons, the and data is discussed with differences in experiment duration in mind. A summary of experiments from which fauna were analysed for labelled amino acids, together with the specimens analysed from each experiment is given in Table 5.1B.

5.2.3 Analytical

Amino acid analysis was preceded by extraction of lipids from the fauna samples by sonication in 9:1 methylene chloride:methanol (v/v) for 30 minutes at room temperature. Amino acid hydrolysis of both fauna and sediments was conducted in double distilled 6N HCl under an N_2 atmosphere for 70 minutes at 150°C, after which charge-matched internal standards (α-aminoadipic acid, norleucine and hydroxylysine) were added and samples were dried. Hydrolysates were then passed through 2ml of cation exchange resin to remove interfering cations (Dowex 50Wx8 50-100 mesh (H^+ form), and amino acids were eluted with 4N NH_4OH. Samples were dried overnight in a centrifugal evaporator, then converted to their trifluoroacetyl isopropyl esters (Darbre and Islam, 1968, Mabbott, 1990), and analysed on a HP 6890 gas chromatograph (GC) equipped with a Sulpelco Equity 5 column (30m x 0.32mm x 0.25μm film thickness, bonded poly[5%diphenyl/95%dimethylsiloxane] stationary phase). The GC oven was held at an initial temperature of 35°C for 1.5 minutes, before being ramped to 100°C at 5.5°C/min, then at 4°C/min to 190°C. Following this, a ramp at 70°C/min to 280°C and a 5 min hold were used to bake the column before the oven returned to 35°C at 30°C/min. This downward temperature ramp, together with a filament-off event when all analytes had eluted, allowed the CI source to cool to its setpoint of 136°C before the start of the next run. The He carrier gas flow rate was 1.5 ml min^{-1}, and the instrument was set in constant flow mode.

Detection was with a HP 5973 Mass Selective Detector (MSD) in positive chemical ionisation mode, with methane as the reagent gas, and using selective ion monitoring (SIM) (for full protocol see chapter 3). A small number of samples were run with the detector in scan mode, as migrating retention times in late peaks caused analytes to elute outside their SIM windows. Trimming the top of the column solved this problem, and most samples were run with the detector in SIM mode.

5.2.3.1 Quantification of ^{13}C-labelled Amino Acids

Labelled compounds were identified using their fully labelled quasi-molecular ions (Dallakian and Budzikiewicz, 1997) that were *n* mass units heavier than the natural version of the ion, where *n* is the number of carbon atoms in the amino acid. In the absence of labelled standards, ^{13}C amino acids were quantified by comparison with their natural abundance equivalents using a calibration technique and corrections described by Sun (2000). Due to a threshold type response of the MSD at low injected analyte amounts, glutamic acid and lysine were often not reliably calibrated, and that data is not reported. Labelled tyrosine also suffered from this problem for a small number of samples, but this had a relatively low impact on data interpretation, and tyrosine data is reported for all samples. For a full discussion of calibration and quantification, see chapter 3.

5.2.4 Data Processing

Amino acid mole percentages (mole %) were calculated by considering the ^{13}C-labelled amino acid suites and the naturally occurring (unlabelled) suites separately, and are discussed separately hereafter. Mole % data was calculated on a glutamic-acid and lysine-free basis, as for some samples calibration of these compounds was problematic.

A parameter to describe the degree of ^{13}C-glycine enrichment in samples (glycine enrichment factor) was calculated by subtracting the mole% of ^{13}C-glycine in the relevant source algae from that in the sample. This parameter was always calculated on a glutamic acid and lysine-free basis.

Absolute yields are presented as moles (of each amino acid) per mg of dry tissue or sediment. Wherever total amino acid yields are given, the moles of each separate compound per mg of tissue (or sediment) were summed.

Principal component analyses were conducted on sub-sets of both ^{13}C-labelled and natural glutamic acid- and lysine-free mole% data.

Correlation analyses were performed using the Microsoft Excel data analysis tool. Correlation coefficients (ρ) thus produced represent the covariance of any two data sets, divided by the product of their standard deviations. For an analysis where n = 9 (as an example), the 5 % significance level for ρ is 0.60, and this reduces with higher values of n.

Student's t-tests were occasionally performed to determine whether the difference between two data sets was significant. Where this was done, a value of P is given. A value for P of 0.05 or less indicates a significant difference at the 5% probability level.

5.3 Results: Fauna

Benthic faunal community structure varied dramatically across the margin in response to steep gradients in bottom water oxygen concentration and temperature, sediment OM quality and abundance, and water depth. At the shallow 140m site, both macrofauna and foraminifera were abundant and diverse (Table 5.1). This site had the highest macrofauna abundance in terms of numbers of individuals per m^2, but lower macrofaunal biomass than the 940m and 850m sites. Diversity was also highest at the 140m site, both within the polychaetes, and between classes, exhibiting as it did many crustaceans, molluscs and echinoderms. The 940m and 850m sites both showed exceptionally high macrofaunal biomass, and low abundances of foraminifera. At these sites diversity was reduced and the polychaetes dominated. The polychaete *Linopherus sp.* was overwhelmingly dominant at the 940m site, but was equalled by a Prionospio polychaete at the 850m site, illustrating how steep gradients in bottom water dissolved oxygen and sediment OM quality were responsible for dramatic benthic community variations over small distances at the OMZ lower boundary. At the 300m site, extremely low oxygen availability dictated that the macrofauna were almost absent, and the community consisted almost exclusively of foraminifera. Here diversity was particularly low, and both here and at the 140m site *Uvigerina sp.* was the dominant taxon (Peter Lamont, Lisa Levin, Stefanie Schumacher, pers. comm.). Sediment texture followed the abundance of

macrofauna, with mixed sediments above and below the OMZ, and laminated sediment within it (Lisa Levin, pers. comm.).

Biochemical analysis was limited to taxa that were particularly abundant in the experiments in question. To a large degree, this produced good comparability of the taxa analysed between different experiments from the same site. In the case of some taxa, particularly the cirratulids, this also allowed comparison between sites. In some cases however, the natural patchiness of faunal communities meant that two experiments from the same site yielded slightly different 'communities' of specimens for biochemical analysis. Thus, at the 940m site in the post-monsoon season, the 5-day shipboard experiment produced samples of *Linopherus sp.* and Cirratulidae polychaetes, whereas the in-situ experiment allowed analysis of specimens of *Linopherus sp. sp.* and Macrochaetae.

Amino Acid	^{13}C-Labelled Amino Acids	Natural Amino Acids
Alanine	7.8	4.2
Glycine	7.9	3.0
Threonine	10.8	9.1
β-Alanine	15.0	6.3
Serine	12.5	7.0
Valine	5.5	5.7
Leucine	8.1	4.7
Isoleucine	10.2	5.0
Proline	23.0	9.0
Methionine	28.2	16.9
Phenylalanine	32.8	7.6
Tyrosine	38.2	24.2
Aspartic Acid	47.1	14.5
Ornithine	28.0	29.8

Table 5.2. Average relative standard deviations of natural and ^{13}C-labelled amino acid data, derived from 6 samples, each of which was run as two or three splits.

The main taxa examined in this study were two polychaete groups Cirratulidae and *Linopherus sp.*, and the foraminiferan *Uvigerina sp.* The cirratulids are free-living burrowing polychaetes. They are surface deposit feeders, are capable of feeding selectively on fresh organic detritus, and have relatively simple gut architecture. *Linopherus sp.* is larger than the cirratulids found on the Pakistan margin, and tends to form more permanent burrows / tubes. It is likely to be omnivorous or a scavenger, and may not be so prone to actively selecting fresh OM for ingestion as the cirratulids (Fauchald and Jumars, 1979, Lisa Levin, pers. comm.). *Uvigerina sp.* is a calcareous foraminiferan that has previously been observed to feed particularly

voraciously on fresh OM (Kitazato et al., 2000 Nomaki et al., 2005 b). Photographs of these taxa are shown in chapter 2.

5.3.1 Data Quality

In several cases, particularly with large animals from the 850m and 940m sites, a single individual was split into several fragments, which were analysed separately (*Linopherus sp.* A, Fig 5.2A, *Linopherus sp.* B and C, Fig 5.3A, and *Linopherus sp.* A and cirratulid A, Fig 5.4A). Even though it was not expected that these splits would be identical (samples were too small to be homogenised), in most cases they returned very similar data. This similarity between sub-samples of the same individual permits confidence that apparent differences between individuals are real. Relative standard deviations of mole percentage data derived from comparing splits of samples range from 7-47 %, for ^{13}C labelled amino acids, and from 3-30 % for the more abundant, naturally occurring amino acids (Table 5.2). Data from pre- and post-monsoon experiments displayed a systematic offset of ~1 order of magnitude in yields (Figs. 5.5A and B compared to 5.5C and D for example) due to the use of different batches of algae (with different C contents and % ^{13}C labelling) and thus different calibration coefficients. Data from different seasons therefore can be compared only on a mole % basis, and not in terms of absolute yields.

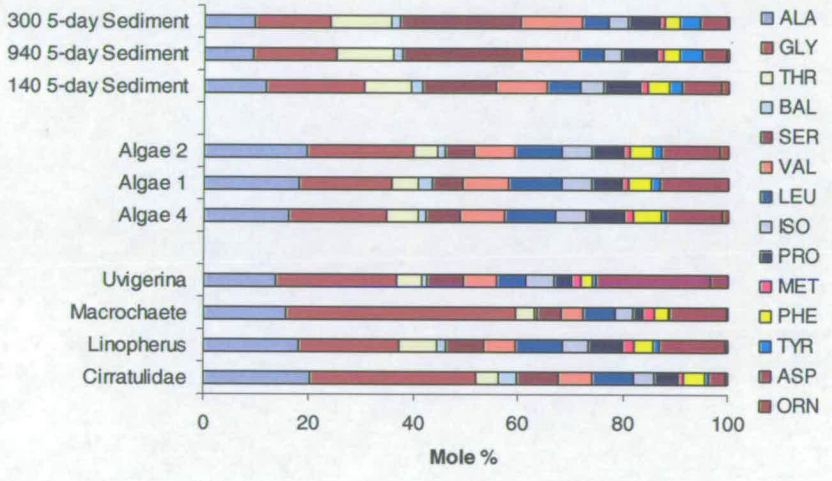
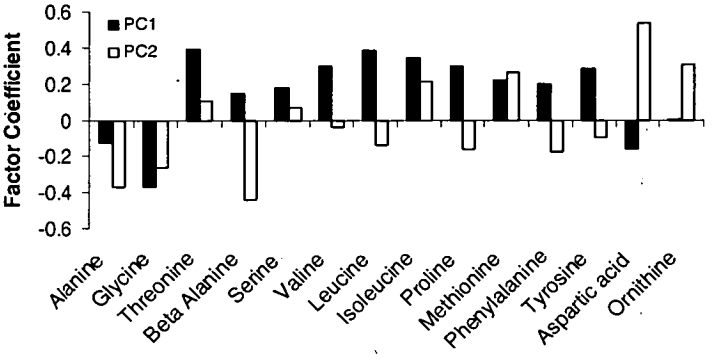


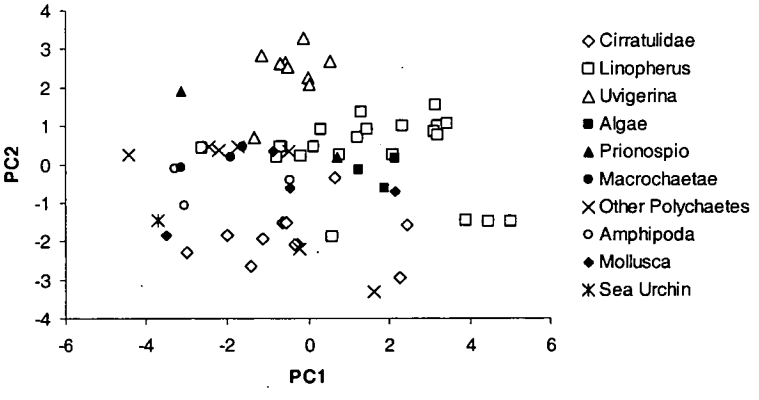
Figure 5.1 Average mole% natural amino acid compositions of sediment, major taxa, and the algae used in labelling experiments. n = 4 for 300 5-day sediment, 7 for 940 5-day sediment, 6 for 140 5-day sediment, 9 for *Uvigerina sp.*, 3 for Macrochaetae, 22 for *Linopherus sp.*, and 12 for Cirratulidae.

5.3.2 Natural Amino Acid Suites

Quantification of natural (unlabelled) amino acids in fauna and algae revealed only subtle differences between fauna (subtler than alterations to the suite of labelled amino acids discussed later) (Fig. 5.1). For all taxa, alanine and glycine were the dominant amino acids, and methionine, phenylalanine and tyrosine were relatively minor. In contrast, the suite of amino acids naturally present in sediments was much less dominated by alanine and glycine, and had less leucine and aspartic acid. Serine, valine and tyrosine constituted a greater proportion of the total amino acids in the sediment than in the fauna.

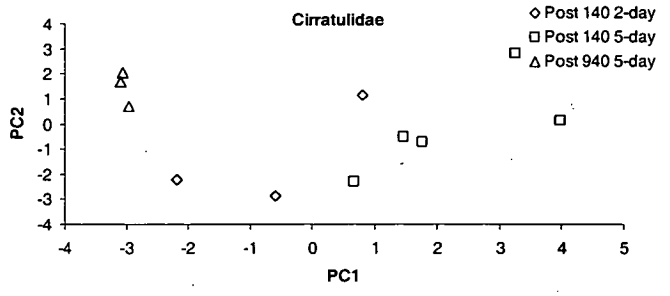


A

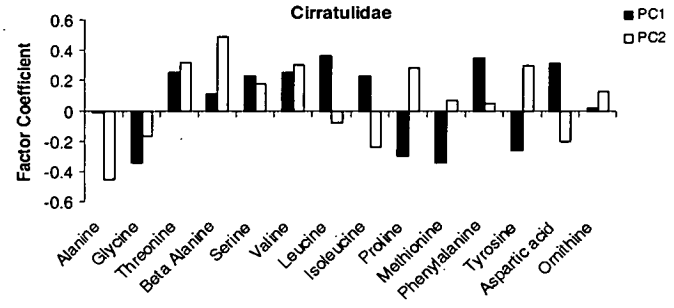


B

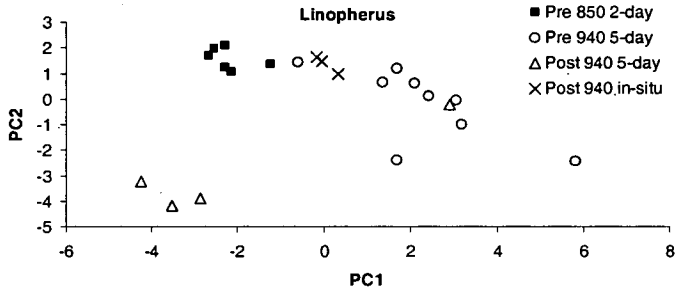
Figure 5.2 A) Principal component 1 and 2 scores derived from the natural amino acid compositions of all fauna. B) Amino acid factor coefficients on the PC1 and PC2 axes for the same PCA.



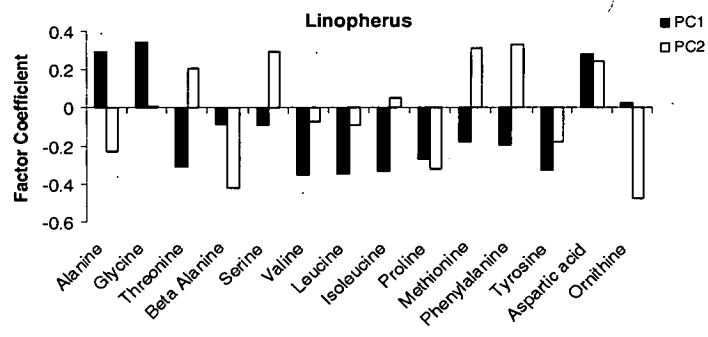
A



B



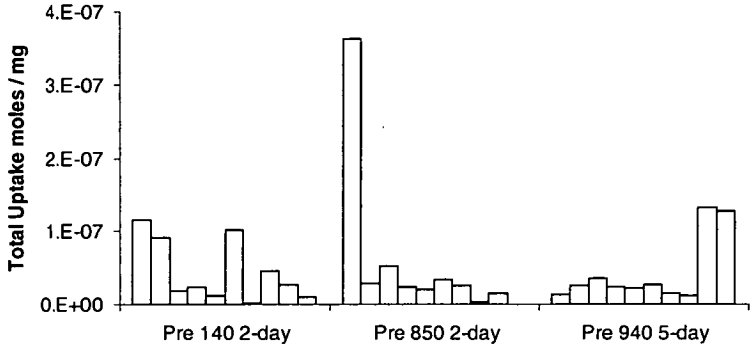
C



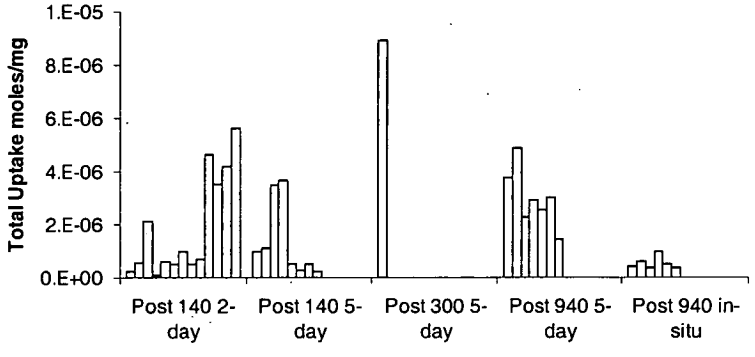
D

Figure 5.3. PC1 and 2 scores (A and C) and amino acid coefficients (B and D) from within taxon PCA of Cirratulidae and *Linopherus sp.* (respectively) natural amino acid data.

Principal component analysis was carried out on the naturally present amino acid suites of all fauna (Fig. 5.2A). Of the three main taxa investigated, the polychaete *Linopherus sp.* had higher PC1 and PC2 scores than the cirratulids, and the



A

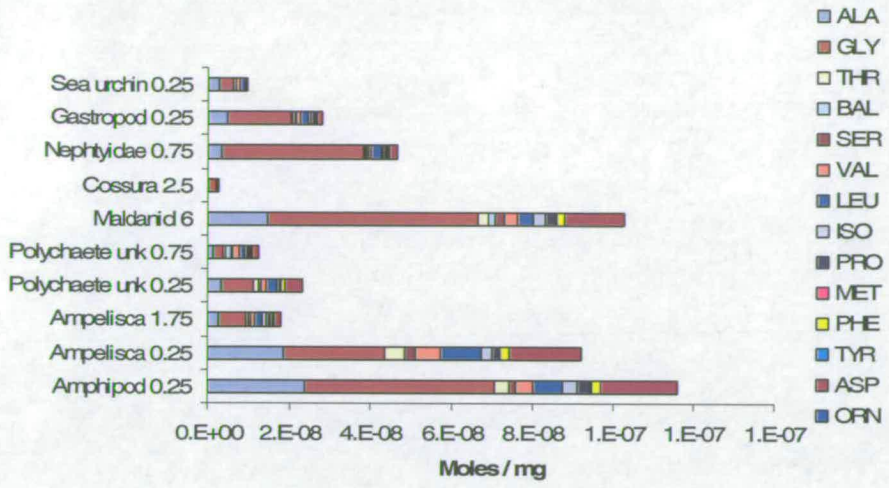


B

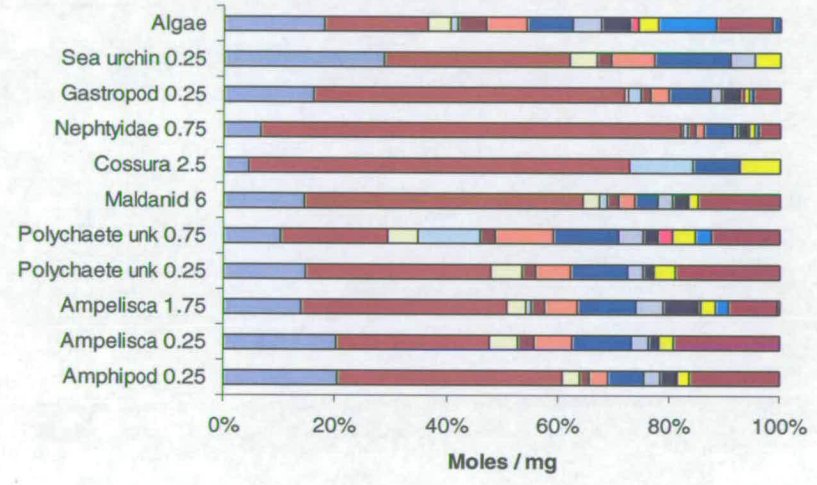
Figure 5.4. Total amino acid uptake in moles / mg of tissue for all samples from each experiment A) pre-monsoon, and B) post-monsoon. Note the different y-axis scales.

foraminifera *Uvigerina sp.* were separated from both by high PC2 scores. High PC1 scores were related to high relative abundances of threonine, leucine and isoleucine, and a relative lack of glycine. High PC2 scores were associated with high relative abundances of aspartic acid, and a relative lack of alanine and β-alanine (Fig. 5.2B). In addition, the Macrochaetae had low PC1 scores and the labelled algae had high PC1 scores, in line with their respectively high and low respective glycine contents (Fig. 5.2).

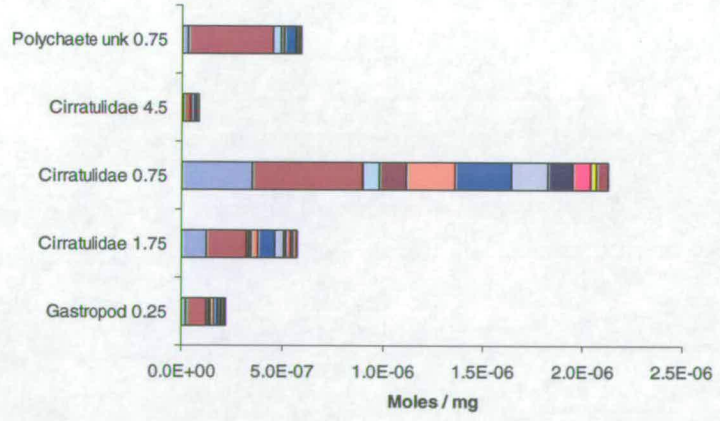
Variation in natural amino acid composition within taxonomic groups was subtler than between them. Cirratulids from the 140m site had higher PC1 scores than those


Sea urchin 0.25
Gastropod 0.25
Nephtyidae 0.75
Cossura 2.5
Maldanid 6
Polychaete unk 0.75
Polychaete unk 0.25
Ampelisca 1.75
Ampelisca 0.25
Amphipod 0.25
0.E+00 2.E-08 4.E-08 6.E-08 8.E-08 1.E-07 1.E-07 1.E-07
Moles / mg
ALA
GLY
THR
BAL
SER
VAL
LEU
ISO
PRO
MET
PHE
TYR
ASP
ORN

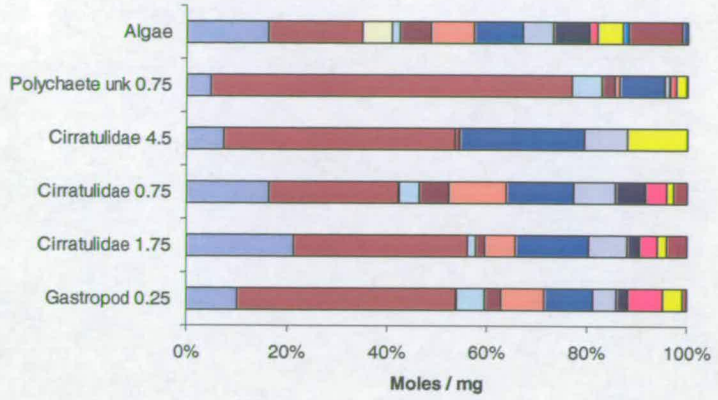
A


Algae
Sea urchin 0.25
Gastropod 0.25
Nephtyidae 0.75
Cossura 2.5
Maldanid 6
Polychaete unk 0.75
Polychaete unk 0.25
Ampelisca 1.75
Ampelisca 0.25
Amphipod 0.25
0%
20%
40%
60%
80%
100%
Moles / mg

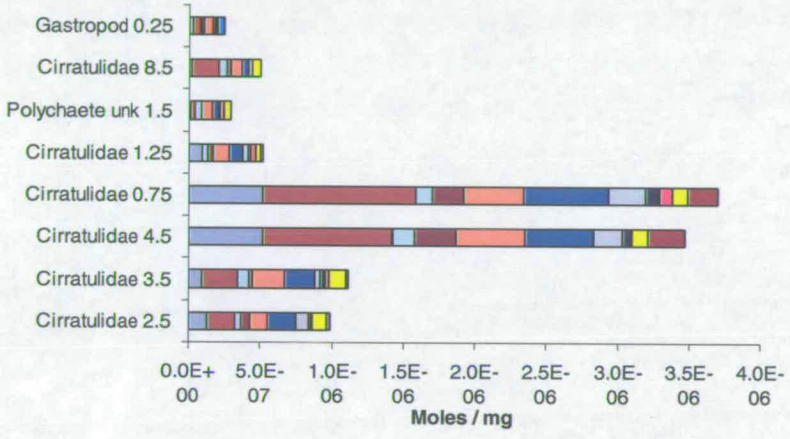
B


Polychaete unk 0.75
Cirratulidae 4.5
Cirratulidae 0.75
Cirratulidae 1.75
Gastropod 0.25
0.0E+00
5.0E-07
1.0E-06
1.5E-06
2.0E-06
2.5E-06
Moles / mg

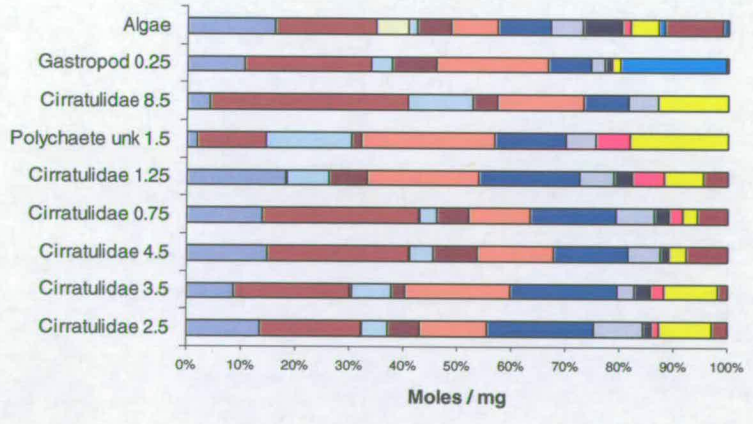
C



D

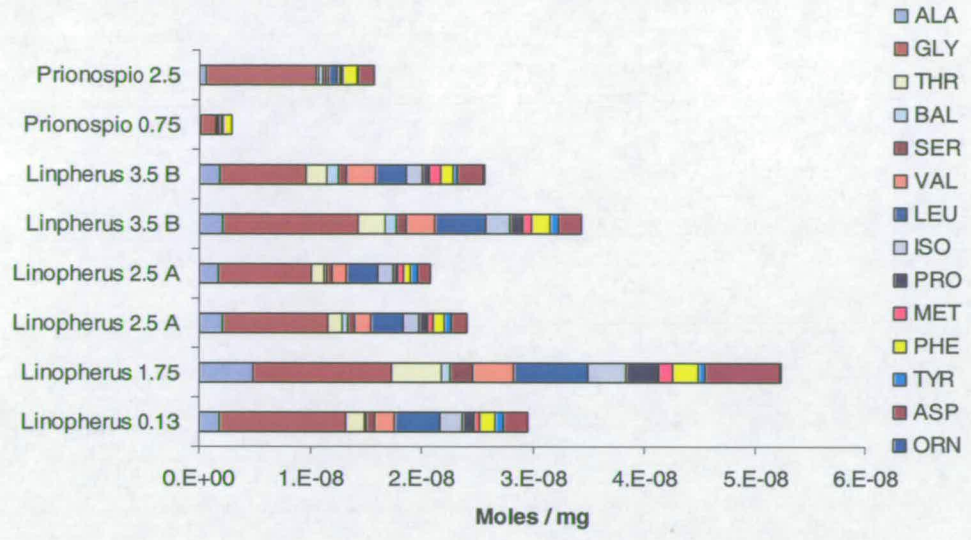


E

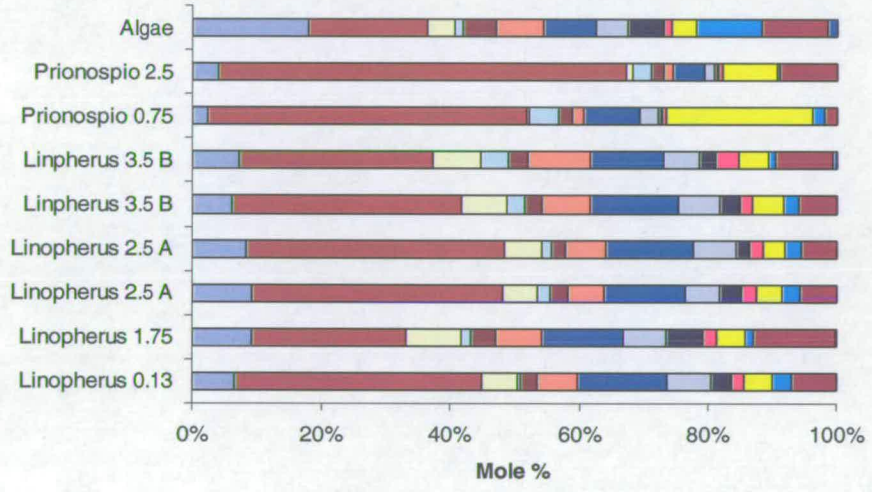


F

Figure 5.5. ^{13}C-labelled amino acid quantities and suites in fauna from 140m site experiments, A and B) pre-monsoon 2-day, C and D) post-monsoon 2-day, E and F) post-monsoon 5-day. The numbers following sample identities indicate the sediment depth (cm) at which samples were found. The key given with A applies to all plots.



A



B

Figure 5.6. ^{13}C-labelled amino acid quantities (A) and suites (B) in fauna from 850m site experiments. The numbers following sample identities indicate the depth (cm) at which samples were found. Where a letter follows sample identities, this indicates splits of the same individual. The key given for A applies to B also.

from the 940m site (Fig. 5.3A), corresponding to higher abundances of leucine and phenylalanine, and less proline, methionine and glycine (Fig. 5.3B). Within the 140m site experiments, specimens from the 5-day experiment had slightly higher PC1 scores than those from the 2-day experiment. This indicates that after 5-days, natural glycine was less significant than after 2 days.

Linopherus sp. compositions varied from high PC1 scores in specimens from the pre-monsoon 5-day experiment, through the post-monsoon in-situ experiment, to the post-monsoon 5-day experiment and the pre-monsoon 850m experiment (Fig. 5.3C). Thus differences in PC1 scores were seen between different sites and seasons, with high scores corresponding to high relative abundances of alanine and glycine, and low proportions of valine, leucine and isoleucine (Fig. 5.3D).

The composition of the foraminiferan *Uvigerina sp.* varied slightly, but there was no obvious separation between specimens from the 140m and 300m sites.

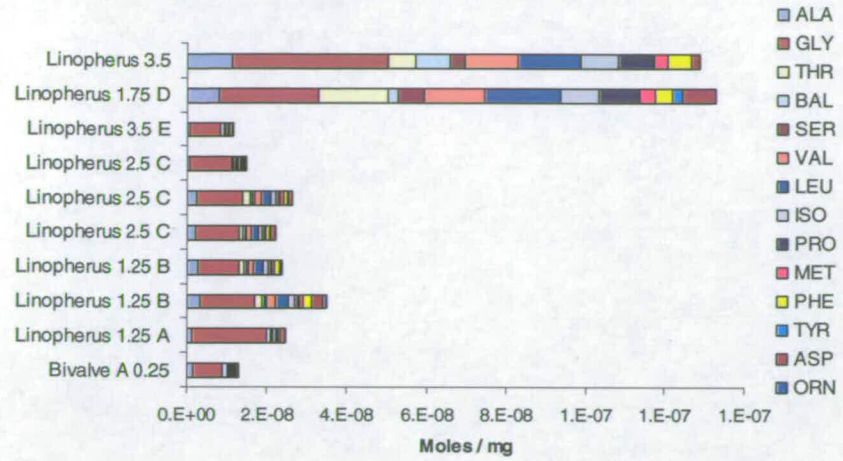
5.3.3 ^{13}C-labelled Amino Acid Yields

The total labelled amino acid uptake by individual macrofauna ranged between 10^{-6} and 10^{-8} moles of amino acid per mg of dry tissue (Fig. 5.4). Cirratulids appeared slightly more efficient at uptake and assimilation than the amphinomid *Linopherus sp.* ($P = 0.01$). However, when post-monsoon data are considered alone (see Data Quality section), this distinction disappears.

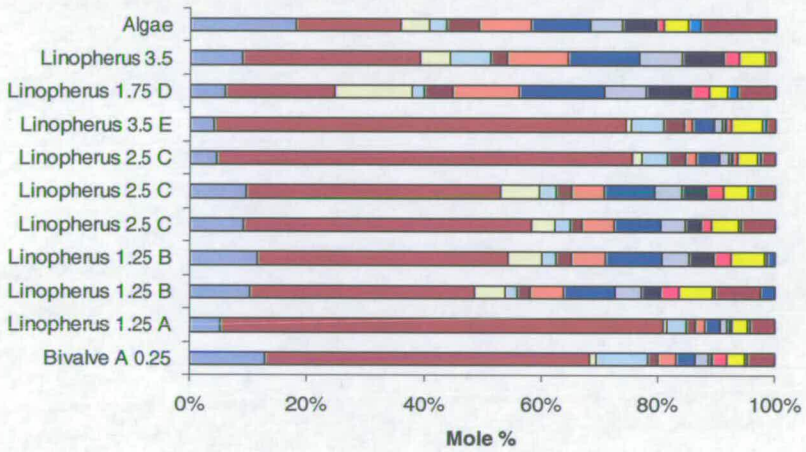
5.3.4 ^{13}C-labelled Amino Acid Suites

Fauna samples were preserved with their gut contents in place; therefore, any labelled amino acids detected may reside either in algae, in the gut, or assimilated into the animal tissue. A change in labelled amino acid suite between the raw algae and the faunal samples must therefore reflect preferential assimilation or egestion of selected compounds.

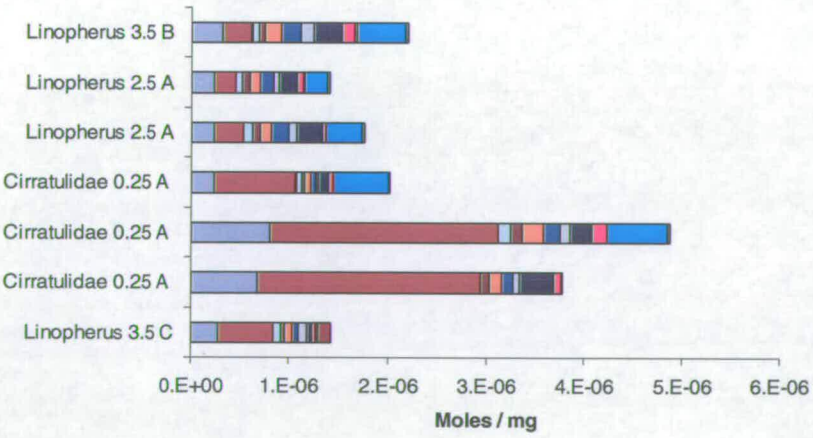
The large majority of samples analysed displayed one obvious change in amino acid composition from that of the source algae, and that was an enrichment in labelled glycine from 15-17 mole% in the algae to as much as 84 mole% in fauna samples (Figs. 5.5B-5.8B, and 5.9). Foraminifera samples did not show this change, and instead tended to become enriched in labelled aspartic acid (Fig. 5.8B).


Linopherus 3.5
Linopherus 1.75 D
Linopherus 3.5 E
Linopherus 2.5 C
Linopherus 2.5 C
Linopherus 2.5 C
Linopherus 1.25 B
Linopherus 1.25 B
Linopherus 1.25 A
Bivalve A 0.25
ALA
GLY
THR
BAL
SER
VAL
LEU
ISO
PRO
MET
PHE
TYR
ASP
ORN
0.E+00
2.E-08
4.E-08
6.E-08
8.E-08
1.E-07
1.E-07
1.E-07
Moles / mg

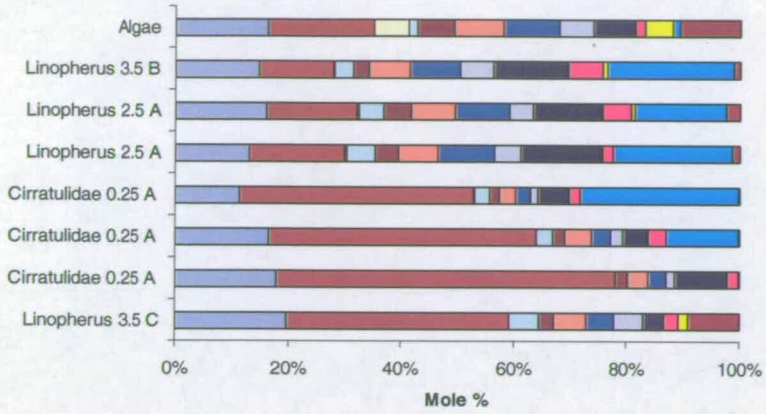
A


Algae
Linopherus 3.5
Linopherus 1.75 D
Linopherus 3.5 E
Linopherus 2.5 C
Linopherus 2.5 C
Linopherus 2.5 C
Linopherus 1.25 B
Linopherus 1.25 B
Linopherus 1.25 A
Bivalve A 0.25
0%
20%
40%
60%
80%
100%
Mole %

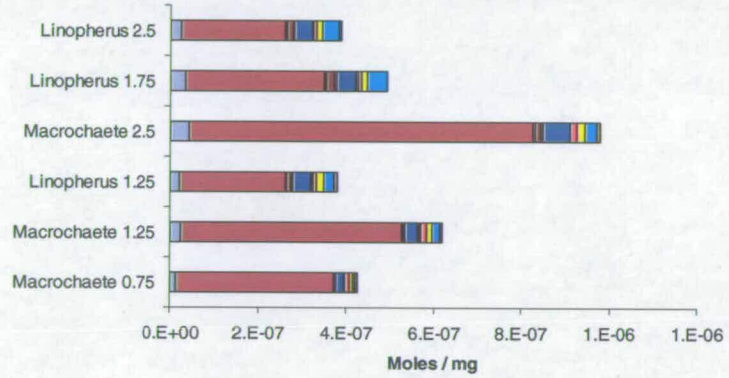
B


Linopherus 3.5 B
Linopherus 2.5 A
Linopherus 2.5 A
Cirratulidae 0.25 A
Cirratulidae 0.25 A
Cirratulidae 0.25 A
Linopherus 3.5 C
0.E+00
1.E-06
2.E-06
3.E-06
4.E-06
5.E-06
6.E-06
Moles / mg

C



D



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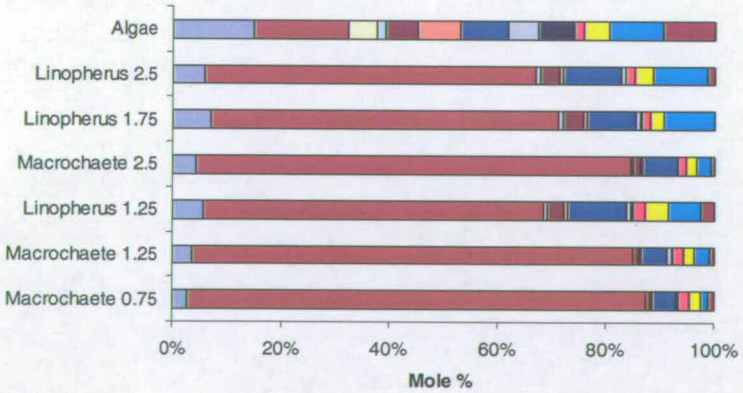
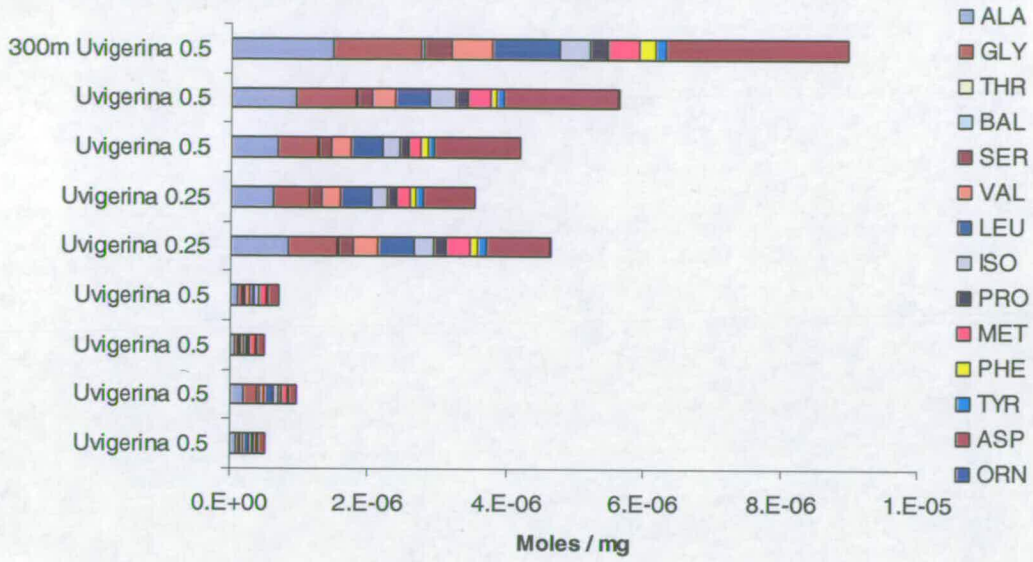
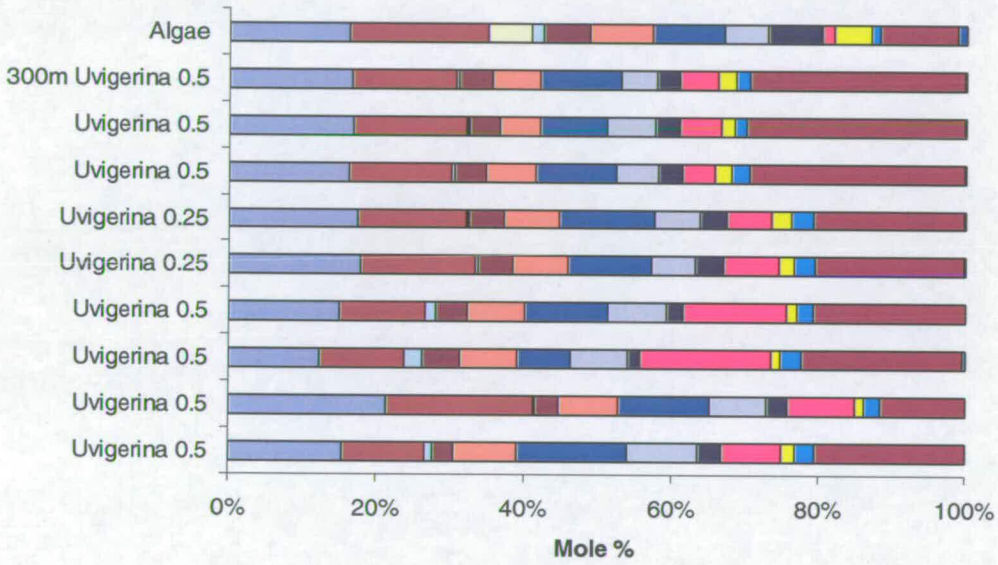


F

Figure 5.7. ^{13}C-labelled amino acid quantities and suites in fauna from 940m site experiments, A and B) pre-monsoon 5-day, C and D)Post-monsoon 5-day, E and F) post-monsoon in-situ. The numbers following sample identities indicate the depth (cm) at which samples were found. Where a letter follows sample identities, this indicates splits of the same individual. The key given with A applies to all plots.



A



B

Figure 5.8. ^{13}C-labelled amino acid quantities (A) and suites (B) in foraminifera (*Uvigerina sp.*) from the post-monsoon 2-day experiment at the 140m site and the post-monsoon 5-day experiment at the 300m site. The numbers following sample identities indicate the depth at which samples were found. Key given for A also applies to B.

5.3.5 Principle Component Analysis

Visual inspection of mole% plots (Figs. 5.5B-5.8B) did not reveal striking changes in ^{13}C-labelled amino acid compositions between algae and fauna, apart from the glycine enrichment described above. In order to asses more subtle trends in minor

amino acids, principle component analysis was carried out on the following sets of the data: all fauna, all sediments, and three taxonomic groups of fauna (Cirratulidae, *Linopherus sp.*, and *Uvigerina sp.*). In addition, the data were analysed in two different forms, as mole%, using all amino acids measured (excluding glutamic acid and lysine), and as glycine-free mole%, thereby eliminating the forcing of minor component mole percentages by the dominant glycine. Glycine-free mole% PCAs did not produce strikingly different results from the glycine inclusive PCAs, thus only glycine-inclusive PCA results will be discussed. For PCAs of glycine inclusive mole % data, the principle component axes 1 and 2 (PC1 and PC2) accounted for 47-66 % of the variance of the various data sub-sets.

5.3.5.1 Factor Coefficients

Most amino acids tended to share similar degrees of influence over sample scores, and the compounds with greatest influence varied between different sample sub-sets. The most significant three amino acids for each sample group are given in Table 5.3. Glycine, isoleucine and leucine were most regularly featured among the most influential compounds, followed by valine, and alanine, with threonine, proline, methionine tyrosine and histidine making one-off appearances. The amino acids that were most significant in determining sample scores thus showed considerable variation; however, it should be noted that similar uniformity of influence among the amino acids, and variation of the most influential compounds was seen by Dauwe and Middelburg (1998) between two sediment sample sets subject to PCA to produce degradation indices. They noted that despite such variation, the two analyses gave samples the same relative scores.

Sample Set	Most Influential Amino Acids		
All Fauna	Proline	Alanine	Isoleucine
New Sediments	Isoleucine	Glycine	Valine
Cirratulidae	Valine	Glycine	Leucine
Linopherus sp.	Isoleucine	Threonine	Valine
Uvigerina	Methionine	Alanine	Glycine
Dauwe and Middelburg North Sea (1998)	Phenylalanine	Glycine	Leucine
Dauwe et al., (1999)	Tyrosine	Leucine	Histidine

Table 5.3. The three most influential amino acids in PCA of various sample sub-sets. Data from this study is from analysis of mole % data. Dauwe and Middelburg data is from Dauwe and Middelburg, 1998 and Dauwe et al., 1999.

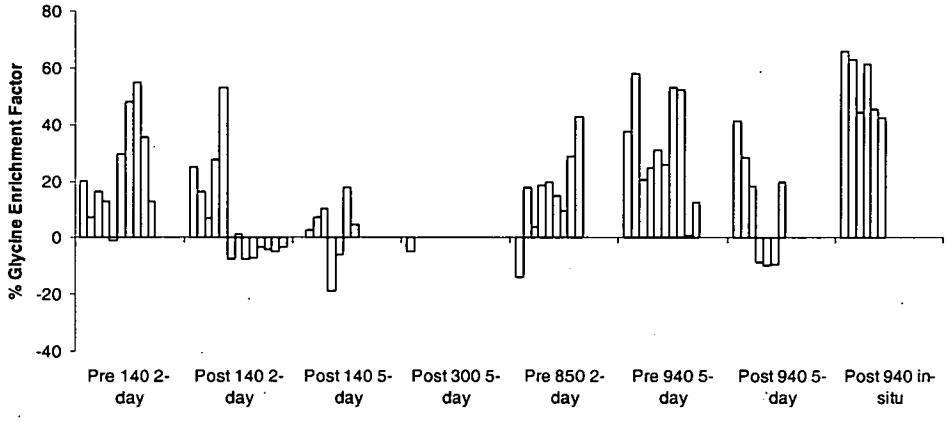


Figure 5.9. Glycine enrichment factors for all samples from all experiments. Note that the negative values for the post-monsoon 2-day experiment are from foraminifera (*Uvigerina sp.*), and should not be directly compared with the rest of the data, which is derived from macrofauna.

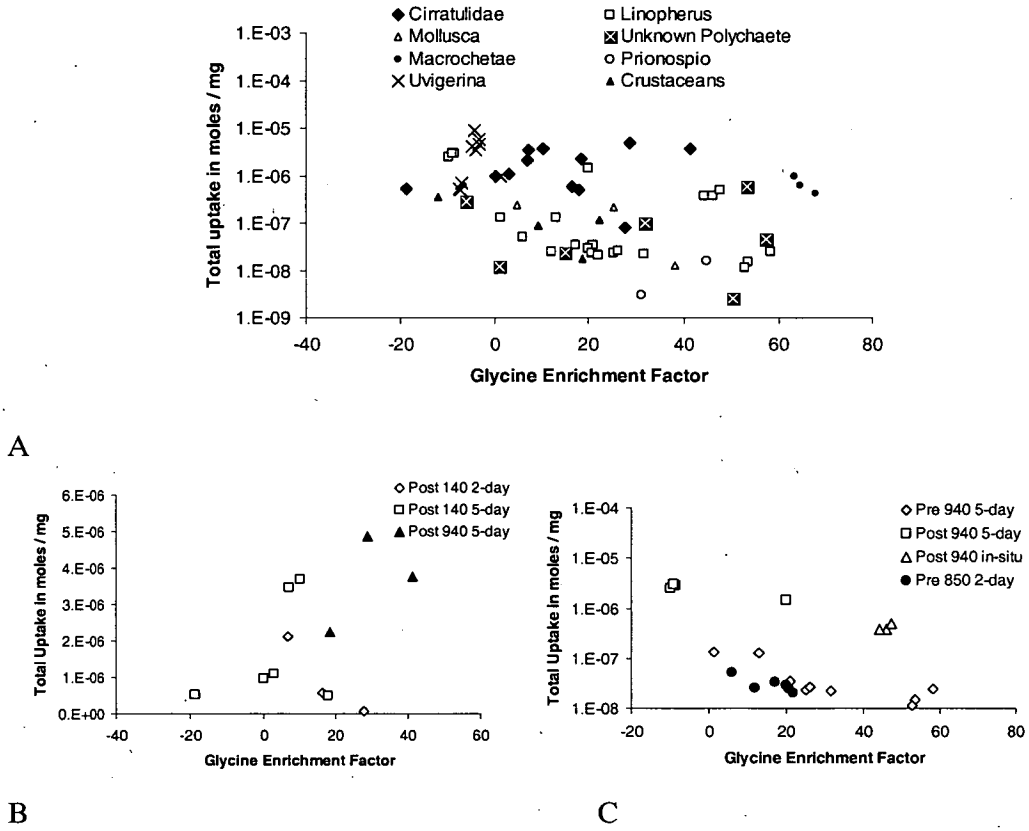
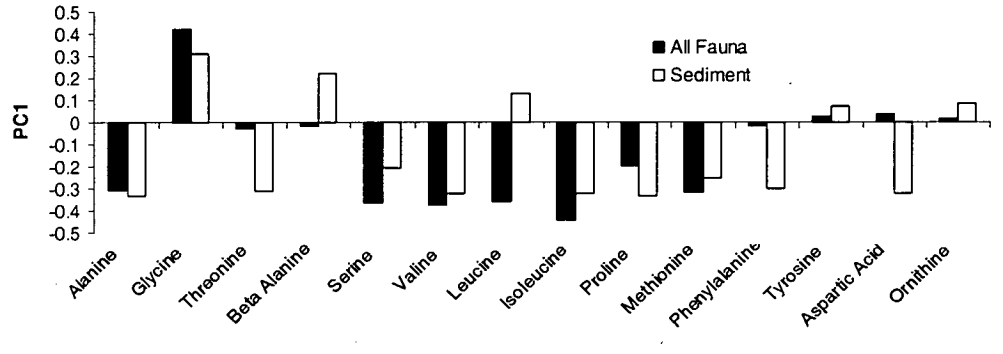
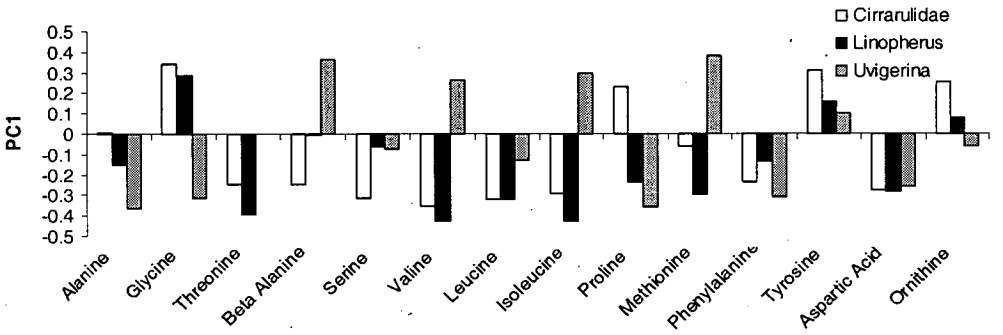


Figure 5.10. Total amino acid uptake (moles per mg of tissue) against glycine enrichment factor for A) all samples, B) Cirratulidae, C) *Linopherus sp.*. Note logarithmic y-axes in A and C.

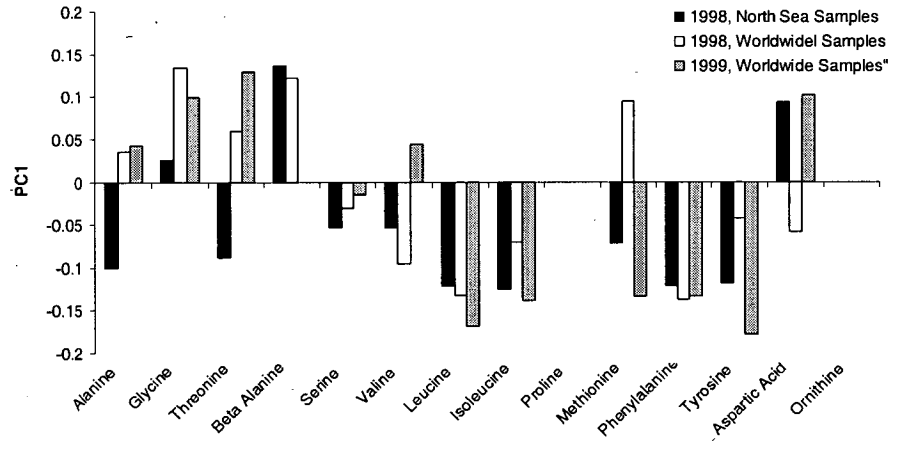
The comparative uniformity of factor coefficients, together with the lack of difference in results between glycine-inclusive and free PCAs indicated that compound selectivity during alteration was comparatively subtle. Nonetheless, PCA results did reveal differences between sites, experiments and taxa.



A



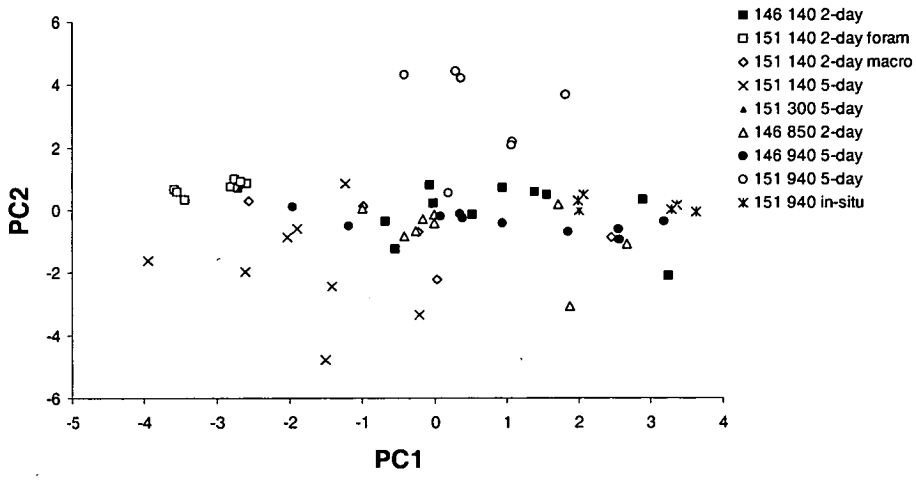
B



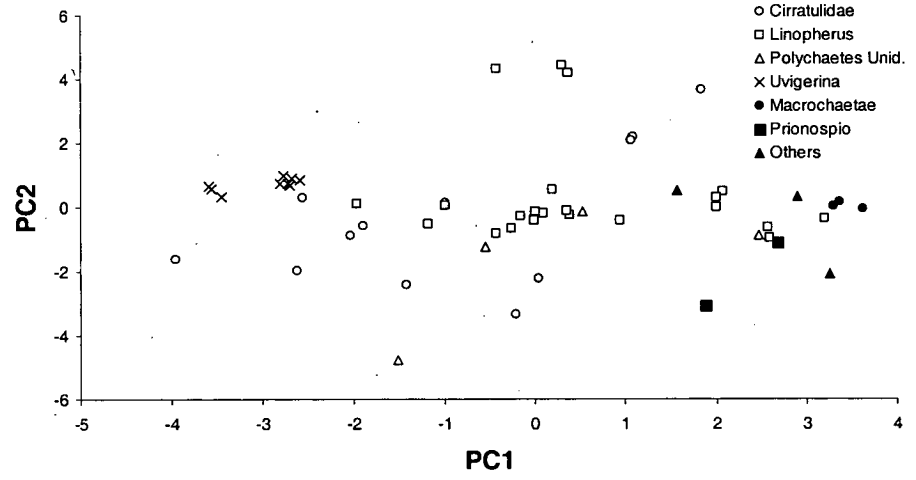
C

Figure 5.11. Amino acid factor coefficients from PCA of several sample sub-sets (A and B), and those from Dauwe and Middelburg (1998, see their Table 4 and Table 1 in Dauwe et al., 1999) (C). Dauwe and Middelburg (1998, 1999) coefficients have been multiplied by –1, as in their studies the PC1 axis was reversed, so that negative values indicated decay.

The most positive principle component 1 (PC1) scores were indicative of the maximal degree of alteration of ^{13}C-labelled amino acid suite away from that of the source algae. Thus, those amino acids with positive factor coefficients tended to accumulate, and those with negative scores tended to be preferentially lost, with increasing degree of alteration. Most amino acids showed negative coefficients, indicating that they were preferentially lost (Fig. 5.11A). These included most of the



A



B

Figure 5.12 Principal component 1 and 2 scores for all fauna from PCA of ^{13}C-labelled amino acid data plotted by A) experiment, and B) taxon.

high-influence amino acids, except for glycine, which along with tyrosine and ornithine, showed positive coefficients and thus accumulated during alteration.

The factor coefficients described above were derived from PCA results of the full fauna data set, but PCAs of other data sets showed broad similarities to these results (Fig. 5.11A,B). One that did not was the ^{13}C-labelled amino acid data from specimens of *Uvigerina sp.* (Fig. 5.11B). In this case, glycine and ornithine had negative coefficients, and valine and isoleucine had positive coefficients (yet inclusion of unaltered algae data in this PCA confirmed that positive values still represented greater degrees of alteration). Thus, glycine, alanine, serine, leucine, phenylalanine and aspartic acid, with their negative coefficients, tended to be preferentially removed during alteration, while valine, isoleucine, methionine, tyrosine and β-alanine tended to accumulate.

Amino acid factor coefficients generated in this study were broadly similar to those published by Dauwe et al. (1999) (Fig. 5.11C), with the exception that threonine, β-alanine, methionine and aspartic acid were sometimes seen to act in the same sense as glycine (Table 4 in Dauwe and Middelburg, 1998).

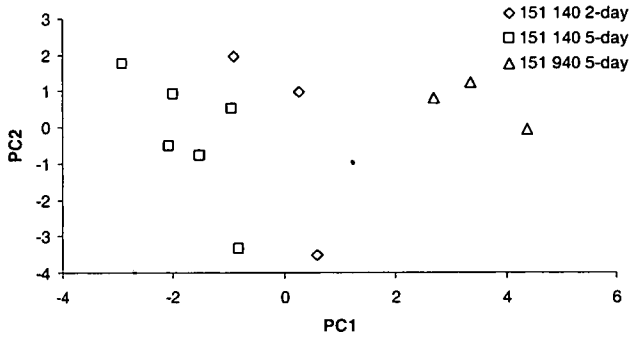
5.3.5.2 Sample Scores

Principle component analysis of all fauna samples produced groupings of fauna both in terms of experiment and by taxon (Fig. 5.12).

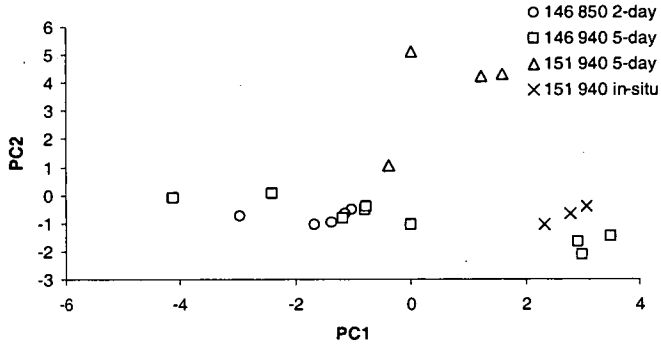
Samples from the post-monsoon 940m *in situ* experiment were grouped at the high end of the PC1 axis, and showed higher scores than those from shipboard experiments at the same site, which also had higher-than-average scores. High scores on the PC1 axis were primarily the result of high relative abundances of glycine, and represented the greatest departure in composition from the source algae. Thus, in general, fauna from the 940m site caused greater biochemical change to fresh OM than those at other sites, and appeared to do so more *in situ* than in shipboard experiments. Some specimens from the post-monsoon 5-day experiment at the 940m site were separated from the rest by relatively high scores on the PC2 axis. This may however be an artefact of calibration problems with tyrosine and ornithine in this small group of samples (they had unusually large mole percentages of those compounds). If the effect is real, then the reason for it is unclear.

Macrofauna samples from the 140m site were separated by season along the PC1 axis, with pre-monsoon samples showing higher scores (Fig. 5.12A). Thus, at this

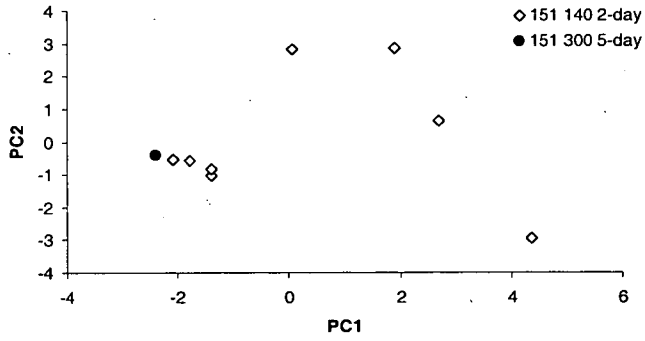
site the macrofauna effected a higher degree of biochemical alteration in the pre-monsoon season when oxygen availability was highest (Table 1).



A



B



C

Figure 5.13.PC1 and 2 scores for samples in single taxon PCAs. A) Cirratulidae, B) *Linopherus sp.*, C) Uvigerina.

Principle component analysis of all fauna samples also produced some grouping of samples by taxon (Fig. 5.12B). Most significantly, the foraminifera from both the 140m and 300m sites all fell within limited ranges on both axes, showing particularly

low PC1 scores and thus a relatively unaltered suites of amino acids, with comparatively low relative abundances of glycine. This PCA produced a slight separation between the cirratulids and *Linopherus sp.* (the latter having the higher scores). Also, Prionospio and Macrochaetae tended to have high PC1 scores.

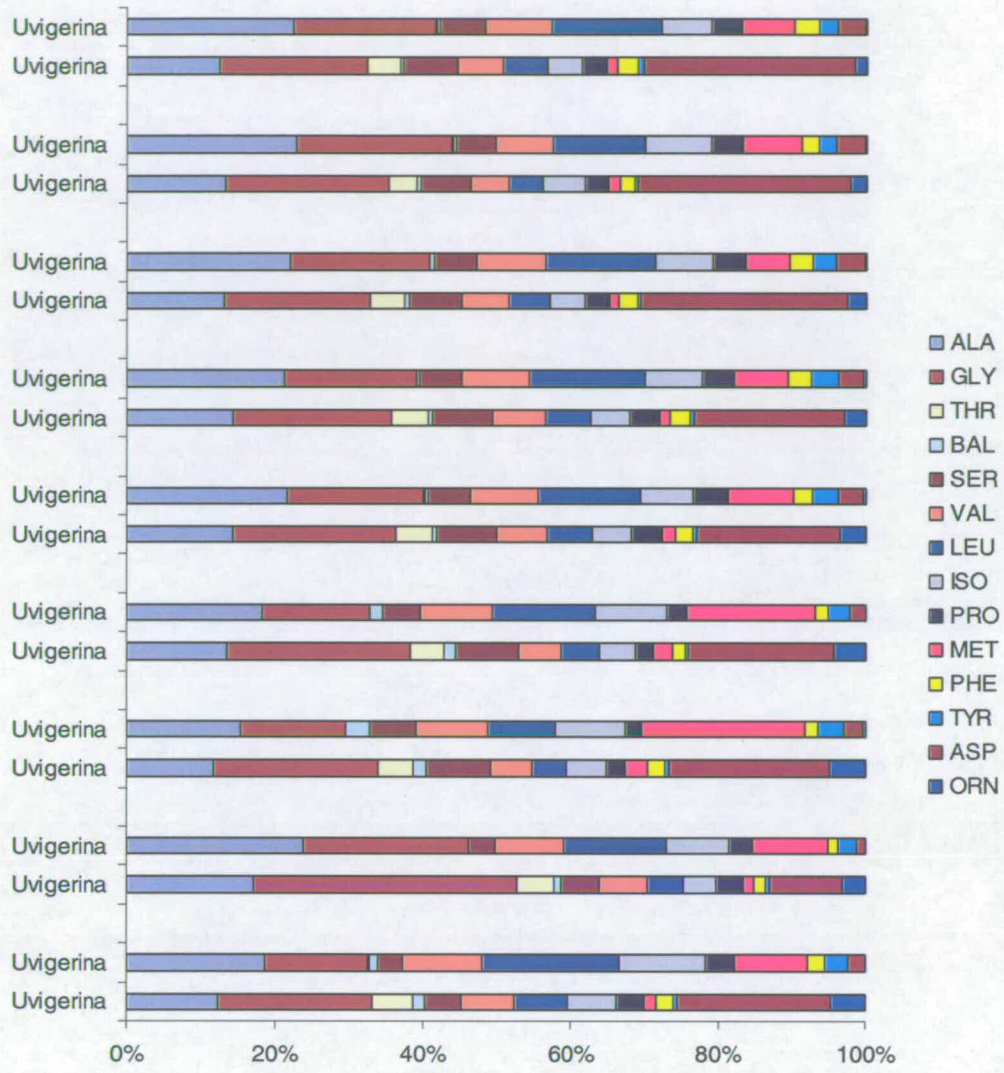
Principal component analysis was also carried out on single taxon sub-sets of the data. Within the Cirratulidae, PC1 scores separated the 140m samples from the 940m samples, the latter group having higher values (Fig. 5.13A), paralleling the results of PCA of the whole fauna sample set. High PC1 scores in this case were related to high relative abundances of glycine and tyrosine, and low relative abundances of valine, serine and leucine (Fig. 5.11B). Cirratulids recovered from the post-monsoon 2- and 5-day experiments at the 140m site were not well separated, and if anything the samples from the shorter experiment showed slightly higher scores.

Among samples of *Linopherus sp.* (Fig. 5.13 B), those from the 940m site had generally higher PC1 scores than those from the 850m site. Samples from the post-monsoon *in situ* experiment at the 940m site were grouped at the top end of the range. The PC1 scores for this group were controlled by the accumulation of glycine and loss of valine and isoleucine (Fig. 5.11B).

Specimens of *Uvigerina sp.* were predominantly from one experiment at the 140m site (Fig. 5.13 C). One sample, however, was from the 300m site, and this lay at the lowest point of the range of *Uvigerina sp.* PC1 scores. Thus, foraminifera at the 300m site appeared to have caused less biochemical change in OM, possibly because of low oxygen conditions. In this case, high PC1 scores were generated by the accumulation of isoleucine and methionine, and by the loss of proline and glycine. This is in stark contrast to the suite changes produced by macrofauna (Fig. 5.11B).

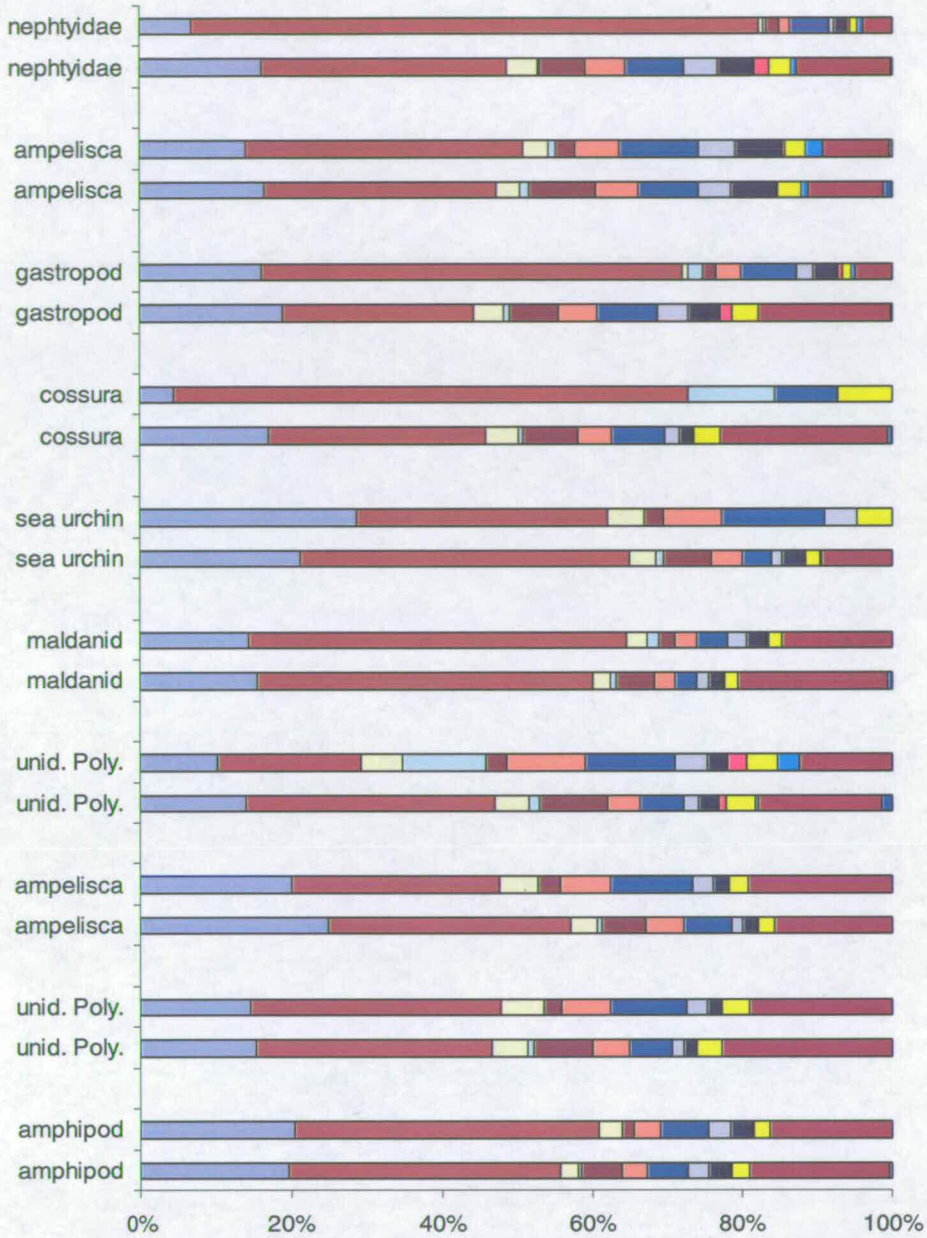
5.3.6 Comparison of the Natural and ^{13}C-labelled Amino Acid Suites of Fauna

A major strength of the method employed here was that naturally present and ^{13}C-labelled versions of each amino acid could be detected and quantified as separate analytes, thus allowing their simultaneous determination. In the present study, this



A

also allowed a comparison between the natural amino acid composition of fauna and the suite of labelled amino acids that they assimilated.



B

Figure 5.14. Comparisons between the ^{13}C labelled amino acid suite and the natural amino acid suite for a sub-set of samples. A) *Uvigerina sp.*, and B) metazoan macrofauna from the pre-monsoon 140m site 2-day experiment. The top bar of each pair is the ^{13}C labelled amino acid suite, and the bottom bar is the natural amino acid suite. The key given for A is also applicable to B.

The suite of ^{13}C-labelled amino acids found in foraminifera tended to be enriched in alanine and slightly depleted in glycine compared to their natural amino acid composition, whereas metazoans showed the opposite effect (Fig. 5.14). Both

classes of fauna however took up aspartic acid in a lower proportion than it was present in their tissues, and greater proportions of valine, leucine, isoleucine and phenylalanine.

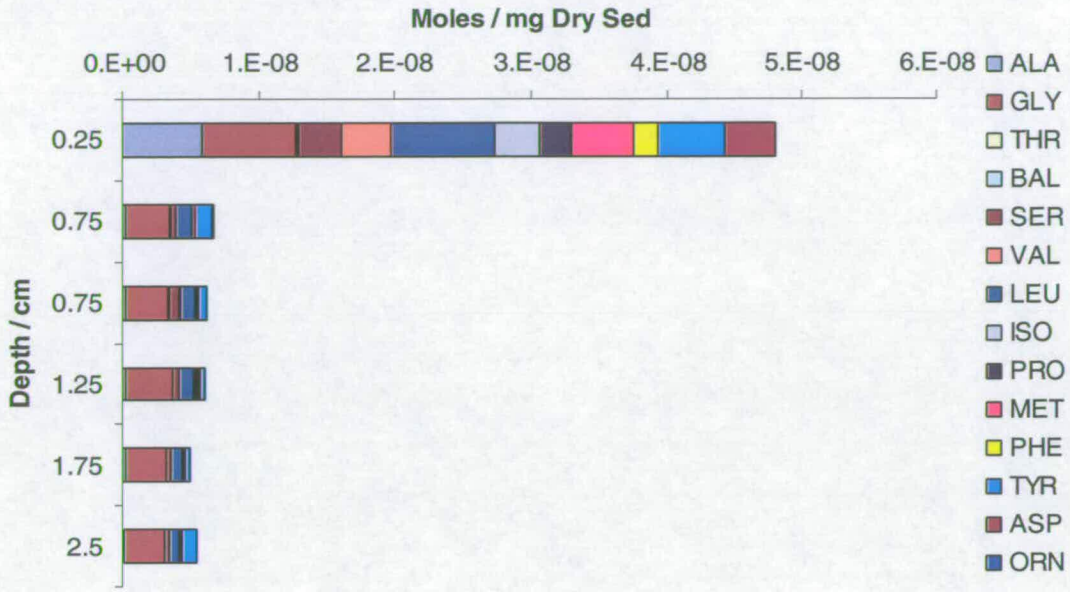
5.4 Results: Sediment

Down-core sediments from the post-monsoon 5-day experiments at the 140m, 300m, and 940m sites were analysed (Fig. 5.15-5.17).

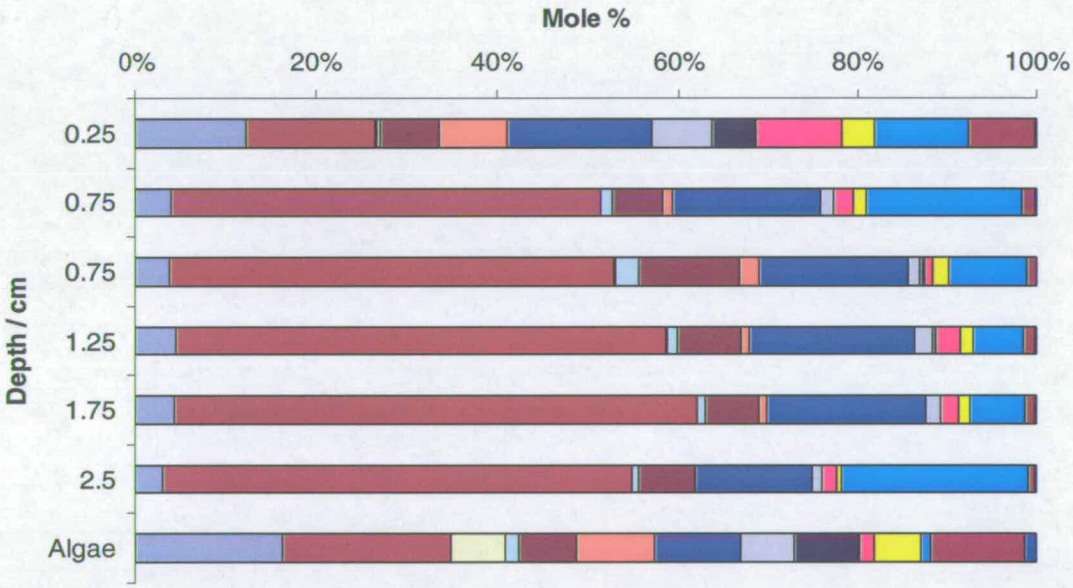
In each case, the concentration of ^{13}C-labelled amino acids was dramatically greater at the sediment surface than below it, consistent with the labelled algae having been introduced as a surface layer. This enrichment fell off immediately with depth, and by 0.5 cm in the 300m core, and by 1.5cm and 1cm in the 940m and 140m cores, respectively, a background concentration was reached (Figs. 5.15-5.17). This was consistent with bulk $\delta^{13}C$ data from the same sediment (chapter 4). The background concentration of labelled amino acids was largely due to down-core smearing of label during core sectioning, nevertheless, above- background concentrations of labelled amino acids did penetrate further at the 140m and 940m sites than at the 300m site, and the suite present below the surface was altered from that in the algae, suggesting a fauna-mediated digestion/transport process within the top 1-2cm. It should be noted that in deeper sections of the cores, where labelled amino acid concentrations became very low, the ^{13}C-labelled amino acid suite will not appear the same as in algae, as many minor compounds will have become undetectable.

5.4.1 ^{13}C-Labelled Amino Acid Suites

The ^{13}C-labelled amino acid suite detected in the surface layer of each core was more similar to the source algae than that of the deeper sediments (Fig. 5.15-5.17). Downcore sediment amino acid suites were consistently enriched in glycine, and depleted in alanine relative to those at the surface. Several minor but reactive amino acids, including valine, isoleucine, proline, methionine phenylalanine and aspartic acid dramatically decreased in mole% values below the top section of all cores (but remained detectable), and leucine, tyrosine and serine remained constant or increased slightly in significance. The non-protein amino acid β-alanine increased in mole% in sub-surface samples.

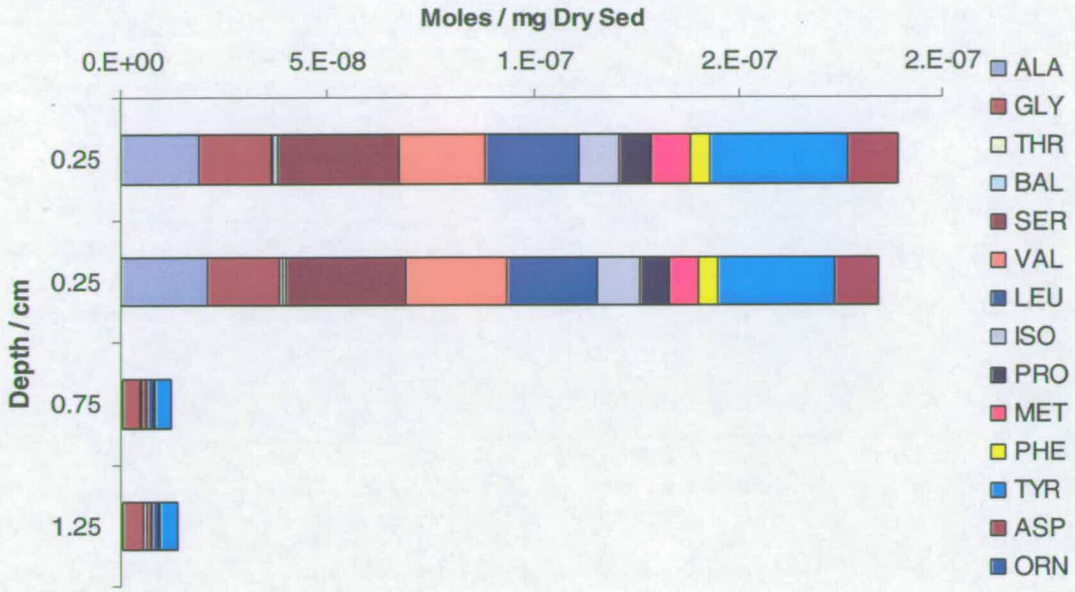


A

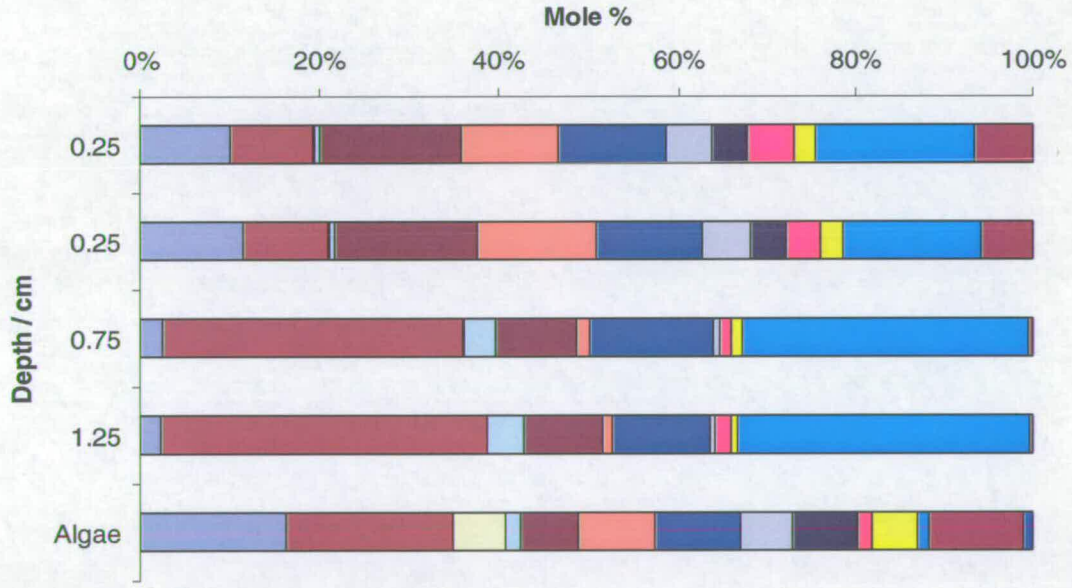


B

Figure 5.15. The ^{13}C-labelled amino acids quantities (A) and suites (B) of sediments from the post-monsoon 5-day experiment at the 140m site.

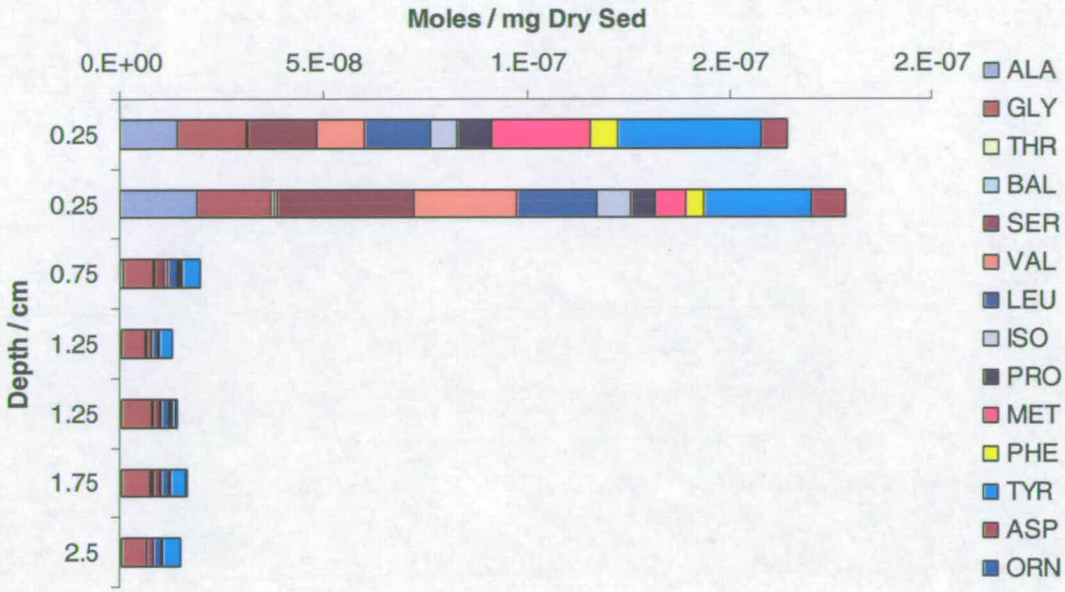


A

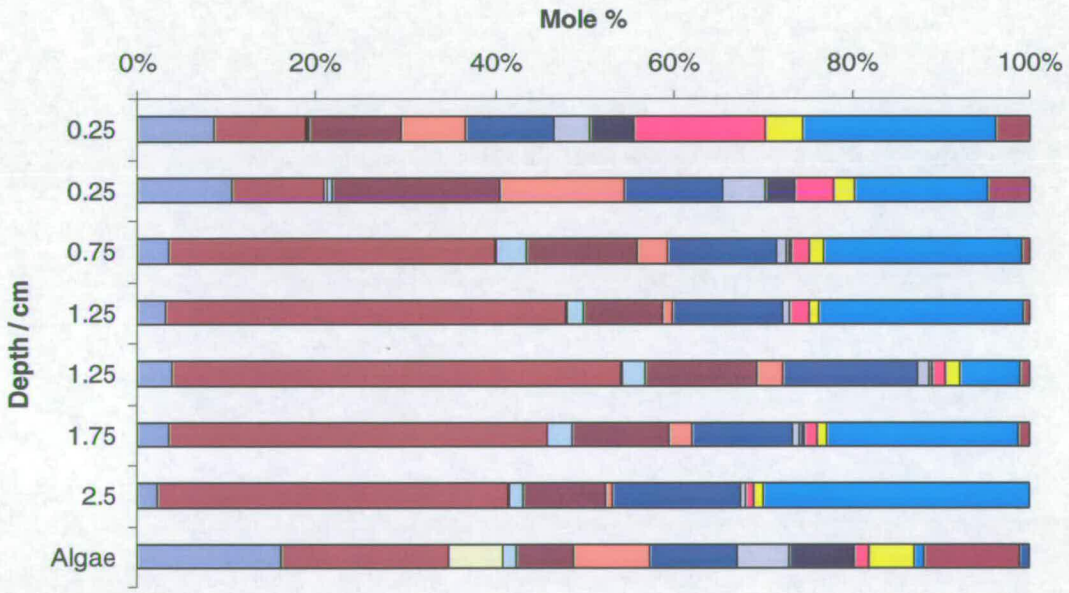


B

Figure 5.16. The ^{13}C-labelled amino acids quantities (A) and suites (B) of sediments from the post-monsoon 5-day experiment at the 300m site.



A



B

Figure 5.17. The ^{13}C-labelled amino acids quantities (A) and suites (B) of sediments from the post-monsoon 5-day experiment at the 300m site.

5.5 Discussion

It should be noted that the fauna analysed in this study were a sub-sample of all samples recovered from the experiments, and the rest were used for bulk carbon tracing, and construction of carbon budgets. While only a small proportion of fauna ($\leq \sim 20\%$) have been analysed for ^{13}C-labelled amino acids, the taxa they represent were always the most abundant and dominant at each site, and replication within taxa was sufficient to support the conclusions drawn (i.e. the main groups, the Cirratulidae *Linopherus sp.* and *Uvigerina sp.*, showed consistent tendencies between experiments, sites and seasons).

5.5.1 Data Summary

The data presented showed unprecedented detail of fauna and sediment natural amino acid compositions. They also showed the preferential uptake and losses of labelled amino acids in tracer experiments conducted on whole natural benthic communities, under *in situ* or near *in situ* conditions, at sites with contrasting redox conditions and benthic communities, and in seasons of differing food supply.

To summarise the primary observations, the natural amino acid compositions of macrofauna were dominated by glycine and alanine, and methionine, phenylalanine and tyrosine were the most minor constituents. Subtle differences occurred between taxa, and even within single taxon groups between different sites and seasons. (Fig. 5.1).

Macrofauna and foraminifera were observed to take up labelled amino acids, with cirratulids being slightly more efficient than *Linopherus sp.*, the other major polychaete on this margin (Figs. 5.5-5.8, 5.10).

In general, the ^{13}C-labelled amino acid composition of fauna was obviously enriched in glycine compared to the source algae (Figs 5.5-5.8). Principle component analysis revealed that fauna also tended to be slightly enriched in tyrosine and ornithine, and depleted in isoleucine, valine, leucine, alanine, threonine, proline and methionine, relative to the source algae. The exact pattern of compound alteration was, however, taxon-dependent, and foraminifera especially exhibited an entirely different pattern of amino acid alteration. (Figs 5.11-5.13)

Within single taxon groups, PCA revealed differences in the degree of alteration between sites and with differing conditions and experiment durations (Fig. 5.13), and the causes of this will be discussed.

Labelled amino acids were also detected in the sediments recovered from the incubation experiments. These data indicated that bioturbation occurred within the upper 1-2 cm of sediment, accompanied by slight biochemical alteration (Figs 5.15-5.17).

5.5.2 Comparison With Previous Studies

In a previous labelled amino acid feeding study, performed in microcosm, Thomas and Blair (2002) made similar observations of changes to a diatom amino acid suite during macrofaunal digestion and assimilation. The four taxa investigated (a maldanid, a spionid, and two terebellids) all exhibited enrichments in labelled glycine, both in their tissues and in their faecal pellets, and the maldanid showed the greatest tissue enrichments. This was accompanied by minor losses of serine, aspartic acid, glutamic acid, valine, proline, alanine and isoleucine in worms compared to the algae, and faecal pellets were enriched in phenylalanine, threonine and leucine relative to the equivalent tissues. The terebellids had different gut architecture from the other taxa (mixed-reactor plug flow as opposed to plug flow reactor, (Penry, 1989)), and this was suggested as the cause for greater concentrations of glycine and threonine in their faecal pellets, as it allowed them to release amino acids from foods more efficiently, but did not give them overall greater efficiency in amino acid assimilation (as had been expected). Glycine and threonine were assimilated in greater mole percentages than their contributions to worm tissues, and the opposite was true for alanine, serine, valine, leucine, isoleucine, aspartic and glutamic acids, and proline and phenylalanine. The authors noted that this could have been a function of the accessibility of those compounds in the food, as well as a reflection of nutritional requirements.

The findings in this study were broadly similar to those of Thomas and Blair (2002), in that enrichment of faunal tissues with ^{13}C-labelled glycine, and depletion in valine, leucine and isoleucine (among others) compared to the algae was also observed here. In addition, glycine was assimilated in greater proportions than those in which it was

present in animal tissues, and alanine in smaller proportions. We also observed variation among the amino acid assimilation patterns of different taxa, although the taxa present on the Pakistan margin were not the same as those studied by Thomas and Blair (2002). Thus, the findings of this and the earlier study are replicated, and are likely generally applicable across many benthic environments and macrofaunal communities.

Purely geochemical studies of seafloor sediments have shown that amino acids display differing relative reactivities, with a consistent pattern. This brings about systematic changes in amino acid composition with the advancing decay (e.g. Cowie and Hedges, 1994). Such changes have been utilised by Dauwe and Middelburg (1998) to produce a degradation index for sediments through PCA of the natural amino acid suite. The majority of studies have found that glycine, serine, aspartic acid and threonine are less reactive than bulk amino acids, and thus become relatively enriched as decay progresses, while glutamic acid, alanine, leucine, iso-leucine, phenylalanine and tyrosine are particularly reactive and are therefore lost. The non-protein amino acids β-alanine and γ-aminobutyric acid are alteration products of aspartic and glutamic acids respectively (e.g. Cowie and Hedges 1994), are of comparatively low reactivity, and also tend to accumulate (e.g. Keil et al., 1998, Lee et al., 2000, Wakeham et al., 1997, Cowie et al., 1992, Sutthof et al, 2000, Horsfall and Wolff, 1997, Dauwe and Middelburg 1998, Henrichs and Farrington, 1987). The accumulation of glycine in sediments during decay is thought to be linked to protection through its association with diatom cell walls (Cowie et al., 1992), and was similarly observed in feeding experiments where diatoms were fed to herbivorous zooplankton (Cowie and Hedges, 1996). This association is thought to make glycine less available for digestion and assimilation, or more resistant to microbial decay. The findings of this study suggest however, that glycine is actually assimilated particularly efficiently, and further suggestions are made below of how it becomes enriched in degraded OM.

5.5.3 General Points

In general (apart from labelled glycine enrichments), the suites of ^{13}C-labelled amino acids recovered from fauna were similar to those found in the source algae. This may

be either because most or all of the ^{13}C-labelled amino acids detected were present as algae in gut contents, or they were all assimilated with similar efficiency, and thus the original suite was preserved. It was clear that in some cases significant amounts of algae were present in gut contents at the time of sampling (visual observations). It is also possible that significant portions of the labelled amino acids detected were present in the dissolved form in gut fluids, which are actively retained in the mid-gut by some polychaetes, and which accumulate unexpectedly high concentrations of simple biochemicals (derived from digestion) compared to the gut contents (Mayer et al., 1997). Finally, it is possible that amino acids other than glycine were selectively assimilated, though glycine enrichment is the only feature that was readily apparent on visual examination of the amino acid spectra.

Principle component analysis provided a more holistic view of amino acid compositional differences, and permitted an assessment of the amino acids that tended to be enriched or depleted in faunal tissues. The degree of alteration and its controls are discussed below.

5.5.4 Controls On Degree of Amino Acid Compositional Alteration

The presented data showed that the scale of amino acid suite alteration varied between specimens. Factors which could account for this include taxon, experiment duration, depth of sample, site, and total amino acid uptake. Examination of the relationships between these factors and various measures of alteration may elucidate which were most significant.

5.5.4.1 Taxon Effects

In addition to different feeding modes, benthic fauna have different biochemical requirements, gut architectures and feeding efficiencies, and gut chemistries are tailored to fit an animals' normal diet (Mayer et al., 1995). Different taxa are therefore likely to cause different degrees of biochemical alteration, and this has indeed been observed among polychaete families (Thomas and Blair, 2002).

When all fauna were considered, glycine enrichment factors varied only slightly with taxon, most groups showing the full range (~ -10-66%) (Fig. 5.10). The macrochaetes always showed glycine enrichments at the top end of the range, and were significantly different from the combined Cirratulidae and *Linopherus sp.*

samples ($P = 8x10^{-9}$). Specimens of *Uvigerina sp.* consistently had the lowest (most negative) values. They also had low, and prionospio and macrochaetes had high PC1 scores, which, consistently with glycine enrichment factors, indicate minor and advanced alteration respectively (Fig. 5.12). Cirratulidae and *Linopherus sp.* specimens did not have particularly different glycine enrichment patterns. Principle component analysis however showed that *Linopherus sp.* polychaetes had higher scores, and thus caused more alteration than the cirratulids (Fig. 5.12).

Variation in the influential amino acids in PCA could be due to variation in diet requirements between different types of fauna. For example, threonine only appeared in the top three influential amino acids for *Linopherus sp.*, and methionine only appeared for *Uvigerina sp.* (Table 3), and these compounds may be particularly required by those taxa. In contrast, the amino acids glycine and isoleucine were influential in determining sample scores for both fauna and sediment samples, both in this study and in that of Dauwe et al., (1999). The changes in relative abundance of these compounds may therefore be related to general nutritional requirements, or the reactivity of these compounds, rather than to any species-specific requirements.

Figure 10A shows that the cirratulids tended to have higher total uptakes than *Linopherus sp.*, while PCA revealed a higher degree of suite alteration in the latter (Fig. 5.12). This result is supported by the results of bulk ^{13}C analyses of fauna from the same experiments, which showed the cirratulids to be more efficient than *Linopherus sp.* at OM uptake (chapter 4). This may indicate selective feeding and large OM throughput by the cirratulids, or it could reflect a relatively large gut volume compared to body size. In contrast, *Linopherus sp.*, showed less efficient OM uptake per unit biomass. It stands to reason then that *Linopherus sp.* would have to bring about more biochemical alteration of OM during digestion in order to flourish on a smaller specific intake. Individual specimens of *Linopherus sp.* were on average larger than the cirratulids (Peter Lamont, pers. comm.), thus the difference in the degree of biochemical alteration brought about by these two taxa is consistent with the observation of Ahrens et al. (2001), that assimilation efficiencies are greater in larger individuals, due to their longer gut residence times.

Thus, ^{13}C-labelled amino acid uptake and bulk ^{13}C uptake data showed consistent taxon-specific effects. The Cirratulidae exhibited greater label uptake, and the lower

label uptake per unit biomass by *Linopherus sp.* was balanced by the fact that they appeared to have a more efficient digestion processes. This is consistent with knowledge of the feeding biology of both the cirratulids and *Linopherus sp.*, which are both thought to be surface deposit feeders. The former group however is thought to be a selective feeder in favour of algal detritus, whereas the latter is possibly an opportunistic omnivore. In addition, cirratulids have been observed to be of simple construction, and thus their digestion efficiency is relatively low (Fauchald and Jumars, 1979, and references therein).

Even considering the taxon-specific effects above, the similarity of the amino acid suites assimilated by various taxa, both in the previous study by Thomas and Blair (2002) and here, is striking. All metazoan specimens exhibited high glycine assimilation (and / or production), and comparatively little selectivity among the other amino acids. The broad conclusion to be drawn is that the different macrofauna assimilated largely the same amino acid suites, and thus will likely have had similar effects on the composition and reactivity of egested OM. This corresponds with the relatively small variation in amino acid composition between various taxa when compared to that of lipid composition, which can be diagnostic of taxa (e.g. Bradshaw et al., 1991).

The most dramatic taxon effect, which must be stressed, is the difference between metazoan macrofaunal and protozoan foraminiferal OM alteration. Principle component analysis showed that *Uvigerina sp.* caused the least alteration to the ^{13}C-labelled amino acid suite of all samples studied (Fig. 5.12B), and factor coefficients derived from PCA of *Uvigerina sp.* data were considerably different from those produced by all other taxonomic groups (Fig. 5.11). The foraminifera appeared not to preferentially assimilate or produce labelled glycine, and thus had negative glycine enrichment factors (Fig. 5.10), while still showing average total labelled amino acid uptake per mg of tissue. Instead of showing glycine enrichment, *Uvigerina sp.* appeared to assimilate glycine in a lower proportion to that in which it was present in their tissues, and alanine and aspartic acid in a greater proportions, the opposite pattern to the metazoan macrofauna (Fig. 5.14). The natural amino acid suite of *Uvigerina sp.* was, however, very similar to that of the macrofauna. Thus fauna amino acid requirements are at least partly determined by factors (such as turnover

times and other metabolic processes) other than tissue protein composition. Alternatively, the similarity between *Uvigerina sp.* and algae ^{13}C-labelled amino acid compositions may be because the foraminifera, not having guts, were not capable of selective assimilation, or because a much greater proportion of labelled amino acids in *Uvigerina sp.* were present as gut contents, compared to that found in macrofauna.

The differences between macrofaunal and *Uvigerina sp.* digestion were reflected in the ^{13}C amino acid suites in sub-surface sediments. Sediments between depths of 0.5 and 2 cm at the 140m and 940m sites, where bioturbating macrofauna were present, showed much larger glycine enrichments (Figs 5.15, 5.17) than those at the 300m site (Fig. 5.16), where foraminifera totally dominated the faunal community.

5.5.4.2 Site Conditions

Glycine enrichment factors for single taxa revealed contrasts between sites. Cirratulids at the 940m site executed both more amino acid uptake and glycine enrichment than those at the 140m site (Fig. 5.10B), and when all fauna data were considered, total amino acid uptake (moles / mg of tissue) was significantly greater at the 940m site than at 140m site in post-monsoon shipboard experiments ($P = 0.002$) (Fig. 5.4). The PC1 scores for the cirratulids and the whole fauna data set also showed more alteration at the 940m site compared to the 140m site (Figs. 5.12, 5.13). The fact that this difference between sites was present even when only one taxon was considered suggests that it was not due to varying faunal community composition, but was instead due to differences in site conditions.

Glycine enrichment values for *Linopherus sp.* (Fig. 5.10C) were higher for individuals from the 940m site than those from the 850m site, while total uptake was roughly the same. This was also reflected in *Linopherus sp.* PC1 scores, which were higher for specimens from the 940m site (Fig 5.10B).

Finally, Uvigerina PC1 scores showed a contrast between the 140m and 300m sites, with alteration being greater at the shallow site, despite greater total uptake at the OMZ site (Fig. 5.13C).

These variations in amino acid uptake and alteration among sites were all present within single taxon sub-sets of the data, and thus must be due to the effects of varying site conditions, as discussed below.

5.5.4.3 Bottom-water Oxygen concentrations

In the post-monsoon season, bulk ^{13}C tracing showed that the macrofauna at the 140m site exhibited suppressed OM uptake compared to the pre-monsoon season, due to monsoon-induced low oxygen conditions (chapter 4, Fig. 4.3). This was also reflected in the ^{13}C-labelled amino acid data in the form lower PC1 scores for post-monsoon macrofaunal specimens (from the 140m site) than their pre-monsoon counterparts (Fig. 5.12). Macrofaunal glycine enrichment factors were also lower ($P = 0.12$) after the monsoon than before it at the 140m site (Fig. 5.9). Thus, the seasonal reduction in oxygen availability at the 140m site resulted in reduced amino acid suite alteration during macrofaunal digestion.

Principle component analysis of *Uvigerina sp.* data showed (Fig. 5.13C) that a sample from the intensely hypoxic 300m site, was associated with less biochemical alteration than samples from the slightly more oxygenated 140m site (Fig. 5.4). Thus there is evidence that the digestion efficiency of foraminifera was also inhibited by low oxygen availability.

Previous work has shown that oxygen influences OM cycling through its role in determining OM decay rates, the composition of benthic communities, the abundance of burrowing fauna, and the faunal groups most active in OM uptake. This study adds to that list the suggestion that low oxygen availability also inhibits the extent of biochemical alteration that accompanies digestion.

5.5.4.4 OM Quality

The natural availability of high food-quality OM is thought to have a strong influence over benthic faunal community composition (e.g. Smith et al., 2000). Tracing of bulk ^{13}C in these experiments (chapter 4) suggested that sites where high quality OM was abundant hosted faunal communities more capable of responding quickly and efficiently to an input of fresh OM. At some sites, a monsoon-induced pulse of OM to the sediment was observed to prime faunal communities, such that they were more able to take up ^{13}C label after the monsoon than before. In this study, both amino acid uptake and alteration were greater at the 940m site than at the 140m site (see above). Sediments at the 940m site had both higher OM abundance and quality than those at the 140m site (Table 5.1). Thus, in addition to hosting communities able to respond quickly to a fresh OM input, sediments rich in high-

quality OM tend to be home to fauna with efficient digestive processes, and which therefore carry out maximal biochemical alteration of ingested OM.

5.5.4.5 Depth in Sediment

The specimens analysed in this study were recovered from a range of depths in experimental cores, from the surface down to 8 cm. Most samples were from the top ~ 2 cm (Table 5.1). If the degree of amino acid suite alteration was dependent on the time elapsed since label consumption, and overall algal throughput, it might be expected that glycine enrichment factors and PC1 scores would correlate with the depth at which fauna were found, as fauna found deep in the sediment may have spent less time feeding on labelled algae at the surface. However, no correlations were found between PC1 scores or glycine enrichment factors, and sample recovery depth (Table 5.1). There was also a lack of correlation between total amino aid uptake and depth in the sediment.

Bulk ^{13}C tracing showed that individuals were capable of significant vertical movement (down to 5 cm depth at the 940m site, and 10 cm depth at the 140m site, much deeper than the ~ 2 cm maximum depth of sediment ^{13}C enrichment, chapter 4), thus the depth from which an individual was recovered may not be representative of where it spent the majority of the experiment. Thus, bulk ^{13}C tracing data, and amino acid uptake and alteration data, were consistent in their lack of correlations with specimen depth. The vertical movement of individual animals through the sediment during experiments is responsible for this absence of correlation, as it disassociates the access to label an individual had, from the depth at which it was found at the end of the experiment.

Interestingly, the Cirratulidae are a slight exception to the above conclusion. When only cirratulids are considered, there are weak correlations between PC1 score or total ^{13}C-labelled amino acid uptake, and specimen depth ($\rho = 0.41$ and 0.50 respectively). These correlations are not statistically significant, but may indicate that the Cirratulidae are more restricted in their vertical movement than other taxa, such as *Linopherus sp.*

5.5.4.6 Experiment Duration

It should be noted that due to vertical movement of fauna through the sediment, experiment duration does not necessarily represent the duration for which any one individual fed on labelled algae. This may have masked relationships between duration of OM processing and degree of biochemical alteration, however, some trends were apparent, and are discussed below.

The degree of uptake and alteration of ^{13}C-labelled amino acids accomplished by any individual organism may be expected to depend on the time elapsed between it beginning algae consumption, and experiment termination. However, neither the PC1 scores from PCA of the whole fauna data set, nor those from PCA of single taxon sub-sets showed correlations with experiment duration (given in Table 5.1). Post-monsoon 2 and 5-day experiments at the 140m site showed no difference in cirratulid PC1 scores (Fig. 5.13A), and no difference in total fauna glycine enrichment factors or total amino acid uptake (Figs. 5.4, 5.9, 5.10).

Similarly, the percentages of total and individual amino acids (natural plus labelled types) present in the labelled form did not increase significantly between post-monsoon 2 and 5-day experiments at the 140m site ($P = 0.34$ for total amino acids), and the slight increase between the post-monsoon 940m site 2-day in situ and 5-day shipboard experiments was more likely to have been due to difference in dosing levels than to the different experiment durations.

The exception to this pattern was that the relative abundance of natural (unlabelled) glycine was lower in animals from the 5-day experiment than in those from the 2-day experiment at the 140m site (post-monsoon) (Fig. 5.3A). This could be due to more replacement of natural glycine with labelled glycine after feeding on the labelled algae of longer in the 5-day experiment.

Total uptake values for single taxon groups revealed slight duration dependency. For the cirratulids at the 140m site, there was a slight increase in total uptake after the longer experiment (Fig. 5.10B). Similarly for samples of *Linopherus sp.*, total uptake was less for the post-monsoon 2-day *in situ* experiment than for the shipboard 5-day experiment in the same season, (Fig. 5.10C).

Thus, the main effect of experiment duration was on the total uptake of labelled amino acids. Longer experiment durations did not produce steadily increasing degrees of compositional alteration. The ^{13}C-labelled amino acid suite may therefore reach a plateau or equilibrium, where assimilation, transformation and loss processes balance to produce an unchanging composition over the timescale of a few days. Loss processes may include a wide range of decay / metabolism, egestion and biochemical transformation pathways.

5.5.4.7 Summary of Controls on the Degree of Biochemical Alteration

Distinct differences in ^{13}C-labelled amino acid suite alteration were observed among faunal groups. Specifically, *Linopherus sp.* caused a greater degree of biochemical alteration than the cirratulids, and the foraminiferan *Uvigerina sp.* showed an alteration pattern very different form that of the metazoan macrofauna. These taxon specific variations, together with the inhibitory effect of low oxygen availability seem to exert the strongest controls on the extent of biochemical alteration during digestion. In addition, the availability of abundant high quality OM seems to support faunal communities capable of the greatest label uptake and alteration, but the depth from which fauna were recovered and experiment duration did not exert strong influences on the degree of suite alteration.

5.5.5 Essential Amino Acid Requirements

Of the 20 protein amino acids, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan in many animals, and also arginine and histidine in mammals, are 'essential', meaning they cannot be synthesised by those animals, and are required in the diet. Studies of flour beetles, carpet beetles and honey bees have confirmed that amino acid essentiality in these species is similar to that in rats, dogs, chicks, mice and humans (Meister, 1965). It therefore seems likely that the same pattern of essentiality will follow for marine polychaetes. The non-essential amino acids are aspartic and glutamic acids, proline, alanine, glycine, serine, tyrosine, cystine and asparagine, which can be synthesised by most heterotrophs using other amino acids and biochemicals. Among microbes, essentiality is variable, with some species capable of synthesising all the protein amino acids just like autotrophs, and some requiring at least half of them in the diet (Meister, 1965).

5.5.5.1 Glycine

The labelled glycine enrichment observed in faunal tissues in this study suggests that glycine was preferentially assimilated during macrofaunal digestion, and is not of low food quality as previously suggested (Dauwe and Middelburg, 1998). Glycine is the most simple of the protein amino acids, and the enrichment of glycine in animal tissues has two possible explanations. Firstly, it may be that as glycine is a required pre-cursor in the synthesis of many important biochemicals (Lehninger, 1982), it was selectively assimilated. Alternatively, the large relative abundances of labelled glycine found in faunal tissues may be an intermediate product of transformation processes involving other ^{13}C-labelled amino acids. The fauna may have assimilated many amino acids with similar efficiencies, and then initiated such processes, which could create large but dynamic pools of labelled glycine.

Glycine is a 'non-essential' amino acid (not required by most animals in their diets), and is synthesised by heterotrophs through the removal of a carbon atom from the backbone of serine (also non-essential). However, the enrichment of faunal tissues with ^{13}C-labelled glycine may be explained by its biochemistry.

Glycine is known to be concentrated in the fibrous tissues of polychaetes (Mayer at el., 1995) and is thought to be an important solute in extracellular fluids, used for osmoregulation (Oglesby, 1978). It is also an ingredient in the synthesis of several fundamental biochemicals including porphyrins, pre-cursors to pigments and haemoglobin, creatine, essential in muscles and nerves, and purine nucleotides which form part of the backbone of DNA (Lehninger, 1982). Therefore, fauna may require more glycine than is evident from its natural mole% contribution in their tissues, although it is unclear why the size of the ^{13}C- labelled glycine dynamic pool would be larger than that which is naturally present (Fig. 5.14).

The existence of a dynamic glycine pool is consistent with the lack of correlation between the degree of glycine enrichment and experiment duration (Figs 5.9, 5.10). It implies that the ^{13}C-labelled glycine concentrations in faunal tissues were not straightforward accumulations of assimilated glycine, which might be expected to increase with time, but dynamic pools, the size of which reaches a maximum when production and transformation processes are equal in rate. The production of labelled glycine in faunal tissues is also consistent with the observation of Thomas

and Blair (2002) that faecal pellets, as well as faunal tissue, become enriched in labelled glycine. In order for this to be the case, labelled glycine must be produced during digestion.

5.5.5.2 Other Amino Acids

Comparison of fauna and algal ^{13}C amino acids suites is not necessarily sufficient to determine which compounds are selectively assimilated. It is possible for an amino acid to constitute a greater proportion of the labelled algae than of the assimilated suite, but for the assimilated proportion to still be greater than the proportion it constitutes of the natural composition of the fauna. This could still be considered preferential assimilation. For example, valine, leucine and isoleucine were never present in fauna ^{13}C-labelled amino acid suites in proportions as great as they were in the source algae, but they are all taken up in greater proportions than those in which they are naturally present in fauna (Figs. 5.5-5.8 and 5.14). Thus, there was selection in favour of these compounds.

Attempts can be made to link the preferential assimilation or egestion of particular ^{13}C-labelled amino acids to their sources and roles in the functioning of organisms. For example, ^{13}C-labelled ornithine was observed to accumulate in faunal tissues (Figs 5.5-5.8), and to have a positive loading in most PCA analyses performed (Fig. 5.11). Ornithine is not a protein amino acid, but is a breakdown product of arginine, and is part of the urea cycle. Thus, the accumulation of labelled ornithine is consistent with active amino acid transformations by the fauna. Also, aspartic acid, which was preferentially assimilated by the foraminifera, is thought to play a major role in providing a pattern for the creation of the calcite test. Thus this aspect of amino acid uptake can also be linked to animal physiology.

It might be expected that essential amino acids would be preferentially assimilated over non-essential amino acids. There is mixed evidence for this. The ^{13}C amino acids which were enriched in animal tissues compared to the source algae were all either non-essential, or non-protein compounds, but the essential amino acids leucine, isoleucine and valine were indeed assimilated in greater proportions that those in which they are present in faunal tissues.

Conversely, essential amino acids may only be preferentially assimilated if their availability is usually limiting. Dauwe and Middelburg (1998) noted the difficulty of gauging whether the availability of some essential amino acids is limiting, due to the possibility that gut bacteria may provide a supply additional to that in the sediment. However, it might be expected that fauna will be most efficient at assimilating compounds that are usually limiting. The availability of glycine in the sediment was less than its relative abundance in faunal tissue (Fig. 5.1), and this may also be a reason why the fauna in this study assimilated glycine more efficiently than any other amino acid. The difference in amino acid suites between sediments and fauna may however be an artefact of the long residence time of glycine in fibrous tissues, which do not require rapid replenishment (Mayer et al., 1995).

Leucine was also relatively rare in the sediments, and was taken up in greater proportions than it was present in tissues. Alanine and aspartic acid were however both less abundant in the sediment than in fauna tissues, yet were taken up as smaller proportions of the ^{13}C labelled suite than those which they constituted of the natural fauna (Figs. 5.1 and 5.14). Other amino acids that are depleted in the sediment compared to fauna (Fig. 5.1), and of which preferential assimilation would be expected, included tyrosine, methionine and arginine. Yet, where information is available, preferential assimilation has not been observed, either in this, or previous studies, except for methionine (Mayer et al., 1995; Thomas and Blair, 2002). Conversely, valine was relatively abundant in the sediment, but tended to be assimilated in greater proportions than that in which it is present in the fauna. Thus there is conflicting evidence for the selective assimilation of limiting compounds. Factors other than fauna and sediment composition, such as individual amino acid turnover time in fauna tissues, specific physiological function, and sources of some amino acids from gut bacteria, must also control which compounds are really limiting, and thus subject to selective assimilation (Dauwe and Middelburg, 1998).

Alanine, aspartate and asparagine are all synthesised using glutamate (which in this study was converted during hydrolytic extraction to glutamic acid). Tyrosine is created by the addition of a hydroxyl group to phenylalanine, and cysteine is the result of adding a methionine S atom to serine. This leads to the suggestion that labelled glutamic acid, phenylalanine, serine and methionine might be assimilated in

comparatively large proportions. Once again, there was no strong evidence for this (Figs 5.5-5.8), but as stated above, other factors, which cannot be measured here, must also play a part in determining demand.

In summary, patterns of ^{13}C-labelled amino acid uptake appear in some cases (glycine and ornithine) to be partially dictated by essentiality, supply versus demand, and other physiological factors. In other cases however knowledge of amino acid availability, and requirements for certain processes, produces expectations of preferential assimilation that are not supported by the data. The synthesis pathways and uses of various amino acids given above are by no means a comprehensive catalogue of the complex biochemistry of amino acids. A combination of other requirements, processes and residence times undoubtedly also determine assimilation patterns, and the data serve to highlight that the static natural amino acid suites of fauna do not necessarily represent their dietary requirements.

5.5.6 Evidence for the Impact of Faunal Digestion on the Composition of Sedimentary OM

Deep sediment mixing appeared to be a comparatively minor process on the Pakistan margin, and was almost un-measurable over the timescale in question, based on the depth of bulk ^{13}C and ^{13}C-labelled amino acid penetration in sediments (Figs. 5.15-5.17 and chapter 4). This is in contrast to previous continental margin isotopic tracer studies, (e.g. Blair et al., 1996, Levin et al., 1997), where significant proportions of the added label were recovered from as deep as 2 cm, and sub-surface peaks were found up to 10 cm downcore after comparable periods. Labelled fauna were however recovered deep as 8 cm (Table 5.1, and chapter 4).

Sediment ^{13}C-labelled amino acid suites and the way these changed downcore must therefore be interpreted with care. Smearing during core sampling means that some labelled amino acids detected below the surface would not have passed through the guts of fauna. In addition, the labelled biochemicals were rapidly diluted downcore, with the result that minor compounds fell below detection limits, allowing major constituents like glycine to become relatively more significant. This is however a comparatively minor problem, as most minor amino acids were detectable in sub-surface samples. These caveats must be considered when examining the data for

evidence of macrofaunal digestion impacting the amino acid geochemistry of the sediments.

In the three cores studied, a down-core increase in glycine mole% (to ~ 1.5-2 cm) was observed, accompanied by a decrease in the mole % of alanine (Figs 5.15-5.17). These two changes were also observed between faunal samples and the source algae, suggesting there may be a link between faunal digestion and sediment geochemistry.

In this study, faecal pellets could not be collected and analysed, but in microcosm studies Thomas and Blair (2002) found ^{13}C-labelled glycine enrichment both in faunal tissues and in faecal pellets, suggesting that the mechanisms for faecal-pellet and sediment glycine enrichment may be the same, and further indicating that faunal digestive processes may impact on sediment geochemistry. In the present study, fauna, as tissues or via OM that had passed through their digestive tracts, may equally have contributed to the observed 13C-labelled amino acid signatures of the shallow downcore sediments.

Downcore sediments form the 300m site exhibited reduced levels of glycine enrichment relative to those from the 140m and 940m sites (Fig. 5.16). This may be due to the absence of macrofauna from this site. The faunal community here was instead dominated by foraminifera, which were observed to cause little or no glycine enrichment.

A comparison of PCA amino acid factor coefficients generated by analysis of faunal data with those published for natural, decaying sediments (Dauwe et al., 1999), allows further assessment of the relationship between digestive alteration and sediment decay processes previously seen as purely or predominantly microbial. The factor coefficients generated by fauna and sediment PCAs in this study were largely similar to those generated by Dauwe et al. (1999) (Figs. 5.8A,C). For example, in all cases, glycine and β-alanine tend to accumulate during decay (positive loading as plotted), and valine, leucine and isoleucine tend to be lost. Thus, the amino acid factor coefficients suggest that early diagenesis due to faunal digestion and overall sedimentary decay processes (generally thought to be predominantly microbial) appear to be fundamentally similar in terms of amino acid suite alteration.

A further way to investigate whether digestion significantly impacts on sediment amino acid geochemistry is to compare preferential assimilation and egestion of

amino acids with typical sediment composition. If the ^{13}C-labelled amino acids preferentially assimilated by fauna match those typically observed to be particularly reactive, then it could be said that a clear macrofaunal signal had been detected in sediment amino acid geochemistry. Glycine and tyrosine were observed to accumulate in faunal tissues, but while the latter is generally reactive and decays comparatively early (Suthhof et al., 2000), the former consistently displays preferential preservation (e.g. Wakeham et al., 1997, Dauwe and Middelburg, 1998). Thus, there is no clear evidence that macrofaunal uptake alone is responsible for the loss of more reactive amino acids from the sediments.

Labelled isoleucine, leucine and alanine did not accumulate in either fauna or sediments (Figs 5.5-5.8, 5.15-5.17). They are all amongst the group of amino acids classically thought to be most reactive and prone to preferential decay (e.g. Dauwe and Middelburg, 1998), and this would account for their apparent disappearance from the "sediment-fauna system".

Labelled valine, threonine and proline did not appear in the fauna in the proportions in which they were present in the algae (Figs 5.5-5.8), but they did constitute the same or slightly greater proportions of the sediment natural amino acid composition as of the fauna (Fig. 5.1). Therefore they showed a preferential egestion signal, but only threonine has been observed to accumulate in the sediment over time. Thus, for a few amino acids, preferential assimilation and egestion patterns were consistent with previously observed accumulations and losses during sediment decay, suggesting that faunal processing and digestion is partially responsible for sediment amino acid composition.

Downcore trends in sediment ^{13}C-labelled amino acid suites were partially consistent with the relative reactivities observed in sedimentary diagenetic sequences (e.g. Dauwe and Middelburg, 1998). The mole percentages of alanine, isoleucine and phenylalanine decreased downcore, that of serine remained roughly constant, and the mole percentage of glycine increased, and these trends are consistent with previous work. However, leucine and tyrosine are typically among the more reactive amino acids, but were observed to remain constant downcore, while the usually stable aspartic acid was preferentially lost. These deviations from normal sedimentary

amino acid decay patterns may be partially due to the unusually fresh nature of the labelled algae, and possible detection problems with deeper samples.

Thus, to some extent, the ^{13}C-labelled amino acid suite alterations observed in sediments in this study are consistent with those observed during decay of natural sedimentary OM. Some of these alterations can be related directly to macrofaunal digestion, however others cannot, and must therefore be related to other, possibly microbial processes.

Both fauna and sub-surface ^{13}C-labelled amino acid suites were enriched in β-alanine (Figs. 5.5-5.8, 5.15-5.17). β-alanine is a non-protein amino acid, and is thought to be a decay product of aspartic acid (Cowie and Hedges, 1994). The accumulation of β-alanine in fauna and sub-surface sediment samples provides direct evidence for it being a decay product, and suggests that its occurrence may be at least partially due to faunal digestive processes.

The analytical technique developed for this study (chapter 3) could be employed in future experiments using labelled aspartic acid and glutamic acid to further elucidate the conditions under which aspartic and glutamic acids decay to produce labelled β-alanine and γ-aminobutyric acid, and so resolve the question of whether they represent just microbial, or other decay processes.

5.5.7 Experimental Technique

Both the experimental and analytical techniques used in this study are novel, and combined, have proved capable of generating detailed and comprehensive information regarding the uptake and alteration of OM by benthic faunal communities.

Comparisons are available between *in situ* and shipboard experiments at the 140m, 300m and 940m sites for a range of measurements. Total ^{13}C uptake by various faunal groups, together with benthic nutrient and oxygen fluxes were similar when measured in shipboard versus *in situ* (Schwartz et al., in prep.) experiments, suggesting the artefact associated with recovering cores did not have a significant impact on experimental results.

Most of the samples available for this study were from experiments conducted aboard ship, with only one in-situ experiment, at the 940m site, providing sufficient sample for specimens to be available for amino acid analysis.

The degree of glycine enrichment displayed by *Linopherus sp.* from the post-monsoon shipboard 5-day experiment at 940m was less than that in animals from the 2-day lander experiment at the same site ($P = 0.003$), despite the fact that overall amino-acid uptake was greater in the shipboard experiment (Fig. 5.10C). Samples of *Linopherus sp.* from the in-situ experiment also had higher PC1 scores than those from the shipboard experiment (Fig. 5.13B). This suggests that the disruption of being brought to the surface may have produced artefacts in faunal behaviour and functioning, and highlights the importance of *in situ* studies.

Thus, while many aspects of sediment community functioning were not significantly impacted by core recovery, other aspects, in this case digestion efficiency may be. These two experimental techniques each have their advantages and weaknesses, and are strengthened when used in conjunction.

5.5.8 Further Work

The data collected in this study potentially contain even more details of taxon specific OM alteration, but the analysis of this aspect of the data was hampered by lack of replication, and the fact that feeding durations could not be controlled or measured. The application of the analytical technique used here to samples from controlled and replicated microcosm feeding studies would allow a more detailed description of digestive alteration. The collection of separate gut contents and faecal pellets would allow construction of quantitative budgets and assimilation efficiencies for individual amino acids, and direct measurements of gut residence and turnover times would also be very useful. In addition, experiments of longer durations (on the order of weeks) should be conducted to further develop the links between faunal digestion and sediment geochemistry, and so link up our knowledge of very early and later diagenetic processes.

5.6 Conclusions

- The experiments conducted in this study were unprecedented parallel *in situ* and shipboard tracer studies of whole-community benthic faunal processes under wide-ranging conditions. These have been accompanied by a novel analytical technique that permits sensitive tracing of OM at the molecular level, producing a detailed description of faunal digestive processes.
- Digestion and processing of organic matter by benthic fauna has been shown to significantly alter the amino acid composition of OM. In particular, the suite of amino acids assimilated was enriched in glycine and depleted in alanine compared to the fresh OM source.
- Many aspects of OM alteration during faunal digestion were consistent with the biochemical requirements and processes of the fauna. In particular, glycine appears to be produced in large amounts by metazoan macrofauna.
- Among the metazoan macrofauna, the pattern of amino acid uptake and assimilation was remarkably constant among taxa, but principle component analysis revealed subtle differences between polychaete families, related to feeding guilds / strategies.
- Foraminiferal OM processing, as reflected by *Uvigerina sp.*, appeared to result in markedly less amino acid compositional alteration, and where foraminifera dominated the benthic community, this signature was carried over into the sediment.
- Low oxygen availability appeared to inhibit the efficiency of biochemical alteration during digestion, while the natural availability of high quality OM tended to enhance it.
- While the exact duration of label processing by any specimen cannot be known, longer exposure to the labelled algae tended to lead to increased total amino acid uptake, but not to increased biochemical alteration, suggesting that the assimilated ^{13}C-labelled amino acid suite reached an equilibrium after a short time.
- The suite alterations seen in faunal tissues and in downcore sediments were broadly similar to those typically observed during natural sediment decay,

thus providing direct links between faunal digestion and the decay of sedimentary OM.

CHAPTER 6

The Distribution Of Pigments In Sediments Across The Pakistan Margin OMZ

6.1 Introduction

Photosynthetic pigments are a ubiquitous constituent of all types of natural organic matter (OM), from forest leaf litter to abyssal marine sediments. The pigments found in photoautotrophs are the coloured compounds used in light harvesting and energy transfer in the photosynthetic production of OM. Pigments in the marine environment are produced by phytoplankton, macroalgae and photosynthetic bacteria, and reach the sediment as sinking organic matter in the form of aggregates or faecal pellets. In coastal settings, terrestrial organic matter may also be a pigment source.

The most common pigments are the chlorophylls, one of which is chlorophyll-a, the primary light-collecting compound in most photoautotrophic organisms. Chlorophylls are composed of a group of five carbon ring structures coordinated by a Mg^{2+} ion, to one of which is attached a phytol side chain (Fig. 6.1A). Slight variations in the functional groups found on another of the rings produce the different chlorophylls a, b and c (Fig. 6.1). In addition to chlorophyll, plants and algae contain carotenoid accessory pigments. The accessory pigments are differently coloured than chlorophyll-a, and serve as supplementary light collectors, or to protect chlorophyll-a from photo damage. The carotenoids consist of two ring structures linked by a long carbon chain with alternating single and double bonds (Fig. 6.1B). Variations in the functional groups attached to the rings mark the differences between the assorted carotenes and xanthophylls. In some cases, photo-catalysed reactions transform one xanthophyll into another, and these compounds protect the light collecting pigments from photo-oxidation (Louda et al., 2002).

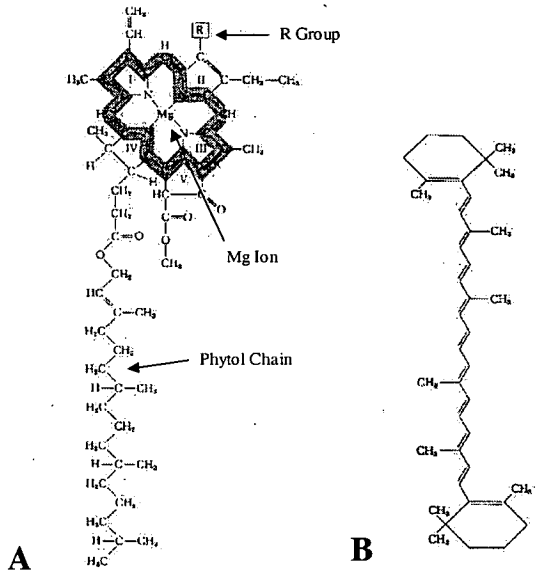


Figure 6.1. The structure of A) chlorophyll, and B) β-carotene (Lehninger, 1982). In chlorophyll-a, the R group is CH_3, in chlorophyll-b it is CHO, and in chlorophyll-c it is COOH.

Pigments are found in plant, prokaryote, fungi and photosynthetic cells. In eukaryotic cells they are arranged in chloroplasts, while in prokaryotic cells they are located on

infoldings of the cell membrane. Pigments do not persist 'free', but rather are incorporated into pigment-protein complexes. The binding proteins force close proximity between chlorophylls and carotenoids, allowing the latter to protect the former from photo-decay, and also alter the chief absorption wavelength, so that one pigment may perform different photosynthetic roles depending on how it is bound. Chlorophylls serve at least two purposes in photosynthesis. They are a constituent of light harvesting centres (LHC), where photon energy is absorbed and transferred into electrostatic energy. Chlorophyll-a is also present, bound to different proteins, in the reaction centres that surround LHCs, where electromagnetic interactions convert photon energy into the reducing power required to convert CO_2 into biochemicals.

The exact suite of carotenoids produced varies among classes of phytoplankton, and pigment assemblages can therefore be diagnostic of OM source (Jeffrey and Vesk, 1997). Bianchi et al (1996) for example used the presence of chlorophyll-b and zeaxanthin to deduce that chlorophytes, cyanobacteria and prochlorophytes were the dominant sources of pigments to Eastern Mediterranean sediments. Higher land plants are a source of the xanthophyll lutein, and green plants are found to contain β-carotene, lutein, violaxanthin and neoxanthin. Sanger and Gorham (1970), on examining the pigment content of lake sediments, found that those with organic matter derived from aquatic algae showed a much wider variety of pigments than those taking their organic matter from the surrounding leaf litter, and also noted that decaying matter yielded a wider variety of pigments as coloured decay products accumulated.

Chlorophyll decay proceeds through the loss of the Mg ion and / or the phytol side chain, producing a range of decay products collectively known as pheopigments. The loss of the Mg^{2+} ion alone results in the decay product pheophytin, the loss of the phytol side chain produces chlorophyllide, and the loss of both of these constituents results in pheophorbide (Yentsch, 1967). Pheophorbide can therefore be produced by two paths, depending on which structural element is lost first. Additional pheopigments exist besides pheophytin and pheophorbide, such as cyclic and pyropheophorbide, but these were not resolved by the techniques used in this study, in which only the more general groups of pheophytin and pheophorbide a, b and c could be resolved. Ultimately the pheopigments also decay to colourless porphyrins, which become preserved in the sediment for geological timescales.

The digestive processes of marine herbivorous zooplankton have been shown to produce pheopigments (Shuman and Lorenzen, 1975, Bianchi et al, 2000), and pheopigment abundances have thus been used as indicators of grazing in the water column. In fact, it is uncertain whether pheophorbide survives passage through the zooplankton gut, or that zooplankton grazing is the primary source of pheopigments. Shuman and Lorenzen, (1975) found 100% conversion of chlorophyll-a to pheophorbide as a result of grazing by *Calanus sp.* (on fresh algae). However, Villaneuva and Hastings (2000) used the correlation of pheophorbide-a with the acid-intolerant chlorophyllide-a to infer that both of these compounds were delivered to the sediments as products of diatom lysis and bacterial attack, not digestion. Also, Louda et al (2002) directly observed the formation of pheophorbide-a and pyropheophorbide-a in pure culture decay experiments in the absence of grazers. The use of these general compound groups to infer grazing is thus complicated by the fact that they are also produced by bacterial decay, and the activity of phytoplankton enzymes during cell senescence. More specific biomarkers for grazing such as steryl pyropheophorbide, carotenol pyrpheophorbide-a, and steryl chlorin esters have now been identified (Squier et al. 2002, Louda et al., 1998, Goericke et al., 1999), but once again, the identification of these compounds requires detection techniques not employed here.

The carotenoids also exhibit multiple decay mechanisms, including the reduction of double bonds in the carbon chain (Fig. 6.1), cleavage of the chain, aromatisation, and hydrolysis of esters attached to the ring structures (Watts and Maxwell, 1977, Repeta and Gagosian, 1984, Repeta, 1989, Hopmans et al., 2005). The last of these produces the fucoxanthin decay products fucoxanthinol and then fucoxanthinol 5'-dehydrate, which have also been shown to be products of grazing (Repeta and Gagosian, 1984). The wide array of compounds produced by these many carotenoid decay paths are generally only identified through the use of mass spectrometry.

Many studies have shown that both the rate and path of chlorophyll decay are dependent on the availability of dissolved oxygen, which is thus at least partially carried out by aerobic microorganisms (Louda et al, 2002, Bianchi et al, 2000, Sun et al 1993a, Sun et al 1993b). Working with relatively fresh OM, Sun et al (1993a) noted that under oxic conditions chlorophyll eventually decayed fully to colourless compounds, whereas in the absence of oxygen the decay product pheophytin was

stable and accumulated. These same authors (Sun et al 1993b) added further detail by observing three pools of chlorophyll in sediments; a), bound (in fresh OM, cells, faecal pellets and non-extractable with acetone), b) free and stable under anoxic conditions, and c) free and labile under anoxic conditions. The first two did not decay under anoxic conditions, possibly due to the exact pigment-protein complex or biopolymer into which they were incorporated, and the third did. Thus, the overall rate and pattern of pigment decay is likely to depend not only on dissolved oxygen availability, but also upon the relative distribution of pigments between bound and free pools, and conditions such as pH, which may affect the partitioning of pigments between the pools.

Louda et al. (2002) used the observation that chlorophyll alteration is retarded at low temperatures to suggest that the process is predominantly biological mediated. Sun et al (1993a) observed this relationship for oxic incubations, but not for anoxic counterparts, prompting the suggestion that only oxic decay paths are biologically mediated.

The decay of pigments in sediments is enhanced by the presence of infauna (Shuman and Lorenzen, 1975, Bianchi et al, 2000). Bianchi et al (2000) found the highest rate of chlorophyll-a decay to occur in oxygenated microcosms populated by a benthic amphipod, although they did not speculate whether the mechanism for this was purely the chemistry of gut passage, or whether irrigation of the sediment by infauna also played a role. By way of clarification of this point, Sun and Dai (2005) found that purely physical mixing did not accelerate chlorophyll-a decay as much as biological mixing, and thus both irrigation and digestive processes were inferred to enhance pigment decay. Sun et al (1993b), after removing meiofauna (all animals retained on a 0.125mm sieve) and microfauna (by freezing or microwave heating) from parallel experiments, concluded that the microfauna were the most active faunal group in pigment alteration (no mention was made of macrofauna).

Studies of deep-sea sediments receiving relatively degraded OM indicate that a certain fraction of sedimentary pigments and their decay products can become preserved, and persist for thousands of years, and have therefore been used as palaeoproductivity indices and measures of OM quality (e.g. Squier et al., 2002, Harris et al., 1996, Dahl et al., 2004).

Due to its relatively short half-life, chlorophyll-a can be used as a tracer of bioturbation, usually over a timescale of days to months. Provided the decay rate of chlorophyll-a for the particular setting is known, fitting a model, which considers only bioturbation and decay, to chlorophyll-a downcore profiles can yield estimates of bioturbation rates (e.g. Boon and Duineveld, 1998). Conversely, if bioturbation rates are independently determined, the same modelling approach can be used to study the way the rate of decay of chlorophyll-a varies between sites with different oxygen availability, benthic community, and OM quality. In addition, inventories of reactive and total chlorophyll-a can be combined with chlorophyll-a decay rates to generate estimates of the flux of chlorophyll-a to the sediment. If the percentage chlorophyll-a content of near-bottom suspended OM is known, this can be converted into a flux of organic carbon to the sediment (Boon and Duineveld, 1998).

The majority of previous studies of pigments in the marine environment have been concerned with the characterisation of new chlorophyll-a decay products, and the processes these products might reflect (e.g. Goericke et al., 1999). Other workers have concentrated on the factors controlling pigment decay rates (e.g. Sun et al., 1993a, b, 1994), but these studies have often been performed using fresh phytodetritus, or on sediments from shallow, coastal settings. Still others have sought to use the concentration of chlorophyll and its decay products in sediment core records as a proxy for past productivity (e.g. Dahl et al., 2004). Relatively few studies have made wide-ranging assessments of both chlorophyll and ancillary pigment concentrations in marine sediments, and still fewer have addressed the surficial and downcore pigment compositions of contrasting deep-sea sediments. Thus, pigment compositions of, and reactivities within, deep-sea sediments are poorly characterised, as are the ways these are influenced by oxygen, OM supply, water depth and faunal processes.

In this study the pigment abundances and compositions of Pakistan margin sediments were investigated. The sites studied exhibited marked contrasts in %Corg, OM quality, oxygen availability, structure and benthic community, and thus the effects of these factors on pigment preservation and decay in the deep-sea are discussed. The above introduction illustrates the range of information to potentially be gained from pigment data, including bioturbation rates, and OM quality, reactivity, source, and flux to the sediment. The acquisition of pigment data for this margin therefore fits

into a wider portrayal of continental margin benthic biogeochemistry, ecology, and functioning, and of the relationships between sediment geochemistry and faunal communities.

6.2 Methods

6.2.1 Study Site

Samples were collected from a series of five main stations (at water depths of 140m, 300m, 940m, 1200m and 1850m) and several additional stations (700m, 850m and 1100m) along a cross-margin transect of the Pakistan Margin. The margin features a mid-water oxygen minimum zone (OMZ) between depths of ~150m and 1000m, roughly coincident with which are maxima in OM abundance and quality, and minima in macrofaunal abundance and biomixing. Study sites were chosen to fall above (140m), within (300m), across the lower boundary of (700m, 850m, 940m, 1100m), and below (1200m and 1850m) this OMZ, in order to allow assessment of the relationships between pigment geochemistry and oxygen availability, OM quality and faunal community composition.

Monsoon-induced upwelling in the Arabian Sea leads to intense productivity and a subsequent pulse of organic detritus to the seafloor (e.g. Haake et al., 1993a). Sampling before and after the monsoon allowed assessment of the effect of this biannual OM pulse on seafloor pigment geochemistry. As pigments are one of the most reactive classes of biochemical (Wakeham et al., 1997), the two sampling periods after the monsoon could potentially allow a study of alterations in pigment chemistry over a time period of just a few weeks.

Station (Depth (m)	Temperature (°C)	Dissolved Oxygen ml L^{-1}	Sediment %OC	OM Quality (DI)	Macrofauna Biomass g(wet) m^{-2} / Diversity	Foraminifera Density / Diversity	Chlorophyll-a $t_{1/2}$ Used in Modelling / days
Pre Monsoon							
140m	22.5	2.05	1.46 ± 0.08		9 / 51 (± 5)	593 / 19	8.40
300m	15.5	0.10	2.36 ± 0.09		0.020 (± 0.022) / 2 (± 0.5)	549 / 18	14.03
940m	9.0	0.13	3.31 ± 0.12		62 (± 45) / 12 (± 1)	80 / 13	22.65
1200m	7.2	0.34	3.27 ± 0.26		0.4 (± 45) / 13 (± 2.6)	77 / 16	26.36
1850m	3.5	1.78	1.40 ± 0.10		9 (± 15) / 53 (± 6)	24 / 8	34.83
Post Monsoon							
140m	18.2	0.11	1.43 ± 0.07	-0.99 ± 0.06	5 (± 2) / 45 (± 3)	1163 / 20	11.44
300m	14.8	0.11	2.56 ± 0.29	-0.40 ± 0.12	0.013 (± 0.019) / 1	839 / 14	14.69
700m	11.2	0.14	2.59 ± 0.01				19.3
850m	10.1	0.14	3.22 ± 0.06				21.1
940m	9.3	0.17	3.40 ± 0.13	-0.48 ± 0.03	45.7 (± 0.02) / 13 (± 1)		22.65
1100m	8.0	0.24	2.96 ± 0.46				24.6
1200m	7.3	0.27	3.27 ± 0.26	-0.49 ± 0.18	7 (± 11) / 14 (± 0.7)		26.36
1850m	3.7	1.7	1.20 ± 0.25	-1.17 ± 0.14	2 (± 0.9) / 44 (± 4)		34.83

Table 6.1. Site conditions. Oxygen concentrations are from CTD casts, %Corg values are for the surface 0-0.5 cm, DI values are averaged over the surface 3 cm (Sandra Vandewiele, pers. comm.). Macrofauna diversity is species number per megacore averaged from 5 cores (Peter Lamont, pers.comm.), foraminifera density data are total number of calcareous individuals in 15 cm^2 of the surface 1 cm of sediment, and diversity data are species number for the same sample volume (Stefanie Schumacher, pers. comm.).

6.2.2 Sampling

Megacore samples were taken during cruises aboard the RRS Charles Darwin before (April-May), during the late stages of, and immediately after (22nd August-15th September) and well after (17th September- 20th October) the summer monsoon of 2003. The five main stations were visited in both pre- and post-monsoon periods, and two of these (140m and 300m) were also sampled in the late monsoon season (i.e. between pre- and post-monsoon periods). Where a site was visited in both of the later periods, it is called 'post site depth m a' for the earlier period, and 'post site depth m b' for the later period. Unless otherwise indicated, all late/post-monsoon samples are from the later period. The additional OMZ lower boundary sites (700m, 850m and 1100m) were visited only in the later post-monsoon period. A summary of site conditions and the sites studied in each season is given in Table 6.1.

Sediment cores were extruded and sectioned at intervals of 0.5cm to 2cm depth, then 1cm to 10cm depth, followed by 2cm to the bottom of the core (usually ~30cm). Samples were placed in plastic sample bags and frozen at -20°C.

6.2.3 Extraction

The sediment horizons 0-0.5cm, 0.5-1cm, 1-1.5cm, 1.5-2cm, 2-3cm, 4-5cm, 6-7cm, 9-10cm, 12-14cm, 16-18cm, 20-22cm and 28-30cm from each core were analysed, except where cores were shorter than 30cm, or where one of these horizons was missing (damaged/lost), in which case the closest horizon was used instead.

Extraction was performed by shaking aliquots of wet (thawed) sediment in a cell homogeniser, with glass beads and 90% acetone (10% Milli-Q), while cooling with rapidly expanded CO_2 gas (~ -20°C). The extracts were decanted away from the glass beads and centrifuged (1300 rpm) to remove sediment, and then analysed by HPLC.

6.2.4 Analysis

HPLC analysis was conducted on a Waters instrument using a Nova-pak C18, 4μm x 15cm reverse phase column with a Nova-pak C18 5μm guard column. The eluents were 0.5M ammonium acetate in 85:15 methanol:Milli-Q, followed by 90:10 acetonitrile:Milli-Q, then ethylacetate (Airs et al., 2001). The details of the

gradients used are given in chapter 2.

Chlorophyll-a	Pheophorbide	Pheophytin	Alloxanthin	Diatoxanthin	Zeaxanthin	β-Carotene	Total
8.6%	4.2%	8.5%	7.4%	8.9%	4.3%	7.3%	4.9%

Table 6.2. Average relative standard deviations for all compounds quantified, n = 33.

Pigments were quantified by integration of peak areas from chromatograms generated with a scanning photodiode array detector, and comparison with a single external standard. Absorption chromatograms were extracted for the wavelengths 450nm and 665nm. The former was used to quantify the accessory pigments, and the latter was used to quantify chlorophyll-a and the pheopigments. Full absorption spectra (200-700nm) from individual peaks were used to corroborate peak identification.

Two horizons from each core were analysed in duplicate, and average relative standard deviations were always below 9%(Table 6.2).

6.2.5 Data Processing

6.2.5.1 Correlation Analysis

Correlation coefficients (ρ) between pigment parameters and site conditions were calculated using the correlation function in the Microsoft Excel data analysis add in. The value ρ is equal to the covariance of two data sets, divided by the product of their standard deviations. In this study 15 separate cores were examined, therefore there were 13 degrees of freedom, and the 5% significance level for ρ was 0.514.

6.2.5.2 Principle Component Analysis

Principal component analyses of surficial and downcore pigment weight percentage data were carried out using Minitab. These analyses generated factor coefficients for each pigment, and scores for each sample, on the principle component 1 and 2 (PC1 and PC2) axes.

6.2.5.3 Modelling

Model curves were fitted to the downcore profiles of chlorophyll-a, pheopigments and accessory pigments. The model assumes first-order pigment decay, and diffusive-type (as opposed to advective) biomixing, and describes the transport-reaction behaviour of a pigment as equation 1:

$$[pig]_x = [pig]_o \, e^{-(k/Db)\,x} \quad (1)$$

where [pig] is the concentration of pigment at the sediment surface (o) or at depth x, k is the pigment decay constant (day^{-1}), and Db is the diffusive mixing rate (day^{-1}). Pigments decayed to non-zero, un-reactive background concentrations, and these were subtracted from the data before model fitting. The model inputs were surface pigment concentration, decay rate, biomixing rate and background un-reactive concentration, and model fitting was achieved using the Excel solver function, which altered a sub-set of the input parameters in order to minimise the difference between the modelled and actual data. It should be noted that the model required a non-zero bioturbation term, and thus was not entirely applicable to the laminated OMZ sites.

The model was used to produce bioturbation rates (Db) from chlorophyll-a data. For this, decay rates were fixed, thus as surface and background un-reactive concentrations were well constrained by the data, model fitting generated an estimate of biomixing rate. The half-life of chlorophyll used for this modelling was adjusted for the temperature at each site using equation 2 (Sun et al., 1994), which was derived from laboratory incubation of estuarine sediments. The half-lives used at each site are given in Table 6.1.

$$ln\ k = 18.34 - 6160\ T^{-1} \quad (2)$$

These decay rates apply to oxic (or suboxic) decay (oxygen present). Sun et al. (1993b) found that chlorophyll-a decay rates were similar in fully oxic and intermittently oxygenated sediments. Thus these decay rates were considered reasonable for all sites where the bottom water dissolved oxygen concentration was non-zero (all except the 300m and 700m sites).

Modelling of pheopigment and accessory pigment profiles was achieved by using the bioturbation rates generated by modelling of chlorophyll-a profiles, and allowing the solver function to alter the other input parameters. Thus estimates of accessory and pheopigment decay rates were generated, although the accuracy of these is dependent upon that of the chlorophyll-a decay rates used to generate bioturbation data.

Due to the shortcomings of the above model when applied to laminated sediments, chlorophyll-a and other pigment profiles from the laminated 300m and 700m sites were also modelled as described by equation 3 (Sun and Wakeham, 1994), using

previously published sediment accumulation rates (S = 0.06 cmy^{-1}) for the Pakistan margin (Cowie et al., 1999). Zero mixing was assumed.

$$[\text{chl-a}]_x = [\text{chl-a}]_0 \, e^{-(k/S)\,x} \quad (3)$$

This allowed the generation of pigment decay rates specific to the OM quality and other conditions found on the Pakistan margin.

Reactive pigment inventories were calculated as in equation 4:

$$Inventory = \Sigma([pig]\, x\, (1-\Phi)\, x\, \rho\, x\, \Delta d) \quad (4)$$

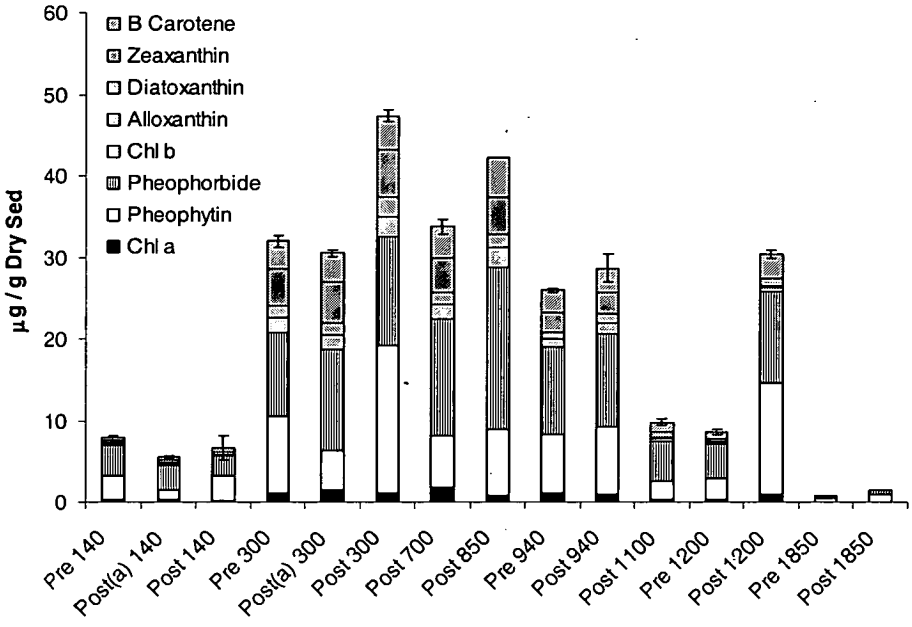
where [pig] is the reactive concentration (total – un-reactive background concentration) of a pigment in a depth horizon of thickness Δd, with porosity Φ, and ρ is the density of solid sediment taken as 2.65 gcm^{-3}. The inventories of each depth horizon were summed to the depth at which the reactive concentration became zero. Inventories were calculated from model-generated data, which had the advantage of being smoothed, and without sub-surface peaks.

6.3 Results

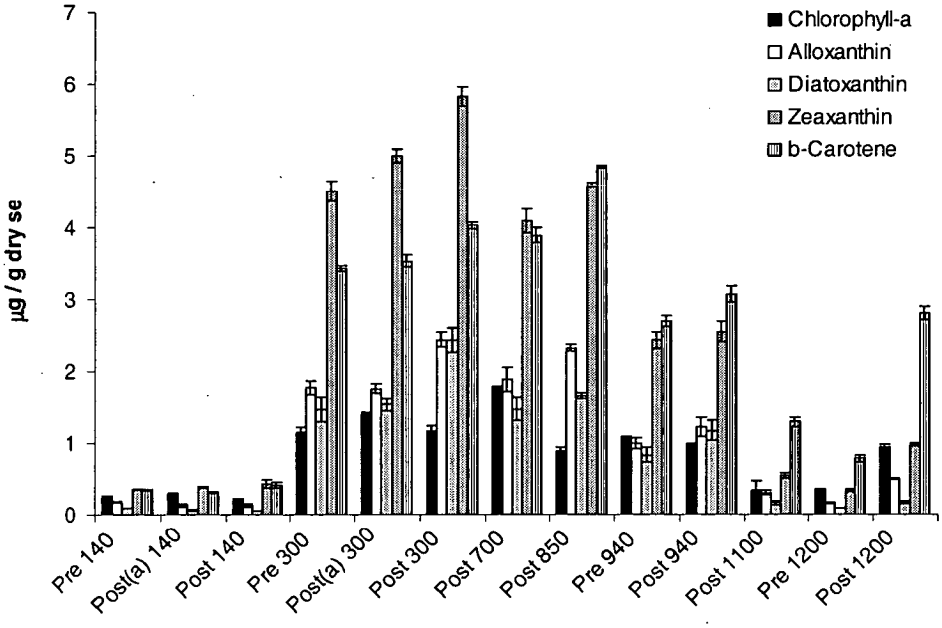
6.3.1 Total Pigment Distributions

The concentrations and suites of pigments averaged over the surface 1.5 cm of sediment varied across the Pakistan margin, and to a lesser degree, between seasons (Figure 6.2 and Table 6.3). The 1.5 cm interval was chosen in order to average out near-surface scatter, without being influenced by downcore changes.

In general, the OMZ sites (the 300m, 700m, 850m and 940m sites) displayed the highest total pigment abundances (average = 35.3 ± 8.0 μg/g dry sed). These were followed by the more oxygenated 1100m, 1200m and 140m sites (9.9 ± 0.2, 8.6 ± 0.3 and 6.8 ± 1.2 μg/g dry sed respectively), and lastly the fully oxygenated, OM-poor 1850m site (1.1 ± 0.5 μg/g dry sed) (Fig. 6.2A). Individual intact pigments showed the same cross-margin pattern (Fig. 6.2B), and the majority of them (chlorophyll-a, alloxanthin, diatoxanthin and zeaxanthin) were below detection limits at the 1850m site. The pheopigments also showed this pattern. The lowest concentrations were found at the 1850m site and then at the 140m site, followed by the 1200m, 940m,



A



B

Figure 6.2. A) pigment concentrations and suites averaged over the surface 1.5 cm, and B) intact pigment concentrations in the surface 1.5cm. All post-monsoon cores are from the later sampling period, except for the 140m and 300m site cores marked 'a', which were taken during the early post-monsoon sampling period Error bars represent 1 standard deviation of total pigment abundance.

700m, 850m and 300m sites, in that order (Table 6.3). Pheophorbide concentrations at the 940m site in both seasons were an exception to the general pattern.

Pheophorbide concentrations at the 940m site were higher than those at the 300m site, and they increased downcore (contrary to all other pigments, see below), with a marked sub-surface peak at 13cm depth (data not shown).

Site	Chlorophyll-a	Pheophytin	Pheophorbide	Total Pigments
Pre-Monsoon				
140	0.25 ± 0.02	3.1 ± 0.2	3.67 ± 0.05	8.0 ± 0.3
300	1.15 ± 0.08	9.4 ± 0.1	10.3 ± 0.4	33.0 ± 0.4
940	1.08 ± 0.01	7.2 ± 0.1	10.70 ± 0.04	26.5 ± 0.2
1200	0.35 ± 0.01	2.50 ± 0.08	4.3 ± 0.2	8.6 ± 0.2
1850	0.00	0.46 ± 0.01	0.35 ± 0.01	0.81 ± 0.01
Post-Monsoon				
140a	0.21 ± 0.01	2.97 ± 0.06	2.44 ± 0.05	5.6 ± 0.1
140	0.29 ± 0.03	1.1 ± 1.3	3.1 ± 0.1	6.7 ± 1.4
300a	1.17 ± 0.03	18.0 ± 0.4	13.4 ± 0.2	31.8 ± 0.2
300	1.41 ± 0.08	4.9 ± 0.1	12.3 ± 0.4	48.8 ± 0.4
700	1.78 ± 0.02	6.4 ± 0.2	14.3 ± 0.2	34.7 ± 0.3
850	0.89 ± 0.05	8.04 ± 0.05	19.90 ± 0.09	43.29 ± 0.06
940	0.98 ± 0.02	8.3 ± 0.2	11.3 ± 1.4	29.3 ± 1.6
1100	0.3 ± 0.1	2.3 ± 0.1	4.9 ± 0.3	9.9 ± 0.2
1200	0.95 ± 0.02	13.7 ± 0.2	11.3 ± 0.2	30.4 ± 0.4
1850	0.00	0.95 ± 0.04	0.52 ± 0.02	1.47 ± 0.06

Table 6.3. Concentrations of chlorophyll-a, pheophytin, pheophorbide, and total pigments averaged over the top 1.5cm of sediment. All are in units of µg/g dry sediment. All post-monsoon data are from the later sampling period, except for the 140m and 300m site values marked 'a', which were taken during the early post-monsoon sampling period.

6.3.2 Seasonal Trends

Total pigment concentrations increased slightly between pre and post-monsoon seasons at the 940m and 1850m sites, but the changes were almost within error. The post-monsoon 300m site core from the later sampling period indicated a marked seasonal increase in concentration, but that from the early sampling period did not. The seasonal variation in surface pigment concentration at the 140m site was within error. Thus, there was a seasonal increase in pigment abundance, but the effect was subtle, except at the 1200m site, where there was a marked increase in concentration (Fig. 6.2).

A similar pattern was evident for the individual intact pigments. All five of these compounds increased in concentration after the monsoon at the 300m and 1200m sites, and at the 940m site diatoxanthin and b-carotene, and at the 140m site

chlorophyll-a, zeaxanthin and β-carotene showed significantly higher concentrations (Fig. 6.2B). These increases were only significant in the surficial sediments, and did not persist downcore, consistent with an addition of fresh OM from the water column.

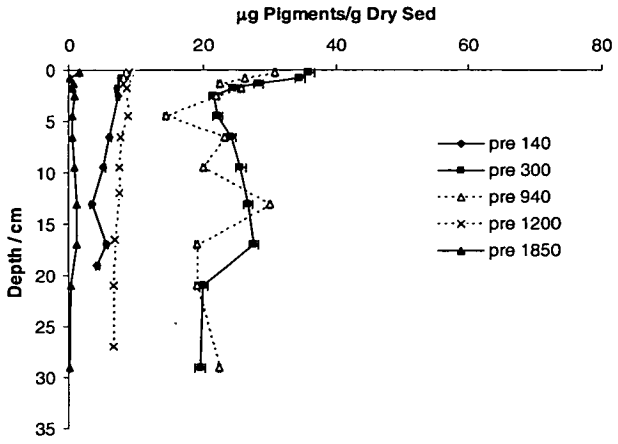
Chlorophyll-a concentrations in surficial sediments from the 140m and 300m sites were higher in early post-monsoon samples than in later post-monsoon samples (Fig. 6.2). Notably, this was not the case for the accessory pigments, which tended to show higher concentrations in the later post-monsoon period compared to the early period.

6.3.3 Downcore Profiles

The concentrations of total pigments down-core showed a similar cross-margin pattern to data for surficial sediments (Fig. 6.3), with the 940m and 300m, 700m and 850m sites having the highest surface values and retaining these throughout the general downcore decrease. The sites displaying low total pigment abundances at the surface (the 140m and 1850m sites) showed the lowest downcore concentrations, and less significant decreases downcore, presumably because the surface concentrations were closer to the un-reactive background values at those sites. During the pre-monsoon season, pigment concentrations at the 1200m site were similar to those at the 140m site both at the surface and downcore. In the post-monsoon season, the 1200m site exhibited high surface enrichment, followed by a marked downcore decrease to values similar to those at the 140m site. Downcore concentrations at the 1100m site were slightly higher than those at the 1200m site.

Downcore profiles of chlorophyll-a, alloxanthin, diatoxanthin and zeaxanthin and the pheopigments were similar to those of total pigments, and showed the same cross-margin patterns (Figs. 6.4-6.7).

β-Carotene was unique in that it did not show significant downcore decreases, except at the 1200m site in the post-monsoon season, due to very high surface concentrations. Pre-monsoon cores from the 1200m and 140m sites showed downcore increases between the surface and ~3cm depth, where concentrations levelled out and become constant with depth (Fig. 6.8).



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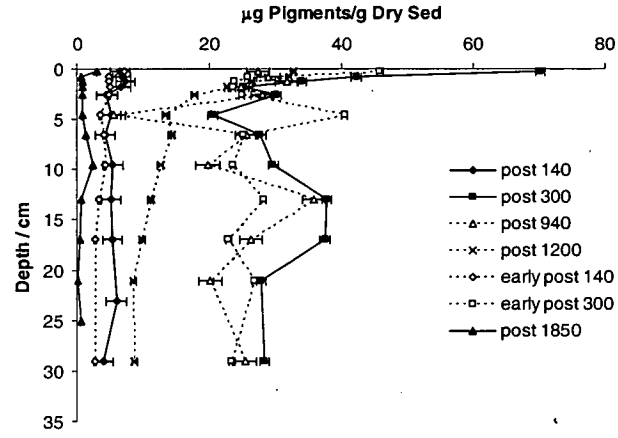

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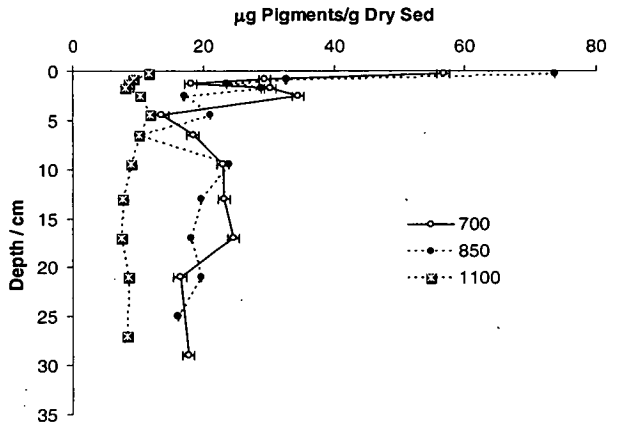
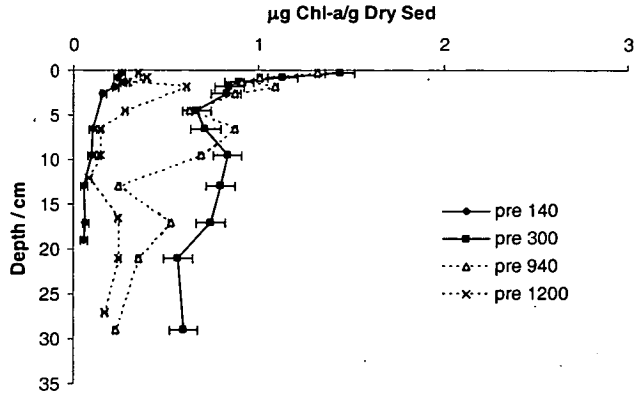


C

Figure 6.3. Downcore plots of total pigment concentrations A) pre-monsoon, main sites, B) post-monsoon, main sites, and C) across the OMZ lower boundary, post-monsoon. All post-monsoon cores are from the later sampling period, except for the 140m and 300m site cores marked 'early', which were taken during the early post-monsoon sampling period. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.



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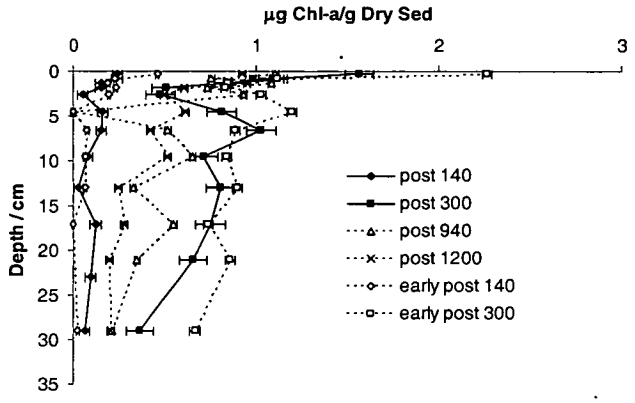

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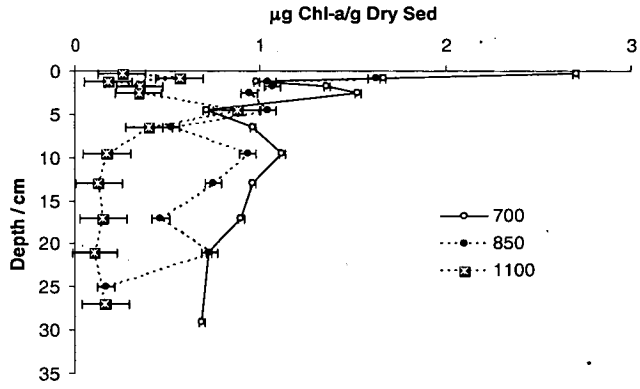
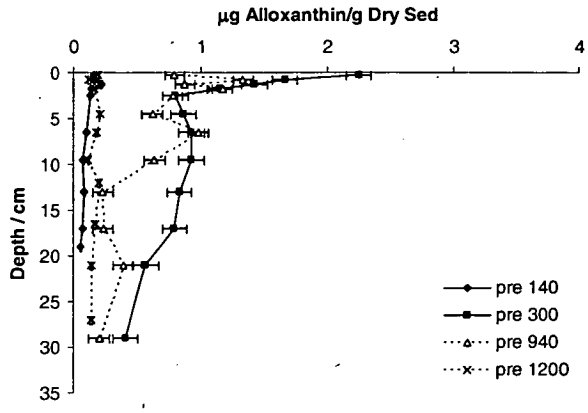


C

Figure 6.4. Downcore plots of chlorophyll-a concentrations A) pre-monsoon, main sites, B) post-monsoon, main sites, and C) across the OMZ lower boundary, post-monsoon. All post-monsoon cores are from the later post-monsoon period, except for the 140m and 300m site cores marked 'early', which are from the earlier sampling period. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.



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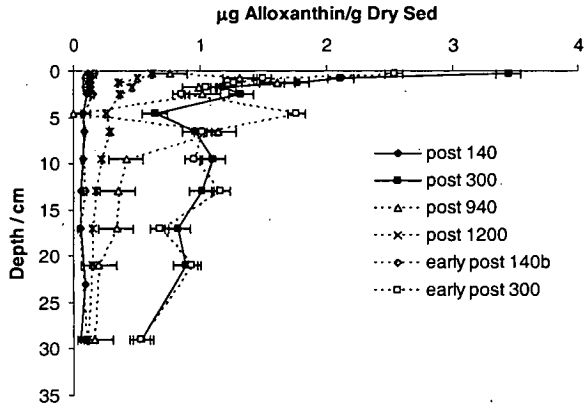

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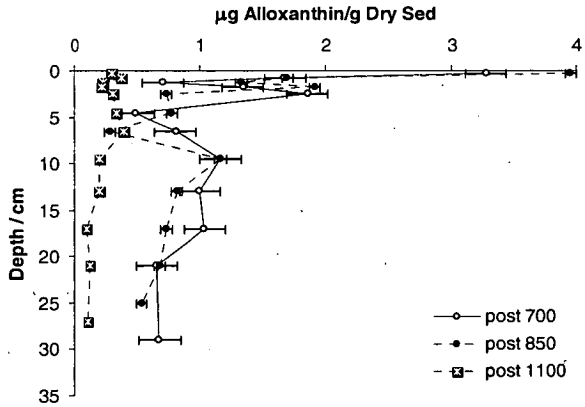
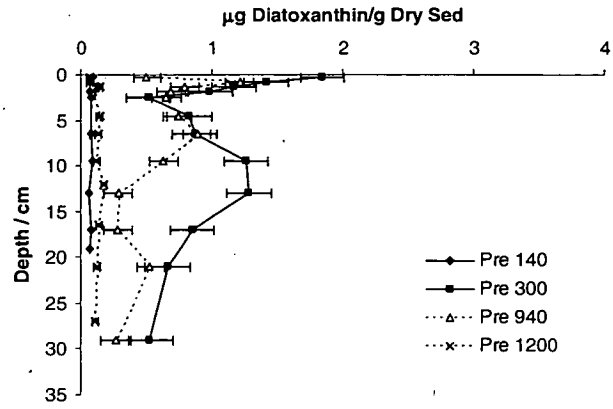


C

Figure 6.5. Downcore plots of alloxanthin concentrations A) pre-monsoon, main sites, B) post-monsoon, main sites, and C) across the OMZ lower boundary, post-monsoon. All post-monsoon cores are from the later post-monsoon period, except for the 140m and 300m site cores marked 'early', which are from the earlier sampling period. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.



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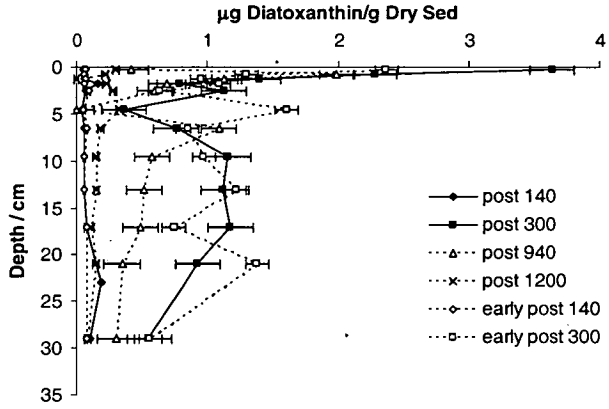

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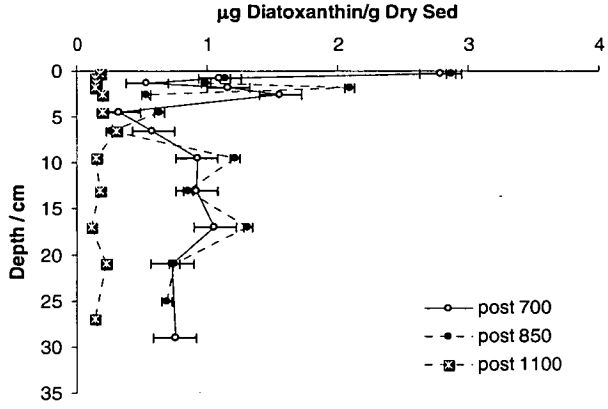
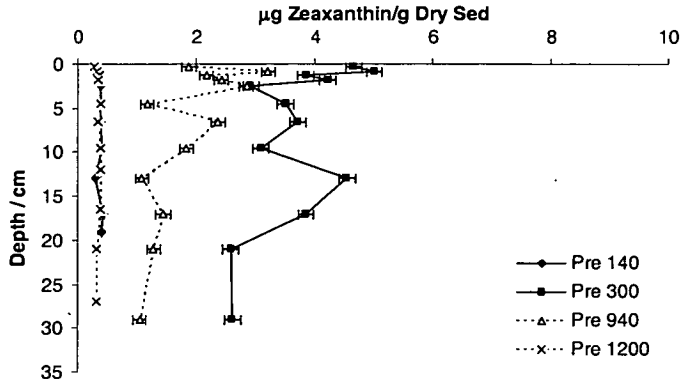


C

Figure 6.6. Downcore plots of diatoxanthin concentrations A) pre-monsoon, main sites, B) post-monsoon, main sites, and C) across the OMZ lower boundary, post-monsoon. All post-monsoon cores are from the later post-monsoon period, except for the 140m and 300m site cores marked 'early', which are from the earlier sampling period. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.



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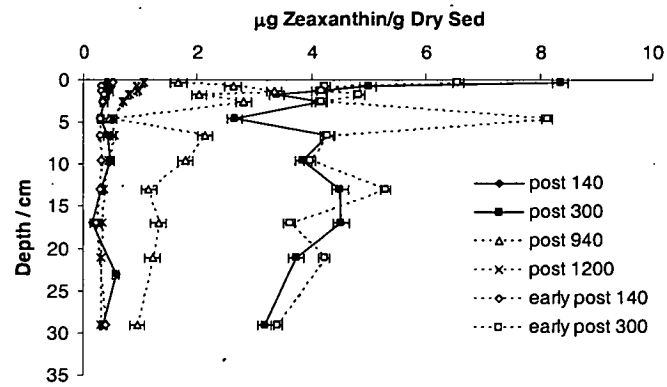

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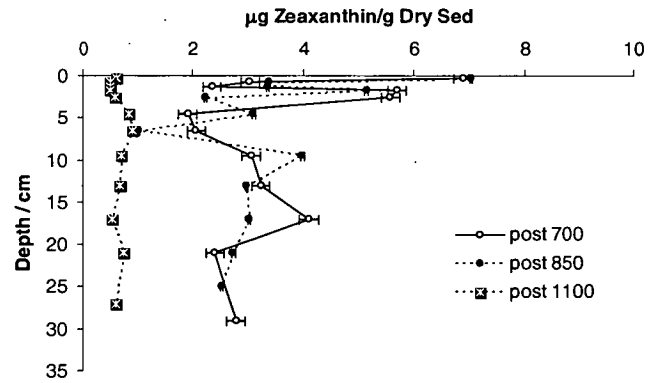
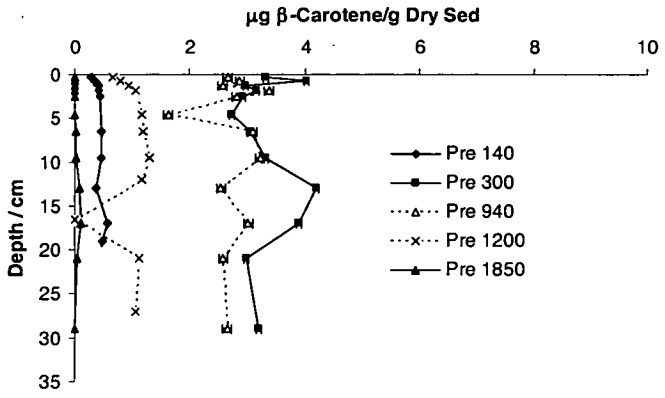
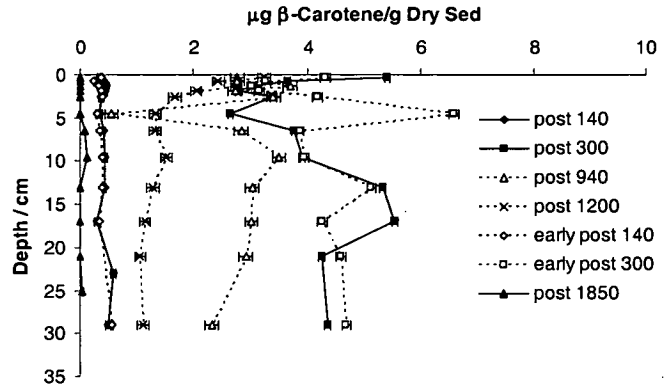


C

Figure 6.7. Downcore plots of zeaxanthin concentrations A) pre-monsoon, main sites, B) post-monsoon, main sites, and C) across the OMZ lower boundary, post-monsoon. All post-monsoon cores are from the later post-monsoon period, except for the 140m and 300m site cores marked 'early', which are from the earlier sampling period. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.



A



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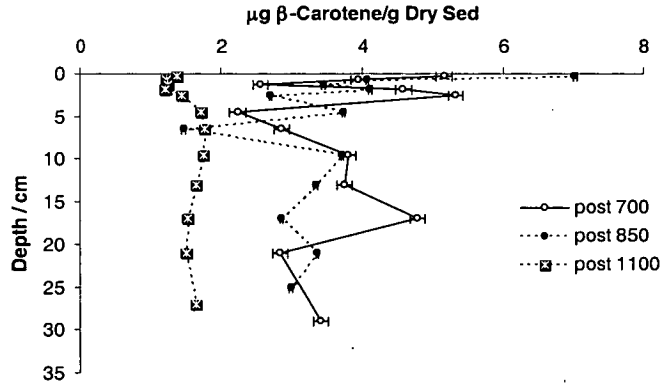


C

Figure 6.8. Downcore plots of β-carotene concentrations A) pre-monsoon, main sites, B) post-monsoon, main sites, and C) across the OMZ lower boundary, post-monsoon. All post-monsoon cores are from the later post-monsoon period, except for the 140m and 300m site cores marked 'early', which are from the earlier sampling period. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.

6.3.4 Pigment Suites

The suites of pigments in surficial sediments on the Pakistan margin were dominated by the chlorophyll degradation products pheophytin and pheophorbide (Fig. 6.2A).

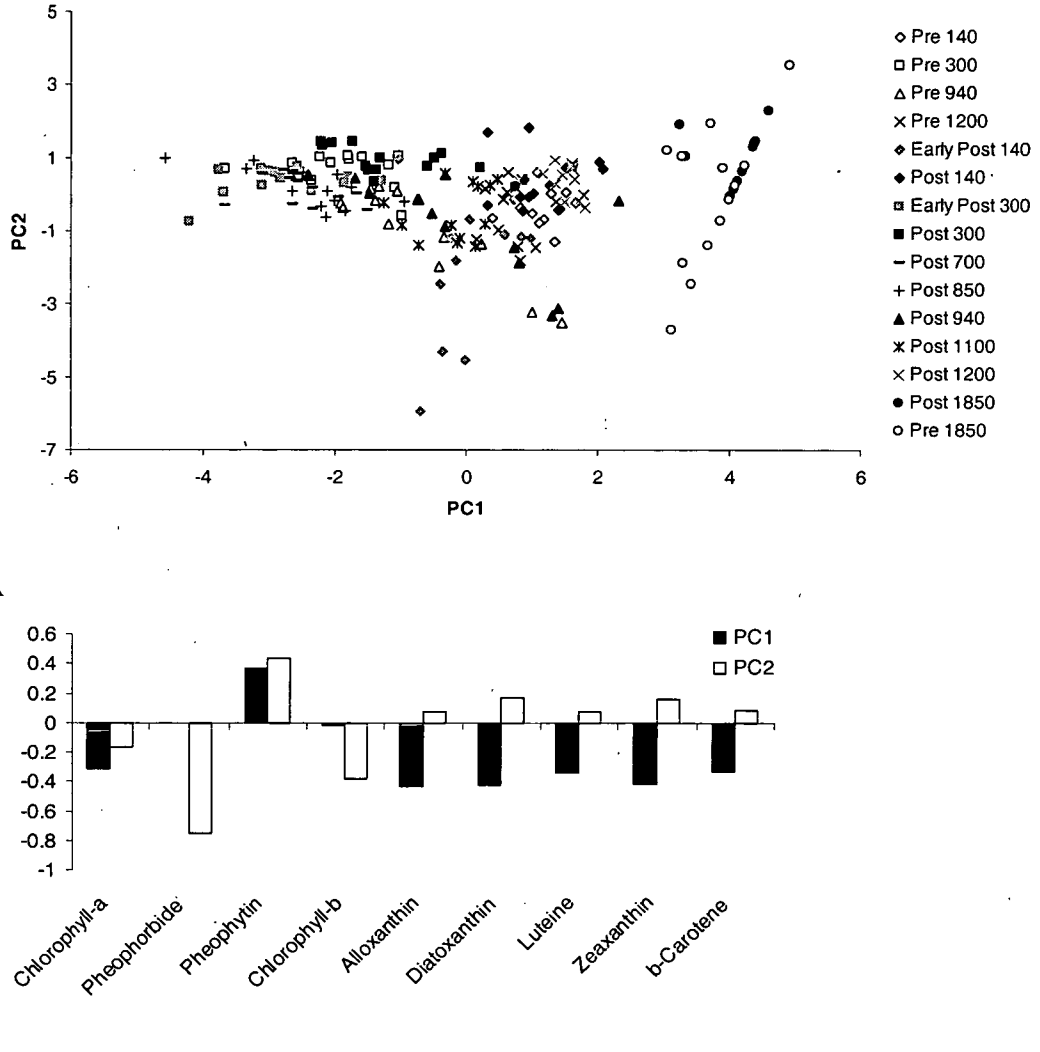


Figure 6.9. Results of PCA of pigment weight percentage data. A) Sample PC1 and PC2 scores, and B) pigment factor coefficients.

These constituted on average 33 ± 15 % and 41 ± 7 % weight percent of the total pigments, respectively. The next most abundant compounds were zeaxanthin and β-carotene, which comprised 7 ± 5 % and 8 ± 4 % of the total respectively, followed by chlorophyll-a, alloxanthin and diatoxanthin which constituted roughly 2-3 % weight percent each. Chlorophyll-b and lutein were minor, rarely-detected components.

The large standard deviations associated with these cross-margin and cross-season averages are a factor of considerable variation in pigment suites among sites.

Principle component analysis of the whole pigment data set produced site scores that separated sites along the PC1 axis (Fig. 6.9A), which accounted for 51.4 % of the total variance. The 300m, 700m and 850m sites had the lowest scores, followed by the 940m and 1100m sites. The 140m and 1200m sites were indistinguishable, and had higher scores again than the 940m site, and the 1850m site had the highest scores of all. Negative scores were a function of relatively high weight percentages of all the intact pigments (chlorophyll-a, alloxanthin, diatoxanthin, Zeaxanthin and β-carotene), and low weight percentages of pheophytin (Fig. 6.9B). Pheophorbide did not have significant influence over PC1 scores, but was the main determinant of PC2 scores. Sites were not separated along the PC2 axis, which accounts for only 16.9 % of the variance. The only feature of PC2 scores was that samples from the 140m and 1850m sites displayed larger PC2 ranges than those from other sites.

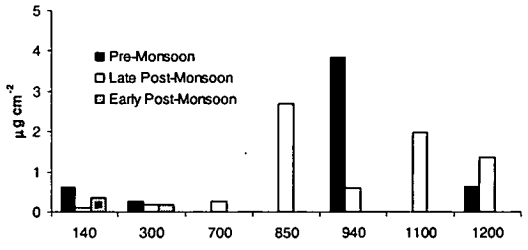
Neither PC1 nor PC2 scores separated samples by season.

Principle component 1 scores were generally constant downcore, except for downcore increases in all cores from the 300m and 940m sites, and very slight downcore increases at the 700m and 850m sites (data not shown).

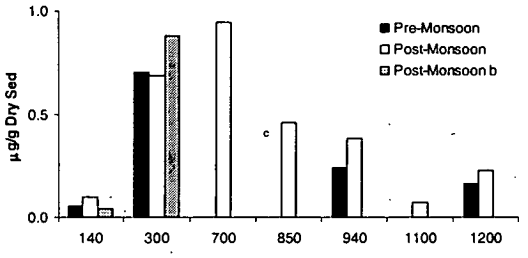
6.3.5 Total And Reactive Inventories, And Unreactive Background Concentrations

Reactive chlorophyll-a inventories were lowest at the 140m and 300m sites, and generally higher across the OMZ lower boundary, with maximal values at the 940m site in the pre-monsoon season (the post-monsoon value at A940 represented an anomalous low). Only the 1200m site showed a seasonal increase in reactive chlorophyll-a inventory that may suggest a seasonal OM pulse (Fig 6.10A).

In contrast, unreactive background chlorophyll-a concentrations were low at the 140m and 1200m sites, and high at the remaining sites, with maximal values at the 300m and 700m sites. Post-monsoon values at the 140m, 940m, 1200m and early post-monsoon 300m sites were higher than in the pre-monsoon season (and the later post-monsoon in the case of 300m) samples (Fig. 6.10B). There is however no



A



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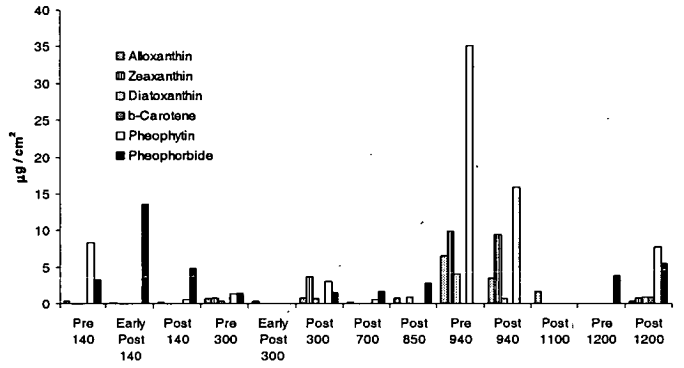

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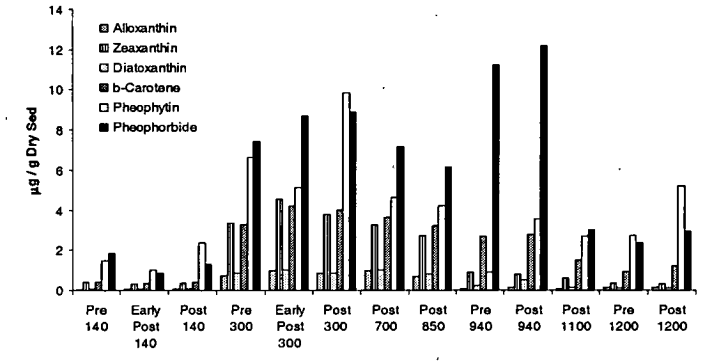


D

Figure 6.10. A) and C) The reactive inventories (µg cm^{-1}) of chlorophyll-a and other pigments respectively, and B) and D) un-reactive background concentrations (µg/g dry sed), of chlorophyll-a and other pigments respectively.

mechanism by which a seasonal pulse of OM could alter downcore un-reactive background concentrations, so these differences are likely to reflect within-site heterogeneity and/or analytical error caused by profile spikiness.

Season/Site	Chlorophyll-a	Alloxanthin	Zeaxanthin	Diatoxanthin	β-Carotene	Pheophytin	Pheophorbide
Pre 140	8	17				22	2
Post 140 a	11	35	12				458
Post 140	11	142				15	
Pre 300	14 / 3658	22 / 4605	31 / 5478	9 / 2907		21 / 4477	11 / 3291
Post 300 a	15 / 1143	45 / 2006	2 / 291	2 / 451		2 / 416	3 / 316
Post 300	15 / 20744	43 / 3551	8 / 1499	23 / 2593		15 / 2102	6 / 1271
Post 700	19 / 2554	7 / 1553	/ 282	/ 889		13 / 2078	7 / 1479
Post 850	21	1		1			
Pre 940	23	47	44	55		24	
Post 940	23	261	471	1		276	
Post 1100	25	29					
Pre 1200	26						13
Post 1200	26	8	12	125	3	7	4
Pre 1850						2	
Post 1850						5	

Table 6.4. Modelled pigment half-lives in days. Chlorophyll-a values are from Sun et al. (1994) (temperature adjusted) / value derived from modelling at the OMZ (at sites A300 and A700) assuming zero mixing and an accumulation rate of 0.06 cmy^{-1} (Cowie et al., 1999). Other pigment values are given as value derived from modelling using chlorophyll-a derived mixing rates / value derived from modelling at OMZ sites.

Reactive inventories of alloxanthin and zeaxanthin were greater than those of diatoxanthin, and reactive β-Carotene was only found at the 1200m site after the monsoon (Fig. 6.10C). Maximal accessory pigment reactive inventories were found at the 940m site, with similar values at the 300m and 1200m sites, and very little present at the 140m site (Fig 6.10C). Only the 1200m site showed a seasonal trend, with no reactive accessory pigments before the monsoon, and the full suite afterwards.

Accessory pigment unreactive background concentrations showed the same trends as chlorophyll-a. The 300m and 700m sites had the highest values, and the 1200m and 140m sites the lowest values (Fig. 6.10D).

Pheophytin showed maximal reactive inventories at the 940m site at the lower OMZ boundary, followed by the 140m and 1200m sites (Fig. 6.10C).

Maximal pheophorbide reactive inventories were found outside the OMZ, where the sediments were mixed, at the 140m and 1200m sites (Fig. 6.10D). Unreactive background pheophorbide concentrations were highest at the OMZ lower boundary 940m site, and throughout the OMZ (Fig. 6.10D).

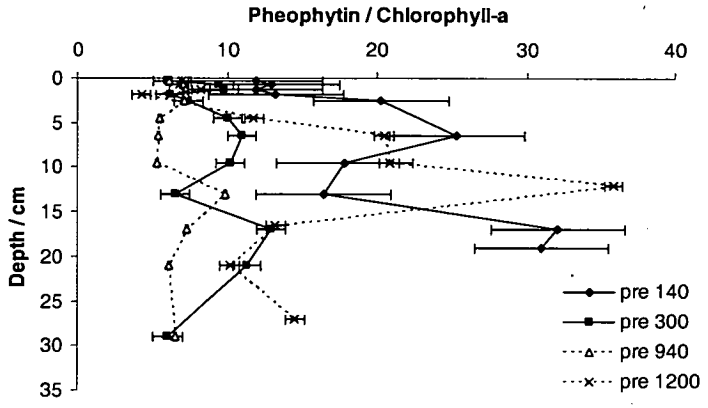
6.3.6 Modelling Results

Intact pigment decay half lives derived from modelling of downcore profiles from OMZ sites with laminated sediments (i.e. assuming zero bioturbation) had a range of ~300-5000 days (Table 6.4). Those for chlorophyll-a were 2 orders of magnitude longer than the half-lives derived from the temperature-dependent relationship proposed by Sun et al. (1994) (Table 6.1).

Mixing rates derived from modelling of reactive chlorophyll-a profiles revealed a cross-margin trend, with intense mixing above, across the lower boundary of, and below the OMZ (Table 6.5). Sites within the OMZ (300m and 700m) showed very low mixing rates. They were perhaps not as low as expected, however, given the laminated nature of the sediments, and this was likely due to the limitations of the model, which required a non-zero bioturbation term.

Site	D_b / d^{-1}
Pre-Monsoon	
140	1.7
300	0.04
940	2.6
1200	0.5
Post-Monsooon	
140a	0.08
140	0.1
300a	0.01
300	0.003
700	0.01
850	1.5
940	0.2
1100	5.9
1200	0.7

Table 6.5. Mixing rates (day^{-1}) derived from modelling of chlorophyll-a profiles. There is no data for the 1850m site as chlorophyll-a was undetectable there. All post-monsoon cores were from the later sampling period, except for the 140m and 300m site cores marked 'a', which were taken during the early post-monsoon sampling period



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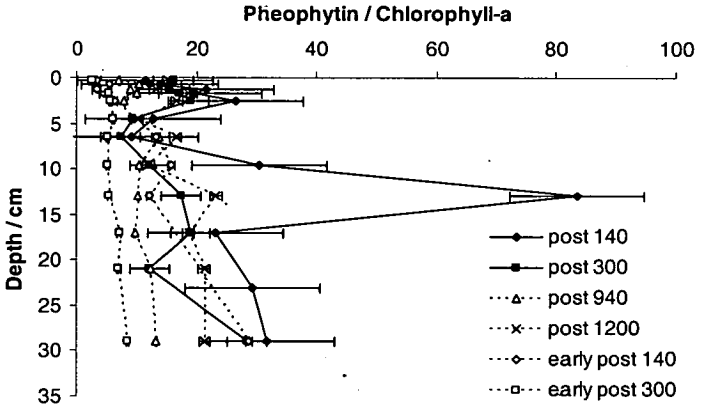

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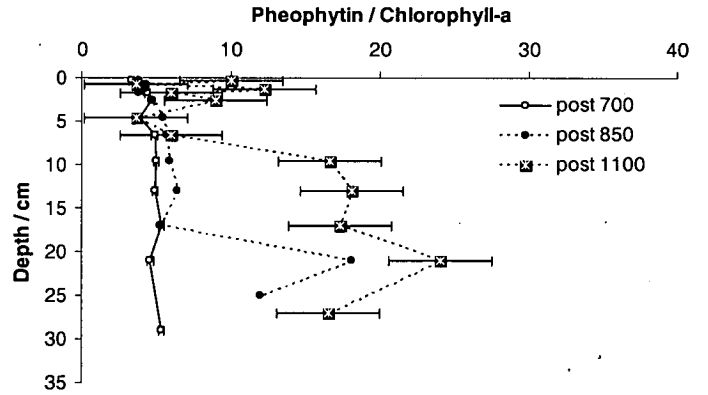


C

Figure 6.11. Downcore ratios of pheophytin : chlorophyll-a in A) pre- and B)post-monsoon seasons, and C) at the OMZ lower boundary. Note different X scale in B. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.

6.3.7 Pheopigment : Chlorophyll-a Ratios

Pheophytin/chlorophyll-a concentration ratios had an approximate range of 3-30, with the occasional sub-surface peak as high as 85. The 300m and 940m sites had similarly low and constant downcore values. The 140m site had the highest values, which increased downcore. The 1200m site had intermediate values, more similar to those at the 140m site than to those at the 940m and 300m sites, and showed a slight downcore increase (Fig. 6.11).

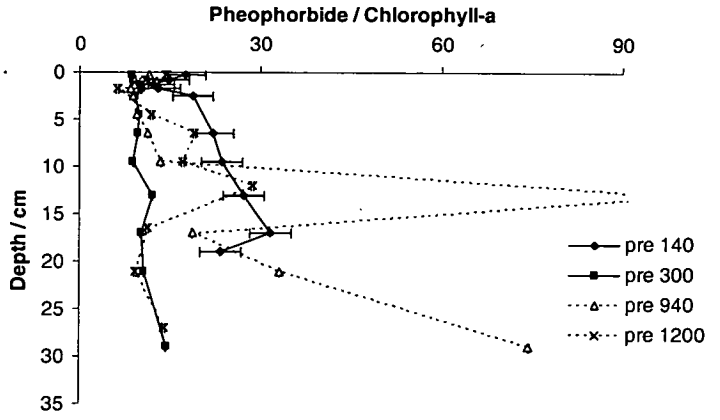
Pheophorbide/chlorophyll-a ratios did not show such marked differences between stations. The range was approximately 2-30, with some significantly higher sub-surface peaks. The 300m and 1200m sites tended to have lower ratios and slighter downcore increases than the 140m and 940m sites (Fig. 6.12)

Across the lower boundary of the OMZ, the surface pheophorbide/chlorophyll-a ratio increased with station depth, with a maximum at the 1100m site. The pheophytin/chlorophyll-a ratio also increased with depth, with a slight hiatus at the 1100m site. (Figs. 6.11 and 6.12).

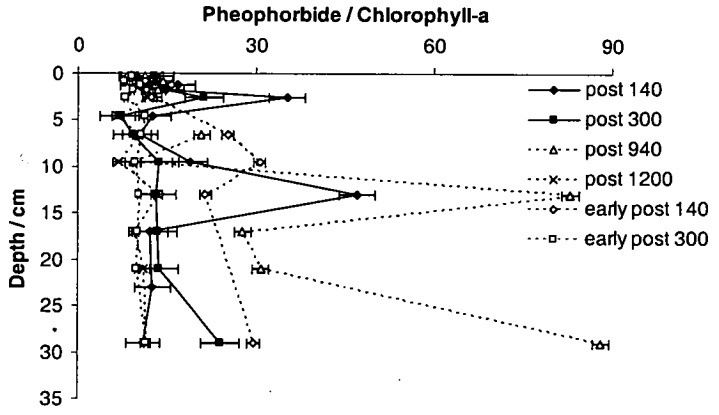
Ratios of pheophytin to pheophorbide were rarely greater than 1 in surface sediments. The 140m and 1200m sites showed the maximal surface values (~ 0.5-0.7 pre-monsoon, and ~ 1-1.5 post-monsoon), which increased downcore (typically to 0.6-0.8, but to ~1.6 in the case of the post-monsoon 1200m site). The 940m site showed minimal surface values (~ 0.5 in both seasons), which decreased with depth (by ~ 0.5 in both seasons). The 300m site had intermediate, depth-constant values, with surface values similar to the 140m and 1200m sites (Fig. 6.13). The early post-monsoon cores from the 140m and 300m sites were exceptions to this pattern, showing low (~0.5) values, which only increased slightly downcore. The 1850m site showed the highest ratios (~ 1.7 in both seasons), and these increased markedly (by ~ 2.6) downcore (Fig. 6.13).

6.4 Discussion

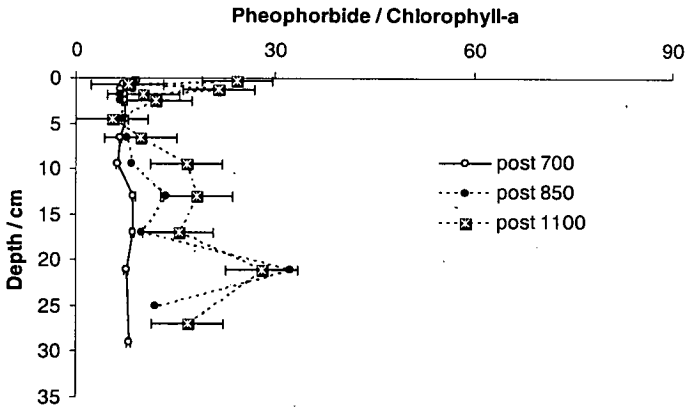
It should be noted that in addition to the controls on pigment preservation discussed below, pigments may become preserved through partitioning into humic associated and other pools. Furlong and Carpenter (1988) found that humic-associated pigments, not extractable using acetone, accounted for up to 75 % of sedimentary



A



B



C

Figure 6.12. Downcore ratios of pheophorbide : chlorophyll-a in A) pre- and B)post-monsoon seasons, and C), at the OMZ lower boundary. Error bars are 1 standard deviation, and where they are not visible, they are smaller than data points.

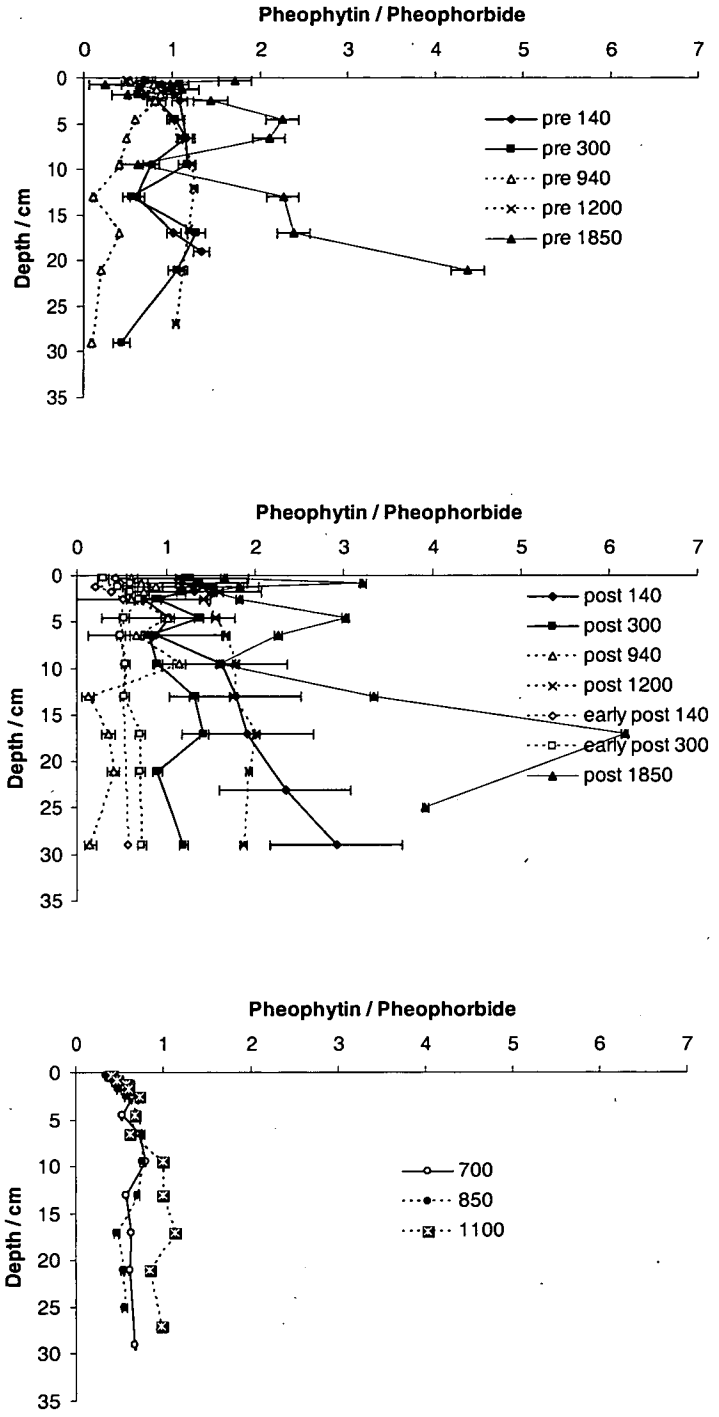


Figure 6.13. Downcore plots of pheophytin/pheophorbide in A) the pre-monsoon season, B) the post-monsoon season, and C) across the OMZ lower boundary. Error bars are 1 standard deviation, where they are not visible, they are smaller than data points.

pigments. King (1995) concluded that HPLC analysis only identified 30 % of pigments, and was unable to resolve high-molecular-weight and acid-extractable phorbins. I was not able to assess the role of this partitioning in pigment preservation on the Pakistan margin as the method used here did not extract humic-associated pigments.

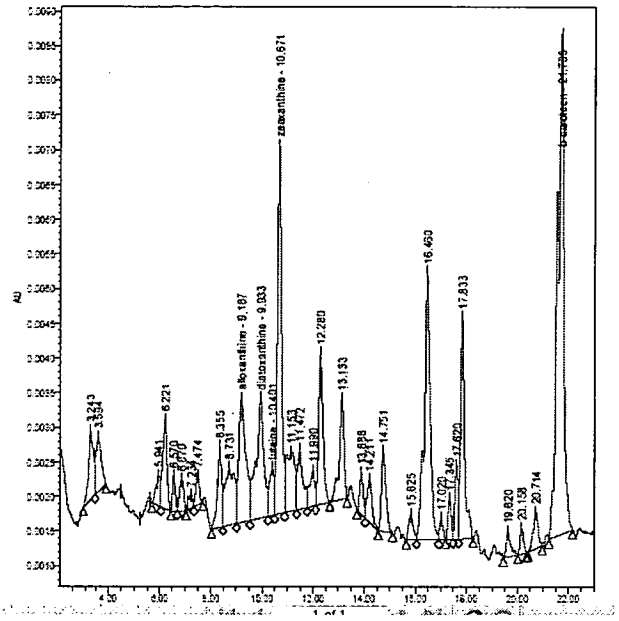
In summary of the data, the abundance of bulk pigments varied across the Pakistan margin between values of 0.8 ± 0.06 and 48.8 ± 0.7 μg / g. Maximal values were found at sites that exhibited high weight percentages of organic carbon (%Corg) and low oxygen concentrations. The distributions of individual intact pigments (chlorophyll-a and accessory pigments) and pheopigments also followed this pattern. The suites of pigments were dominated by pheophytin and pheophorbide, with comparatively low concentrations of chlorophyll-a, and the accessory pigments alloxanthin, diatoxanthin, zeaxanthin and β-carotene, indicating relatively degraded OM compared to other settings (see later section).

6.4.1 Comparison With Other Marine Settings

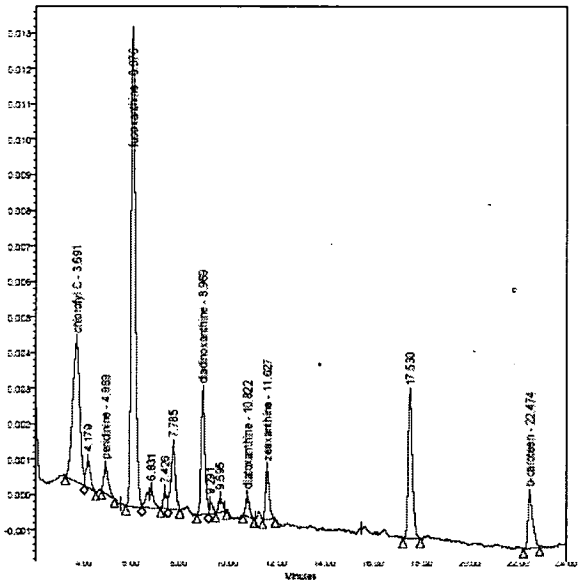
The chlorophyll-a concentrations presented here were approximately 3 orders of magnitude lower than those reported by Sun et al. (1994a) for Long Island Sound surficial sediments and, this highlights the degraded nature of the pigment suite on the Pakistan margin. This is further emphasised by comparing a typical pigment chromatogram from the Pakistan margin with one from a sample of estuarine sediment (courtesy of Jan Sinke and Marco Houtakamer) (Fig. 6.14). The relatively degraded nature of OM on the Pakistan margin is highlighted by the relatively noisy baseline and greater peak widths in the chromatogram from this study compared to that derived from an estuarine sample. Pakistan margin sediments also stand in stark contrast with those from the anoxic Mariager Fjord, where the pehopigment/chlorophyll-a ratio was 0.61, indicating that chlorophyll-a was more abundant than its degradation products (Reuss et al., 2005).

Surface pheophorbide concentrations were however similar to those recorded in 180m of water in Dabob Bay, a coastal fjord (Furlong and Carpenter, 1988). Surface chlorophyll-a and zeaxanthin concentrations were surprisingly similar to and up to on order of magnitude greater than (respectively) those found in the eastern

Mediterranean below just 25-50 m of water (Bianchi et al., 1996), although these authors remarked that their samples exhibited exceptionally low pigment concentrations..



A



B

Figure 6.14. A comparison of pigment chromatograms from A) Pakistan margin sediments, and B) an estuarine sediment (courtesy of Jan Sinke and Marco Houtakamer).

Notably, the suites appear to reflect "fresher" OM than that found on the Oman continental slope, where pheophytin-a, pyropheophytin-a, pyrophaophorbide-a, cyclopheophorbide-a and carotenol and steryl-chlorin esters, all chlorophyll and other pigment decay products, were detected, but intact pigments (i.e. chlorophyll-a and accessory pigments) were absent (Shankle et al., 2002). Total chlorin (chlorophyll-a + pheophytin + pheophorbide) concentrations in this study were however roughly an order of magnitude lower than those found on the Oman margin, implying perhaps a lower OM supply, but a greater degree of preservation on the east side of the Arabian Sea (consistent with lower oxygen availability and faunal abundance there compared to the west side). Shankle et al. (2002) also observed a lack of pigment suite change, both in surficial sediments across the Oman margin and downcore at any given site, which also indicates more degraded OM than was found on the Pakistan margin. Sediments from the Equatorial Pacific showed a similar balance of chlorophyll-a to pheopigment abundance to the Pakistan margin, but overall concentrations were an order of magnitude lower. This is not surprising, given the depths (~ 4000-5000m) and relatively high oxygen concentrations of the study sites, but does not fit with the authors' observations of layers of fresh (even intact) phytoplankton on the sediment surface (Stephens et al., 1997).

Therefore the pigment contents of Pakistan margin sediments represent relatively degraded OM. This is consistent with their continental margin setting, and also with previously observed contrasts in oxygen availability and faunal abundance (Lisa Levin, pers. comm.) between the western and eastern Arabian Sea margins.

6.4.2 Pigment Suites And Sources

The suite of pigments present in a sediment can be indicative of the source and diagenetic history of its OM. Many accessory pigments are only produced by a limited range of phytoplankton classes, for example fucoxanthin is a diatom biomarker (Jeffrey and Vesk, 1997), and pheopigments are often used to infer grazing (e.g. Shuman and Lorenzen, 1975). The interpretation of pigment suites in these terms must however be carried out with caution, as by the time pigments reach the sediment they have already been subject to considerable compound selective

decay and re-cycling processes, which can lead to the complete loss of biomarkers for important OM sources (Repeta and Simpson, 1991).

Pheophorbide and pheophytin dominated the pigment suite on the Pakistan margin, with relatively minor contributions from chlorophyll-a, alloxanthin, diatoxanthin, zeaxanthin and β-carotene. Intact pigments constituted a greater portion of pigment suites than on the Oman margin, where none were detected (Shankle et al., 2002). On the other hand, the suites were markedly different from shallower settings, where chlorophyll-a has been observed to account for ~20-70% of sedimentary chloropigments (Sun et al., 1994). Thus, the pigment suite of Pakistan margin sediments was indicative of a relatively advanced state of decay, and this must be considered when inferences of pigment source are made.

The presence of pheophorbide and pheophytin has been shown to be the result of grazing of phytoplankton by zooplankton in the water column (Shuman and Lorenzen, 1975, Bianichi et al., 2000), and the particular pheopigment formed during digestion has been observed to be species-specific (Louda et al., 1998). Their use as indicators of grazing has however been questioned, as diatom lysis, bacterial attack, and microbial decay have also been observed to produce pheopigments from chlorophyll-a in the absence of grazers (Villaneuva and Hastings, 2000, Louda et al., 2002), and it has been suggested that pheophorbide may not even survive gut passage (Louda et al., 1998). Thus, the presence of pheopigments in Pakistan margin sediments most likely indicates grazing within the water column, but it does not follow that this is their only source. Recently developed analytical techniques have allowed the identification and use of other, more specific, grazing indicators, such as steryl chlorin esters (e.g. Squier et al., 2002) and accessory pigment decay products (Repeta and Gagosian, 1984).

The accessory pigment suite is potentially diagnostic of the types of algae from which phytodetritus is derived (e.g. Bianchi et al., 1996). The dominant accessory pigments present on the Pakistan margin were alloxanthin, diatoxanthin, zeaxanthin and β-carotene. Diatoxanthin and β-carotene are not particularly diagnostic, as they are present at trace or minor levels in many classes of algae (Jeffrey and Vesk, 1997). Alloxanthin, however, is strongly diagnostic of the class Cryptophyta (nanoplanktonic flagellates), and zeaxanthin is a major pigment for Rhodophyta,

Prochlorophyta and Cyanophyta. Rhodophyta are sparsely studied red algae, Prochlorophyta are picoplanktonic prokaryotes, and Cyanophyta are cyanobacteria (Jeffrey and Vesk, 1997).

This diagnosis of the Arabian Sea phytoplankton community is consistent to some extent with previous studies. The prokaryotic prochlorophyte *Synechoccus sp.* has been shown to be abundant in Arabian Sea surface waters, especially in more oligotrophic areas of the basin (Tarran et al., 1999). The same study also confirmed the presence of abundant nanoplanktonic flagellates, here indicated by the presence of alloxanthin. Barlow et al. (1999), as in this study, used the presence of zeaxanthin to indicate the presence of *Synechoccus sp.* They however also recorded fucoxanthin and 19'-hexafucoxanthin in surface waters, indicative of diatoms and prymnesiophytes respectively, which were particularly abundant during monsoons. These plankton groups were also observed by Tarran et al. (1999), but this study did not find their characteristic pigments in the sediment. This illustrates a shortcoming of the use of sedimentary pigments as indicators of phytoplankton communities in the deep sea, as the biomarkers of two important groups of phytoplankton did not appear to survive transit through the water column. Previous studies have also observed the disappearance of these compounds from sinking OM within the photic zone (Repeta and Simpson, 1991), and have shown that they are more reactive than other accessory pigments (Repeta, 1989).

In summary, the pigment suites of Pakistan margin sediments indicated relatively degraded OM, which has been subjected to some amount of grazing. The phytoplankton OM sources indicated by the accessory pigment suites were consistent with previous studies of Arabian Sea phytoplankton communities, but the absence of diatom and coccolithophorid biomarkers from the sediments highlights the limitations of the approach.

6.4.3 The Relationships Between Pigment Distribution and Site Conditions

In general (Fig. 6.2), total pigment abundances appeared to co-vary with bottom water oxygen concentration, and to a lesser degree with sediment %Corg. This pattern was also apparent for all the individual pigments, and leads to the question

whether redox-controlled preservation or OM supply are primarily responsible for the observed pigment distributions.

6.4.3.1 Oxygen

Correlation analysis showed a negative correlation between surficial total pigment abundance and bottom water dissolved oxygen concentration ($\rho = 0.58$). This relationship was also present for all individual pigments (chlorophyll-a, pheophorbide, alloxanthin, zeaxanthin and β-carotene), excluding pheophytin and diatoxanthin, and the range of correlation coefficients (ρ) was 0.53 (alloxanthin) to 0.61 (β-carotene and chlorophyll-a). In addition, PC1 scores derived from the pigment suite, which were indicative of OM quality or freshness (see later section) correlated strongly with oxygen concentrations ($\rho = 0.72$), and showed that lower quality OM (higher PC1) was associated with greater availability of oxygen. Therefore oxygen availability seems to influence the preserved concentration and compositional freshness of sedimentary pigments. The lack of significant correlation between pheophytin concentrations and oxygen availability implies that oxygen does not play a dominant role in determining the distribution of this pheopigment.

Reactive chlorophyll-a inventories showed no relationship with bottom water oxygen concentration, and unreactive background chlorophyll-a concentrations had only a weak negative relationship ($\rho = 0.36$), with generally higher values at OMZ stations (the 300m and 700m sites, Fig. 6.10B). The same pattern was followed by the relationship of accessory pigment inventories with oxygen availability. Only unreactive background concentrations showed negative correlations with oxygen availability, (although these relationships were generally weak, $\rho = 0.32$-0.62).

Low oxygen availability seems therefore to influence pigment distributions by maximising the total amount of unreactive pigment ultimately buried, rather than by facilitating the preservation of reactive chlorophyll-a in near-surface sediments. This is consistent with observations of unreactive pigment concentrations at low oxygen sites in Long Island Sound (Sun et al., 1994), and with the observation that the decay rate of refractory OM is more oxygen dependent than that of fresh OM (Hulthe et al., 1998). Sun et al. (1993 b) found that naturally present chlorophyll-a in sediments was partitioned between free and particle-bound pools, which had different reactivities under different redox conditions. The observation that oxygen seems to

influence unreactive pigment concentrations more than reactive concentrations suggests that oxygen availability may influence chlorophyll-a preservation through its varying effect on the reactivity of different fractions of OM. Sun et al. (1993b) also found that release of chlorophyll-a from the bound pool into the free pool as the first step in decay was slowed in the absence of oxygen.

It is interesting to note that even though the 140m site displayed a dramatic reduction in bottom water oxygen concentration (imposed by the water column) between pre- and post-monsoon seasons, this was not accompanied by a proportionate increase in total or individual pigment concentrations. Consequently, while oxygen may exert some control over pigment distribution, it does not do so on this seasonal timescale; either because there was not a significant input of fresh OM during the monsoon at this site, or because continued faunal activity (ingestion, mixing and irrigation; see chapter 4) was sufficient to cause rapid pigment turnover.

Principle component analysis revealed that the pigment suite changed across the margin, with intact pigments more dominant at low oxygen sites. Low PC1 scores indicated fresh OM. Oxygen concentration and PC1 scores showed a strong positive correlation ($\rho = 0.73$) indicating that fresh pigment suites were associated with low oxygen conditions. Thus, oxygen availability not only influences pigment concentrations, but selective decay of chlorophyll-a and accessory pigments versus pheopigments under oxic conditions means that oxygen availability also influences pigment composition.

The relationships between pigment parameters and oxygen concentrations were slightly weaker than might be expected, and this could be because oxygen concentrations are not necessarily indicative of oxygen exposure times, which may have a stronger overall influence on OM.

Despite this, in general, pigment abundances appear to some extent to be influenced by oxygen availability. Low oxygen availability seems to influence the unreactive, rather than the reactive fractions of pigments. This has been previously observed, and the absence of oxygen is even posited to reverse the relative reactivities of chlorophyll-a decay products and total organic carbon, such that the pigments are preferentially preserved (King, 1995). Low oxygen conditions also seem to enhance the preservation of intact pigments, and therefore lead to fresher pigment suites.

6.4.3.2 OM Supply

On the Pakistan margin sediment %Corg and oxygen availability are mechanistically linked, thus the independent influences of oxygen and OM supply on pigment distributions are difficult to de-convolve. In addition, as the pigments constitute a part of sedimentary OM, it is unsurprising that they co-vary with %Corg, and are controlled by the same factors as %Corg, a dominant one of which is oxygen availability.

Total pigment concentrations indeed showed a relatively strong positive relationship with sediment %Corg ($\rho = 0.66$). The individual pigments also showed this relationship, although the correlation was only significant for chlorophyll-a, pheophytin, pheophorbide and β-carotene. Thus, the distribution of pigments is likely to be controlled by similar factors to those which control the overall abundance of OM, the dominant of which are likely to be oxygen availability and delivery effects.

An examination of the 1200m site provides insight into the role of OM delivery in determining pigment abundance. During both seasons, this site, despite being relatively well oxygenated and bioturbated (and ventilated), had the highest %Corg values anywhere on the margin. In the pre-monsoon season this was not reflected in total or individual pigment concentrations, which were similar, both at the surface and downcore, to those at the relatively OM-poor 140m site (Fig. 6.2, 6.3). During the post-monsoon season however, the surface pigment concentrations (but not those downcore) at the 1200m site were dramatically increased to values similar to those shown by the OMZ sites. The best explanation for this is a seasonal pulse of fresh OM to the 1200m site, demonstrating a clear local effect of OM supply. Sediment %Corg data did not however show this increase, and PC1 scores did not indicate fresher OM in the post-monsoon season (Table 6.1 and Fig. 6.9), therefore other evidence did not support the suggestion of a seasonal input of fresh OM to the 1200m site.

Comparisons of reactive inventories and unreactive background concentrations of chlorophyll-a and accessory pigments provide a potential distinction between OM delivery and redox-related preservation effects as influences on pigment distribution.

Maximal chlorophyll-a and other pigment reactive inventories were found across the lower boundary of the OMZ (Fig. 6.10). These were not significantly related to oxygen availability, but showed a positive correlation with surface sediment %Corg ($\rho = 0.58$). This suggests that reactive pigment inventories are determined more by OM delivery at a given site than by redox-related preservation, and that delivery is focused onto the lower boundary of the OMZ. This conclusion must however be treated with caution. Oxygen exposure times (OETs), which are determined by both sedimentation rate and oxygen concentrations, are not yet known, and the maximal reactive inventories at the OMZ lower boundary may be the result of rapid sediment accumulation and thus minimal OETs. Similar suggestions of a concentration of OM supply at the OMZ lower boundary have however been made previously, based on %Corg and lipid data (e.g. Cowie et al., 1999, Schulte et al., 2000), and such concentrations of supply have been attributed to winnowing in the OMZ and downslope movement of OM (Schulz et al., 1996).

In contrast, as stated above, unreactive background concentrations showed weak negative correlations ($\rho = 0.32$-0.62) with oxygen availability, and were not related to sediment %Corg, suggesting that the amount of unreactive pigments eventually buried is more dependent on redox-related preservation than OM supply.

The suggestion of larger OM/pigment fluxes to the OMZ lower boundary sites may offer insights into the ecology of this part of the margin. The OMZ lower boundary sites were characterised by low-diversity, high-biomass macrofaunal communities, which altered in composition over small distances. Their presence may not only be controlled by the high quality OM preserved in hypoxic conditions, but may also reflect an OM input greater than anywhere else on the margin, consistent with previous studies which have observed stronger relationships of faunal abundance with OM flux than with sediment %Corg (e.g. Pfannkuche et al., 2000).

In general, total and individual pigment concentrations in surface sediments correlated with both oxygen availability and sediment %Corg. The correlations of un-reactive background concentrations with oxygen suggest that redox-related preservation leads to higher values within the OMZ, while correlations between reactive inventories and %Corg, together with the seasonal signal at the 1200m site suggest that high OM delivery influences reactive pigment abundance across the

OMZ lower boundary. This is consistent with the findings of Shankle et al. (2002), who, in a study of the Oman margin sedimentary pigments, found their abundance to be controlled by both water-depth-regulated delivery, and oxygen- regulated preservation.

6.4.3.3 Faunal Effects

Macrofaunal abundances (Table 6.1) and bioturbation varied considerably across the Pakistan margin. At the 140m site, the macrofauna were most abundant and diverse, but relatively small, so constituted a mid-range biomass. The 1850m site had a lower macrofaunal biomass, but similar diversity. Relatively small macrofaunal biomasses were recovered from the 1200m site, but at the 1100m site they were more abundant (Lisa Levin, pers. comm.). The 940m and 850m sites, exhibited particularly large macrofaunal abundances and biomass, but low diversities (Peter Lamont, pers. comm.). Sediment x-radiographs (chapter 2, Lisa Levin, pers. comm.) showed that the 1200m and 1850m sites were so well mixed that individual burrow structures could not be seen. The 140m and 1100m sites were also well mixed, and sediments at the 940m and 850m sites contained visible, discrete burrows in x-radiographs (to depths of ~5cm), but retained some lamination. The OMZ 300m and 700m sites displayed finely laminated sediments and almost no metazoan macrofauna.

Total pigment concentrations in surface sediments were generally minimal at the 140m, 1100m, 1200m and 1850m sites, which exhibited most bioturbation (Fig. 6.2). These sites also exhibited the most constant downcore pigment profiles (Figs. 6.3-6.8), the highest pheopigment to chlorophyll-a ratios (Figs. 6.12, 6.13), and the lowest pigment unreactive background concentrations (Fig. 6.10). In addition, they showed the highest PC1 scores, which were indicative of relatively low weight percentages of intact pigments compared to decay products. Therefore, sites that were bioturbated exhibited minimal, and OMZ sites where metazoan macrofauna were absent exhibited maximal, quantities and qualities of sedimentary pigments.

This is consistent with previous mesocosm experiments, in which the presence of fauna was shown to increase the rate at which pigments decayed (Bianchi et al., 2000, Sun and Dai, 2005, Ingalls et al., 2000). There are likely to be several mechanisms for this. Simple physical mixing increased decay rates by producing fluctuating sediment redox conditions, but mixing by live fauna had a greater effect,

suggesting that active burrow ventilation, digestion, and microbial stimulation processes also act to accelerate pigment degradation (Sun and Dai, 2005).

The presence of macrofauna affected not only pigment concentrations, but also downcore profiles. Where macrofauna were absent, downcore profiles of total and individual pigments were smoother, and reached background concentrations at shallower depths than when macrofauna were present (Figs. 6.3-6.8). Thus macrofauna were shown to increase the depth penetration of reactive pigments, and also to produce spiky profiles, due to the spatial heterogeneity of burrowing.

Correlation analysis did not show the expected negative relationship between macrofaunal biomass and total pigment abundance. This is likely due to exceptional sites such as the 940m and 850m sites where, due to the interaction of oxygen availability and sediment %Corg, both macrofaunal biomass and pigment concentrations were high. Different faunal communities therefore clearly have different effects on pigment and OM cycling. Those effects can be hard to de-convolve from the effects of OM supply and oxygen availability both on each other, on faunal abundance itself, and through all these other interactions, on pigment distribution. This may however be attempted by considering the following examples.

Chlorophyll-a surface concentrations, reactive inventories and unreactive background concentrations in the post-monsoon season corresponded in showing low points at the 1100m site (Figs. 6.2, 6.10), which were, surprisingly, lower than those at the more oxygenated 1200m site. This could be due to the variation in the abundance of burrowing animals across the OMZ lower boundary. Such fauna became more abundant as oxygen levels increased with increasing water depth below the OMZ, and showed a maximum around 1100m (Lisa Levin, pers. comm.), at which location modelled bioturbation rates also showed maximal values (Table 6.5). Thus, more intense burrowing and sediment irrigation at the 1100m site may account for the low surface chlorophyll-a and un-reactive background concentrations observed there. In addition, across the OMZ lower boundary, pheophorbide- and pheophytin-to-chlorophyll-a ratios both increased with increasing water depth as oxygen concentrations increased, (Figs. 6.11, 6.12). The former ratio however showed a maximum at 1100m, and the latter ratio a minimum. This is consistent with maximal *in situ* pheophorbide production as a result of faunal activity at the

1100m site. If some of that production were through transformation of pheophytin, then this would also explain the minimum in the latter ratio.

Correspondingly, the absence of burrowing fauna from the 300m and 700m sites could contribute, along with low oxygen availability, to the high pigment concentrations found there. It should be noted that reduced macrofaunal abundance within the OMZ is attributable to low oxygen availability, and thus these factors are linked, and work together to enhance pigment preservation.

Post-monsoon pigment concentrations, and reactive inventories of all pigments at the 1200m site showed enrichments attributable to the delivery of a monsoon induced fresh OM pulse. The reason this effect was not observed at other stations may be linked to faunal activity. The 140m site, for example, in the pre-monsoon season had a pigment signature similar to that of the 1200m site, and the post-monsoon low oxygen conditions observed there might be expected to preserve a pulse of fresh OM. However, no seasonal increase in pigment concentrations, such as was seen at the 1200m site, was observed at the 140m site. The difference between the 140m and 1200m sites was that the 140m site supported a much greater density and biomass of macrofauna (Peter Lamont, pers. comm., Table 6.1), and their actions could have caused a rapid loss of any seasonal OM pulse associated with the monsoon.

It has been suggested that digestion by pelagic metazoans is an important mechanism for pheophorbide formation (e.g. Shuman and Lorenzen, 1975, Bianchi et al., 1988). This is supported by my data, in that the 140m and 940m sites showed larger macrofaunal biomass and larger surface and downcore pheophorbide/chlorophyll-a ratios than the 300m and 1200m sites (Fig. 6.12). The ratio of pheophytin/chlorophyll-a was also higher, and showed greater downcore increases at oxygenated, bioturbated sites. This also could be a result of macrofaunal digestion, the acidic conditions of which are thought to strip the magnesium ions out of chlorophyll-a.

In general, and in line with previous studies, the actions of burrowing fauna on the Pakistan margin appeared to alter the pigment suite, and reduce pigment preservation. In this study, these effects are demonstrated for natural samples from a wide range of different environments, thus providing real-world support for conclusions reached in laboratory studies. Fauna also altered downcore pigment

profiles by mixing reactive pigments to depth in the sediment, and by introducing sub-surface peaks.

6.4.3.4 Water Column Depth

The observed general lack of marked post-monsoon increase in pigment concentrations suggests the possibility that water-column processes, including microbial decay, zooplankton grazing and solubilisation, may have removed the majority of pigments from sinking OM before it reached the sediment. Sinking depth would thus influence the abundance and composition of pigments in Pakistan margin sediments.

Evidence for an influence of water column decay on the pigment contents of sediments is provided by the lack of detectable intact pigments (chlorophyll-a or accessory pigments) at the 1850m site, either before or after the monsoon, implying that they are fully removed before OM reaches the seafloor at that site. The suggestion that sinking depth may partially control sediment pigment abundance and composition is consistent with observations of decreasing total concentrations and chlorophyll percentages down through the water column in the Equatorial Pacific (Lee et al., 2000). It is also supported by previous studies, which have shown that only ~ 1% of chlorophyll-a produced in surface waters reaches abyssal sediments (Pfannkuche et al., 2000), and that this causes water-depth related de-coupling between surface water productivity and sediment pigment contents (Watts et al., 1992, Shankle et al., 2002). No pigment parameters however (concentrations or inventories) showed significant negative correlations with water depth, thus its influence was mostly obscured by the influences of varying redox-related preservation, as well as faunal, and cross-margin OM supply effects.

6.4.4 Seasonal Effects

Past records of particle fluxes in the NE Arabian Sea (Haake et al., 1993a) have shown that the SW monsoon and resultant upwelling and productivity caused an increase in bulk particle and OM flux to the sediment. This was observed in this study as increases in sediment lipid and carbohydrate contents, and through the expansion of the OMZ between pre and post-monsoon sampling periods (Rachel

Jeffreys, pers. comm., chapter 7, and the CD 150 cruise report). It was not however reflected in surficial sediment %Corg values (Table 6.1).

There were only subtle increases in pigment concentrations between pre and post-monsoon seasons (Fig. 6.2). Two sites were exceptions to this general subtle seasonal increase. These were the 140m site, which displayed no change beyond analytical error, and the 1200m site, which was the only site to exhibit a marked seasonal increase.

The general lack of pronounced post-monsoon pigment enrichment could simply highlight the extremely labile nature of pigments. The later post-monsoon (September/October) sampling (CD 151) seems to have been sufficiently after the summer monsoon for most of any pigment pulse to have to decayed. The lability of chlorophyll-a is further illustrated by comparison of early and later post-monsoon samples from the 140m and 300m sites, both of which showed decreases in chlorophyll-a (but not accessory pigment) concentrations over a period of only a few weeks (Fig. 6.2). Such an explanation for a lack of monsoon-induced increase in pigment concentration is supported by the fact that Pfannkuche et al. (2000) found highest pigment concentrations at abyssal Arabian Sea sites during the February-April NE monsoon, and lowest concentrations in October, after the SW monsoon.

Further possible explanations for the lack of seasonal signal include suggestions that the monsoon induced increase in particle flux in the eastern Arabian Sea is not particularly pronounced (Haake et al., 1993a), and that the majority of the extra OM flux is degraded within the water column, both of which imply that relatively little OM was delivered to the sediments as a result of the monsoon. This is particularly applicable to OMZ stations, where low oxygen availability was associated with high pigment concentrations, which may not be appreciably altered by a relatively subtle addition of OM. Conversely, at the OM poor 1850m site, even a minor pulse of OM would impact sediment geochemistry, however the long water column above this site means that pulse was more likely to have decayed before it reached the sediment.

6.4.5 Organic Matter Preservation State

The "quality" (or preservation state or freshness) of organic matter in Pakistan margin sediment has been independently assessed using the amino-acid-based

degradation index (DI) (Table 6.1, Vandewiele, pers. comm; Dauwe and Middelburg, 1998). This index (where higher values = high quality or freshness) indicated higher quality OM within the OMZ, than above and below it, and displayed strong positive correlations with total pigments ($\rho = 0.87$) and all individual pigments. Thus, pigments are associated with, and could therefore be indicative of, relatively high quality OM.

A more detailed impression of the relationship between pigments and OM quality is achieved by examining pigment suite changes across the margin. Surface pheophorbide/chlorophyll-a ratios showed a negative relationship with DI (albeit only weak; $\rho = 0.49$). Thus, fresher sediment OM generally contained more chlorophyll-a compared to pheophorbide, consistent with the classic use of this ratio as an index of OM alteration in the water column. Similarly, reactive inventories of chlorophyll-a, zeaxanthin and pheophytin showed significant correlations with DI, and these may also be indicative of OM quality.

Instead of using OM quality indices that are based on individual compounds, principle component analysis of pigment weight percentages produced a single parameter (PC1 score) for each sample, based on the relative concentrations of all the pigments. Maximal PC1 scores were associated with low weight percentages of intact pigments, and high weight percentages of pheopigments, and so were indicative of maximal decay. Surficial sediment PC1 scores showed a strong negative correlation with DI ($\rho = 0.78$), thus, they showed the same quality relationships between samples as DI, and so are a robust measure of OM quality. As an indicator of OM quality, pigment based PC1 scores have the same advantage as DI of being based on multiple parameters. In addition, the compositional changes which serve to indicate reduced OM quality, loss of fresh pigments and build up of decay products, are intuitively pleasing, and this lends support to the proxy.

Thus both the abundance and suite of pigments present in a sediment are indicative of the bulk OM quality. This is relatively unsurprising, given that pigments are sourced only from fresh phytodetritus, and are often found to be one of the most reactive biochemical classes in the marine system (Lee et al., 2000). Although several single compound based parameters appear to be indicative of OM quality, the

most robust indicator is a pigment composition based parameter, derived by performing principle component analysis on pigment weight percentage data.

At most sites, PC1 score were constant downcore (data not shown), therefore decay in the sediment was non-selective among the pigments. The only sites where downcore increases in PC1 score were apparent were the least well oxygenated 300m and 940m sites (and possibly the 700m and 850m sites). Selective decay among the pigments may therefore only occur in low oxygen conditions. It seems more likely however that selective decay only occurs among the pigments when the starting point for decay is relatively labile OM, which was only present at the low oxygen sites.

6.4.6 Modelled Pigment Decay Constants

Published decay rates for chlorophyll-a in a range of marine environments and laboratory experiments reveal half lives ranging from a few days to several hundred years, depending on oxygen availability, the freshness of the OM studied, and whether reactive, surficial OM or downcore preserved/refractory pools are considered (Stephens et al., 1997, and references therein).

The chlorophyll-a decay rates used in bioturbation modelling in this study were derived from laboratory studies of relatively fresh sedimentary OM, and the resultant half lives ranged from ~8 to 34 days (Sun et al., 1993 a, b). Modelling of chlorophyll-a profiles at OMZ sites (300m and 700m, where zero bioturbation could be assumed) however produced half lives of ~ 3-10 years (Table 6.4).

Previously published measured and modelled chlorophyll-a half lives from algal decay studies and surface marine sediments show similar results to those produced by Sun et al. (1993 b), with values typically falling in the range ~ 4-90 days (Stephens et al., 1997, and references therein, Chen et al., 2005). Multi-G modelling of sediment chlorophyll-a profiles, however, has shown much longer half lives for buried chlorophyll-a, ranging up to 2000 days for the marine sites mentioned above (Chen et al., 2005). These rates are much more comparable to modelled chlorophyll-a decay rates from the Pakistan margin. The values derived from modelling OMZ profiles are undoubtedly better indications of chlorophyll-a decay rates on the Pakistan margin than those used in the modelling of bioturbation, which were 2 orders of magnitude faster.

The relatively slow rate of chlorophyll-a decay on the Pakistan margin may be due to low oxygen availability (e.g. Sun et al., 1993 b), and the relatively refractory nature of OM delivered to the sediments (e.g. Chen et al., 2005). In Studies of chlorophyll-a decay rates using fresh algal detritus, rates were slower, and decay ceased earlier in the absence of oxygen (e.g. Sun et al., 1993 b, Bianchi et al., 2000). Chlorophyll-a decay rates have also been found to be dependent on the partitioning of chlorophyll-a between pools of varying reactivity. This partitioning may be altered by oxygen availability and OM state of decay, and thus will vary depending on site conditions and the history of sedimentary OM. In the deep-sea, OM is subjected to considerable decay during sinking, and the most reactive fraction may never reach the sediments.

The dominant reason for the relatively slow modelled chlorophyll-a decay rates at the 300m and 700m sites, is likely to be that even the most reactive pool here is comparatively refractory (Chen et al., 2005). Although oxygen availability and the absence of fauna (Bianchi et al., 2000) may also influence chlorophyll-a decay rates at these sites, decay during sinking appears to have heavily altered the form, and reduced the reactivity, of OM delivered to the sediment. In previous studies, only variations in OM degradation state have appeared sufficient to generate variations in chlorophyll-a half-life on the order of 2 orders of magnitude (e.g. Chen et al., 2005). In contrast, anoxic and fully oxygenated experiments have only yielded roughly twofold differences in fresh chlorophyll-a decay rates (Sun et al., 1993b).

From this follows the likelihood that chlorophyll-a decay rates across the Pakistan margin are much slower than suggested by the decay constants used in modelling. Unfortunately it will not be possible to perform detailed study of the way chlorophyll-a decay rates varied across the Pakistan margin until bioturbation rates are independently constrained from ^{210}Pb profiles (this work is ongoing).

The model equation used assumes an unreactive background concentration of chlorophyll-a, which is constant downcore. If, however, chlorophyll-a decay proceeds through a kind of geopolymerisation, or other mechanism bringing about protective preservation, then the unreactive fraction may in fact increase downcore, and such an increase would be obscured by the simultaneous decrease in reactive chlorophyll-a. In such a scenario, the rate of decay of reactive chlorophyll-a would in fact be faster than that modelled using independently-derived mixing rates.

The multiple-G model of OM decay proposes that OM consists of fractions of different reactivity, which decay at different rates (Jorgensen, 1978). Previous studies have shown that multiple-G type modelling of chlorophyll-a profiles produced much better model fits (Shankle et al., 2002, Stephens et al., 1997) than the use of a single, constant decay rate. The very presence of an unreactive background chlorophyll-a or other pigment concentration indicates that degradation does indeed proceed through decay of two or more portions of OM with different reactivities (Stephens et al., 1997). As a result of the multiple-G type behaviour of chlorophyll-a (and other pigments), decay rates derived from just the surface sediment (or mixed layer) have been shown to be 1-2 orders of magnitude faster than those found for the buried, refractory component (Chen et al., 2005, Stephens et al., 1997). Ingalls et al. (2000) noted that chlorophyll-a might, in fact, exhibit a continuum of decay rates, as redox conditions, faunal abundance and age of OM change down through the sediment. Independent quantification of bioturbation rates will also allow multiple-G type modelling of chlorophyll-a decay rates, leading to better model fits, and more detailed insight into chlorophyll-a decay dynamics across the Pakistan margin.

Pheopigment and accessory pigment decay half-lives were obtained by fitting the decay/bioturbation model described in equation 1 to downcore profiles, using bioturbation rates derived from modelling of chlorophyll-a data (Table 6.5). Decay rates modelled in this way all carry an error introduced by the fact that the chlorophyll-a decay rates used to generate bioturbation data were probably at least 2 orders of magnitude too fast, thus both bioturbation and other modelled decay rates are maximal values. Pheopigment and accessory pigment decay rates were also modelled at the laminated OMZ sites by assuming zero mixing, and these values are thought to be considerably more representative of the whole margin.

As for chlorophyll-a, pheopigment decay rates modelled at laminated OMZ sites, were ~2 orders of magnitude slower than those produced for non-OMZ sites (Table 6.4). It is likely that pheopigment decay across the whole margin proceeds at similar rates to those modelled within the OMZ (half lives 300-4500 days), and that these rates are primarily controlled by OM quality/reactivity. These half lives are consistent with the upper end of the range of values derived by Stephens et al. (1997) from Equatorial Pacific sediments of ~22 to 3000 days. The shortest of these half-

lives were associated with only the very surface and most reactive sediments, and the longer ones with downcore refractory pheopigments. Short pheopigment half -lives (~ 40-50 days) were also found in the coastal setting of Dabob Bay (Washington State, USA; Furlong and Carpenter, 1988). Shankle et al. (2002) found the half lives of buried chlorins on the Oman margin to be as long as ~ 250000 days. Thus, while the most reliable pheopigment decay rates derived from this study are long compared to Pacific surface sediments, they show that, overall, sedimentary pigments on the Pakistan margin are more reactive than those off Oman.

Accessory pigment half lives derived from modelling of 300m and 700m site data (assuming zero bioturbation) did not show systematic differences in reactivity among the four accessory pigments (Table 6.4). Similarly to chlorophyll-a and the pheopigments, they all displayed half-lives in the range of ~300-5500 days. As with the other pigment groups, it is thought that decay rates were primarily a factor of the degradation state of Pakistan margin OM, and rates across the margin were probably similar to those modelled at OMZ sites.

In summary, where accurate modelling was possible, the pigments present on the Pakistan margin were found to be relatively refractory compared to those in fresh algal or estuarine OM used in laboratory decay experiments. The preservation state of OM, rather than low oxygen concentrations or faunal abundances is thought to be the reason for slow modelled chlorophyll-a and other pigment decay rates within the OMZ. Once bioturbation on the Pakistan margin has been independently constrained, more detailed modelling of pigment decay dynamics will be performed, such that rates can be related to OM quality, oxygen availability and faunal activity.

6.4.7 Pheopigments

6.4.7.1 Pheopigment Source

In sediments from Long Island Sound (20-100m water depth, 0.29-4.69 % organic carbon), Sun et al. (1994) found that while pheophorbide concentrations decreased exponentially, pheophytin concentrations increased downcore. This was interpreted as indicating that pheophorbide was sourced from the water column, whereas pheophytin was being produced *in situ* from chlorophyll-a decay. On the Pakistan margin, both pheopigments were observed to decrease downcore (with relative rates

differing between stations), and by the same reasoning, they must both have been predominantly sourced from the water column. There may also have been minor *in situ* production of pheophytin from chlorophyll-a, and of pheophorbide from pheophytin.

This is as expected, as the water column in this study was comparatively long, and chlorophyll-a concentrations in the sediment were extremely low compared to those of the pheopigments, thus decay of the former was insufficient to influence the concentrations of the latter (pheophorbide- and pheophytin/chlorophyll-a ratios in Long Island Sound sediments were ~ 0.3-3, compared to ranges of 3-30 in this study).

At the 940m site, a large sub-surface pheophorbide peak was seen at 13 cm depth in both seasons. This peak was thus persistent, having been sampled on two occasions several months apart, and was probably related to a past deposition event rather than an isolated burrowing event. The event could have been an unusually productive season, which delivered a large pulse of OM to the sediment, or a downslope movement of OM rich sediment originating in a shallower region (i.e. a turbidite). Decay, either at source, or in its current hypoxic location, seems to have favoured production and/or preservation of pheophorbide, as no similar peak was found in either chlorophyll-a or pheophytin. A relict turbidite layer, marked by low porosity and %Corg (but which was not analysed for pigments) was seen at ~5-6 cm depth in all cores from this and nearby sites, and Pfannkuche et al. (2000) also observed an unusually pigment-rich turbidite in cores from the abyssal Arabian Sea. Thus, turbidite deposition is a feature of this part of the margin, however the one observed at 5-6cm was OM poor, and was thus unlikely to have produced a pheophorbide peak. Furthermore, the pheophorbide-rich layer at 13cm was not observed at the nearby 850m or 1100m sites, as would be expected if this was a regional turbiditic deposition event.

6.4.7.2 Pheopigment Decay Rates And Oxygen Availibility

The sites with maximal oxygen availabilities and maximal macrofaunal abundances, exhibited maximal surface values and downcore increases in pheophytin/pheophorbide ratio (Fig. 6.13). Downcore increases in this ratio suggested that, at most sites, pheophorbide decayed more rapidly than pheophytin,

and that the discrepancy in decay rates was greater in the presence of oxygen and bioturbating fauna. This difference in pheopigment decay rates is supported by modelled half lives (Table 6.4). The preferential preservation of pheophytin over pheophorbide in oxic conditions has been observed (Sun et al, 1993 a), however oxic conditions have also been suggested to lead eventually to the dominance of pheophorbide as the main chlorophyll-a decay product (Louda et al., 2002).

These downcore trends and cross-margin patterns are an illustration of the suggestion that pheophorbide decay rate is more oxygen-sensitive than that of pheophytin (Sun et al., 1993a). Under oxic conditions pheophorbide decayed more quickly than pheophytin, and in the absence of oxygen they either decayed at roughly the same rate (producing approximately constant downcore pheophytin/pheophorbide profiles at the 300m and 700m sites), or pheophorbide was preferentially preserved. Consistent with this, surface pheophorbide concentrations showed a significant negative correlation with oxygen concentration ($\rho = 0.59$), while those of pheophytin did not. In addition, modelled pheophorbide decay rates (Table 6.5) showed a positive correlation with oxygen availability ($\rho = 0.70$), whereas those for pheophytin did not. Pheophorbide has previously been observed to be the preferred chlorophyll degradation product in the seasonally hypoxic setting of Dabob Bay (Furlong and Carpenter, 1988).

At the 940m site, pheophorbide was the dominant pheopigment, and that dominance increased with depth. Thus, at this site pheophytin was more reactive than pheophorbide, and decayed more quickly downcore. The 940m site differed from the OMZ sites as it had abundant macrofauna of a type found only locally, and differed from the deeper sites in its relative lack of oxygen and its semi-laminated sediments. It appears that in these unique circumstances, oxygen availability was sufficiently low to make the pheopigment decay rates very similar, and pheophorbide was also produced *in situ* through the digestive alteration of pheophytin. Together, these circumstances produced decreasing pheophytin/pheophorbide ratios downcore. Comparing the 940m site with other sites illustrates that in the presence of oxygen, the combined irrigation and digestion effects of macrofaunal activity result in the preservation of pheophytin, but in the absence of oxygen, digestion leads to pheophorbide production and preservation.

This same trend would be expected at the 850m site, due to its low oxygen availability and abundant macrofauna. The fact that this was not seen could be due to its slightly different faunal community compared to the 940m site, or to within-site heterogeneity and the fact that only one core from this site was studied.

Thus, in oxygenated, bioturbated environments, pheophytin was less reactive than pheophorbide, and when oxygen and fauna were scarce, the decay rates of pheophytin and pheophorbide were very similar. The combined effect of low oxygen availability and pheophorbide production by fauna (at the 940m site) produced pheopigment dynamics that were distinctly different from any other site.

6.4.8 Accessory Pigments

An examination of the cross-margin and downcore variations in accessory pigment weight percentages (of the total intact pigments) revealed relative reactivities among the intact pigments. Downcore profiles of weight percentages (data not shown) showed that chlorophyll-a and alloxanthin accounted for roughly the same proportion of the total intact pigment suites at all sites, and generally decreased in significance downcore. Thus, these two compounds were more readily degraded than the other intact pigments. Diatoxanthin and zeaxanthin showed roughly constant weight percentages downcore, so were neither selectively decayed nor preserved compared to the other pigments.

The weight percentage of the intact pigment suite accounted for by β-carotene increased downcore in all cores analysed from surface values of ~20-40% to ~40-60% at ~30cm depth. Thus β-carotene was selectively preserved compared to the other pigments. This selective preservation of β-carotene is in contrast to a study by Louda et al. (2002), in which β-carotene in fresh algal detritus was observed to decay faster than chlorophyll-a, but is consistent with the finding of Repeta (1989) that carotenes were among the accessory pigments least likely to decay.

Across the margin, weight percentages of β-carotene increased, and those of zeaxanthin decreased with increasing water depth. These relationships with water depth suggest that water column decay processes may influence the accessory pigment composition of sediments. This is consistent with the earlier observation that two accessory pigments (fucoxanthin and 19'hexafucoxanthin), thought to be

produced in the surface waters of the Arabian Sea, were not found in the sediment, and so must have decayed entirely during sinking.

Surface weight percentages of alloxanthin and diatoxanthin showed maximal values within the OMZ. This implies that these pigments were primarily influenced by oxygen availability.

Studies of the products of accessory pigment decay have highlighted several mechanisms by which this occurs (including reduction of carbon double bonds, carbon chain cleavage, and hydrolysis reactions), and have found a consequently wide range of degradation products (Watts and Maxwell, 1977, Repeta and Gagosian, 1984, Repeta, 1989, Hopmans et al., 2005). None of these products were detected in this study (for most of them mass spectrometric detection is required), thus preventing comparisons between chlorophyll-a-pheopigment and xanthophyll-decay product dynamics.

In summary, downcore plots showed chlorophyll-a and alloxanthin to be the most readily degraded intact pigments, and β-carotene the least. Variations in intact pigment suite across the margin showed zeaxanthin to be preferentially lost, and β-carotene to accumulate during water column decay, and that weight percentages of diatoxanthin and alloxanthin were influenced by redox-related preservation.

6.4.9 Calculated Chlorophyll-a Derived Bioturbation Rates

Published chlorophyll-a decay rates from oxic coastal sediments were imposed in modelling of chlorophyll-a profiles, and bioturbation rates were then adjusted to achieve the best model fits. The degradation state of OM on the Pakistan margin however makes it very likely that actual chlorophyll-a decay rates were considerably slower than those used in modelling. Consequently, the bioturbation rates modelled using rapid decay constants are maximal values (see equation 1). It is thought, however, that modelled decay rates still produce a valid comparison between sites and seasons within this study.

The 140m site and the lower OMZ boundary sites (850m, 940m, and1100m) showed the highest mixing rates (Table 6.5), and this is in accordance with the abundance of macrofauna at those sites. The 1200m site showed relatively low mixing rates

compared to sites close by, and this also corresponds with a comparative lack of macrofauna at that site.

The 140m site showed a dramatic post-monsoon reduction in mixing rate that corresponded to a reduction in oxygen availability and macrofaunal activity, as detected in isotope tracing experiments (chapter 4). This illustrates that by using chlorophyll-a as a mixing tracer ($t_{1/2}$ = 8-35 days for oxic decay of fresh OM), it is possible to resolve changes on a seasonal timescale that may not be resolved by the use of longer-lived tracers such as ^{210}Pb ($t_{1/2}$ = 22.3 years).

The 940m site also showed a reduction in mixing rate in the post-monsoon season. This was not associated with a dramatic change in oxygen availability, sediment %Corg or macrofaunal biomass, and was either a product of within-site heterogeneity, or was due to a change in macrofaunal behaviour.

Thus, in general, modelled mixing rates were consistent with the macrofaunal abundances observed across the margin. The actual mixing values are however probably not accurate, due to the use of rapid chlorophyll-a decay rates during modelling.

6.4.10 Further Work

Once bioturbation rates on the Pakistan margin have been independently constrained, further modelling will be carried out. This will produce more accurate decay rates for all pigments at all sites, and allow an assessment of how they are influenced by oxygen availability and exposure time, faunal activity, and OM quality. This modelling will ideally also consider non-local transport, as well as diffusive mixing, and will treat pigment decay in as a multiple-G type process (Soetaert et al., 1996). In addition, accurate chlorophyll-a decay rates, together with forthcoming data regarding the chlorophyll-a content of sedimenting particulate matter, will be used to convert chlorophyll-a inventories into flux rates of OM to the sediment (Boon and Duineveld, 1998).

6.5 Conclusions

- Maximal pigment concentrations in Pakistan margin sediment were found at sites exhibiting minimal oxygen concentrations and macrofaunal populations.

- The pigment suite was dominated by pheopigments, which suggested that OM on the Pakistan margin was relatively degraded.
- The distribution of most pigments was influenced by oxygen availability. Low oxygen conditions favoured the preservation of relatively large unreactive pigment pools.
- Maximal pigment abundances and fresh pigment suites were associated with, and indicative of, relatively fresh OM.
- Pigment concentrations provided relatively little evidence of a monsoon induced flux of fresh OM to the sediment, and this may have been an illustration of the relatively reactive nature of pigments.
- A seasonal increase in pigment abundance was seen at the 1200m site, leading to the suggestion that OM deposition on the Pakistan margin is focused on the OMZ lower boundary.
- The presence of abundant macrofauna was associated with minimal pigment abundances, and with the deeper penetration of reactive pigment pools.
- Modelling of pigment decay at OMZ laminated sites produced half-lives that ranged between approximately 500 and 3000 days.
- Pheophorbide was observed to be more reactive than pheophytin in oxic conditions, and this difference in decay rates was reduced in hypoxic conditions.
- β-carotene was the least reactive of the intact pigments. The distribution of alloxanthin was particularly influenced by oxygen availability, and that of zeaxanthin appeared to be partially dependent on the extent of decay during passage through the water column.

CHAPTER 7

The Occurrence Of Carbohydrates In Sediments From The Pakistan margin

7.1 Introduction

The carbohydrates are a large and important class of biochemicals. They are the primary product of all photosynthesis, and include cellulose, the most abundant polymer on Earth. The functions of carbohydrates in plant cells can be broadly divided into structural and storage roles. Each group contains certain polysaccharide polymers, made up of contrasting distributions of monosaccharides. The differences between storage and structural polysaccharides result from these compositional contrasts, as well as differences in molecular weight, ease of hydrolytic breakdown, and the numbers and types of long- and cross-chain linkages.

Carbohydrates have been found to constitute 21-49% of the carbon in marine phytoplankton (Biersmith and Benner, 1998 and references therein, Cowie and Hedges, 1984), and 5-16% of identifiable organic matter (OM) in marine sediments (e.g. Cowie et al, 1992, Vichkovitten and Holmer, 2005, Danovaro et al., 2001, Wakeham et al., 1997). Organic carbon (Corg)-normalised aldose yields of sinking material have been observed to decrease with depth in the water column (e.g. Hamilton and Hedges, 1988, Ittekkot et al., 1984 a, b, Hamanaka et al., 2002), and the concentration of dissolved aldoses in the ocean also follows this trend (Pakulski and Benner, 1994). Aldoses are also degraded in preference to bulk organic carbon across the sediment-water interface, and this transition can cause an up to 75% loss of carbohydrates (Hedges et al., 1988). Thus, in general, they appear to be comparatively reactive components of marine OM (Hedges et al., 1999, Haake et al., 1993b), being preferentially degraded in the water column (Cowie et al., 1992 and references therein). As such, they may represent a high quality food source to heterotrophic bacteria and other organisms.

Conversely, previous studies (e.g. Hedges et al., 1999) have shown a lack of variation in sedimentary carbohydrate yields or composition, either downcore or between contrasting benthic environments (e.g. across the Washington margin, or estuary versus ocean floor, Burdige et al, 2000), and that they generally appear less readily degraded in the sediment than, for example, amino acids (e.g. Cowie et al., 1992, Wakeham et al., 1997).

The difference in carbohydrate reactivity between water column and sediments suggests the existence of different carbohydrate pools, likely to be split between soluble and reactive intracellular storage polysaccharides, and more resistant structural polysaccharides. Indeed, the reactive component in the water column (e.g. Hamanaka et al., 2002) and during zooplankton digestion of algal OM (Cowie and Hedges, 1996), has been clearly linked to glucose-rich starch-like polymers and other intracellular sugars, while the more resistant structural polymers, such as cellulose and hemicellulose (rich in mannose, galactose and xylose) have been shown to be preferentially preserved in surficial sediments.

Within the sediments, there is evidence for an influence of organic-mineral interactions on carbohydrate cycling and fate. For example, Bergamaschi et al. (1997) showed that aldose composition differed between sediment grain size fractions on the Peru margin, due to the protective adsorption of diatom cell wall carbohydrates onto fine-grained sediments. Also, Keil et al. (1998) demonstrated that sorption of aldoses onto mineral surfaces, and subsequent protective preservation, showed "certain preferences between compounds and mineral types", with clay associated carbohydrates having the largest weight percentages of rhamnose and fucose (Keil et al., 1998). Ultimately, sediment studies indicate that some carbohydrates, either inherently or through association with a protective matrix, are comparatively refractory, and that they could contribute significantly to buried OM. In addition, it has been suggested that uncharacterised sedimentary OM, which becomes an increasing proportion of the total residual OM as decay progresses, is partly composed of altered or condensed carbohydrates that fall out of standard analytical windows (Klok et al., 1984).

Much previous study of sediment carbohydrate geochemistry has focused on estuarine and coastal settings (e.g. Hedges et al., 1994, Cowie et al., 1992, Hamilton and Hedges, 1988), where vascular plant remains and other terrestrial OM are a significant portion of the available OM, water columns are short, and sediments are suboxic to anoxic. Relatively few studies have focused on continental margin and deep-sea sediments, and there has been no previous characterisation of sedimentary carbohydrates across an oxygen minimum zone (OMZ), where sediments show dramatic contrasts in redox conditions, OM abundance and quality, benthic

communities and microbial process rates. Even fewer previous studies have related the behaviour of carbohydrates to that of other biochemicals and source/degradation state indices, and none have been conducted in parallel with studies of benthic communities and their turnover of organic matter.

In summary, few studies to date have investigated how the carbohydrate content or composition of sediments varies over a wide range of environmental conditions. Thus, the controls on sedimentary carbohydrate geochemistry, and how these relate to factors determining bulk OM cycling and preservation, remain poorly constrained. The interplay and relative importance of factors including oxygen availability, faunal communities, bioturbation, and the quantity and quality of OM delivered to the benthic interface, is yet to be defined. Thus, it is unclear what physical, chemical or biological mechanisms result in preservation of refractory carbohydrates, which are similar in composition to labile carbohydrates. Conversely, it is not known if or how the reactive carbohydrate content of sediments affects the benthic community, and whether the carbohydrate abundance or composition in sediments may be indicative of OM bioavailability.

In this study, the carbohydrate concentrations and compositions of Pakistan margin sediments were investigated. The Pakistan margin features an intense and permanent OMZ, where steep gradients in bottom water oxygen concentration are accompanied by variations in sediment OM contents and microbial processes, and in benthic faunal communities (size, composition and function). It therefore provides a natural laboratory for addressing the following questions:

- What is the carbohydrate abundance and composition of Pakistan margin sediments, and how does this relate to OM source and state of decay?
- What environmental and biological factors control the carbohydrate content of marine sediments?
- Does carbohydrate abundance control benthic community size or behaviour?
- How reactive are carbohydrates in relation to other biochemicals, where and when does sedimentary carbohydrate decay occur, and is it compound-selective?

- In what way are carbohydrates indicative of OM quality (either bioreactivity or degradation state), and how do they relate to other indices?

In addition, this study was carried out alongside studies of other biochemical distributions, which allowed independent assessments of the abundance and quality of sedimentary OM, and comparisons of carbohydrate dynamics with those of other major biochemicals. A parallel biological survey of all study sites was also conducted, therefore allowing direct links to be made between biological and geochemical processes.

7.2 Methods

7.2.1 Field Area

Sediments were sampled at sites along an offshore transect of the Pakistan margin of the Arabian Sea. In this region, a combination of monsoon-driven upwelling and intense productivity, sluggish renewal of intermediate water, and an aged mid-water source produce a permanent zone of oxygen-depleted waters between depths of roughly 150m and 1000m (an oxygen minimum zone, OMZ). Where these waters impinge on the continental slope, the variation in oxygen conditions brings about variations in faunal community, and sediment structure and geochemistry (Cowie, 2005, and references therein). Above and below the OMZ, oxygen availability supports diverse macro- and meiofaunal communities and results in bioturbated, homogeneous sediments. Within the OMZ, the OM contents of the sediments is greater (Cowie et al., 1999) and of higher quality (as defined by the amino acid based degradation index (DI), Dauwe and Middelburg, 1998, Suthhof et al., 2000, Schulte et al., 2000). Under the prevalent hypoxic conditions at the core of the OMZ (oxygen ~ 0.1 ml L^{-1}), macrofauna are almost entirely absent, and foraminifera dominate the benthic community.

Monsoon-induced upwelling and productivity in the Arabian Sea generate biannual fluxes of fresh OM to the sediment (Haake et al., 1993a). Thus the food source on which the benthic food chain depends is delivered in a pulsed manner.

Sampling across the Pakistan margin in pre- and post-monsoon seasons therefore allowed an assessment of the way sediment carbohydrate concentration and

composition varied between sites with different oxygen availability, bioturbation rate, water depth and benthic faunal community, and of how these changed in response to a seasonal input of fresh OM.

7.2.2 Sampling

Sediment megacores, 30-50 cm in length, were collected from sites along an offshore transect of the Pakistan Margin, which forms a roughly NE-SW diagonal across the area defined by 23°17'N and 22°52'N; and 65°59E and 66°43' E.

The study sites were at water depths of 140m, 300m, 940m, 1200m, and 1850m. The first of these was above the OMZ in the pre-monsoon season, and within it after the summer monsoon (due a monsoon-induced to shoaling of the OMZ upper boundary). The 300m site was within the OMZ, the 940m site was at its lower boundary, and the 1200m and 1850m sites were below it. Sampling was conducted during three cruises, one before (CD 146, April-May), one during the closing stages of (CD 150, August-September) and one after (September-October) the summer monsoon of 2003.

Cores are named hereafter as pre- or post-monsoon, followed by the water depth of the site. The pre-monsoon 940 B core is a replicate from the 940m site taken at the same time as the pre-monsoon 940m site core. The post-monsoon 300 A core was taken on cruise CD 150, during the closing stages of the monsoon, and earlier than the other post-monsoon cores.

Cores were stored at seafloor temperature until sectioning, and were sectioned at intervals of 0.5 cm to 2 cm, then 1 cm to 10 cm, and 2 cm thereafter. Samples were stored frozen in plastic bags until freeze-drying.

7.2.3 Analytical

Gently homogenised sub-samples were weighed into tubes for H_2SO_4 hydrolysis, adapted from Cowie and Hedges (1984b). This was followed by the addition of an internal standard (myo-inositol), anion and cation exchange cleanup of the extract, and the conversion of the sugars to their alditol acetate derivatives (see Fox et al., 1989 for a discussion of this method, and chapter 2 for the full protocol).

Monosaccharide	Average RSD (%)
Rhamnose	9.8
Fucose	10.8
Ribose	16.5
Arabinose	8.7
Xylose	10.1
Mannose	10.5
Galactose	11.6
Glucose	12.4
Total	10.2

Table 7.1. Average relative standard deviations, n = 10.

Derivatives were analysed by gas chromatography, using a flame ionising detector. The instrument was a HP 5890 equipped with a SP 2330 60m, 0.32mm I.D. capillary column from Supelco. The GC oven program started at 90°C and was ramped to 190°c at 20°C/min, then to 250°C at 4°C/min. This was followed by a ramp to 270°C at 20°C/min, where the temperature was held for 7 minutes to bake the column. The injector and detector temperatures were 280°C and 250°C respectively, and the He flow rate was 2 ml min^{-1}. All monosaccharides eluted within 35 minutes. Peak identities were confirmed by comparison with single compound standards. See chapter 2 for further details.

For each core analysed, at least one depth horizon was analysed in duplicate or triplicate. Average relative standard deviations for the monosaccharides ranged between 8.7% and 16.5%, and are given in Table 7.1.

7.2.4 Data Processing

Monosaccharide yields are reported both as mass per weight of dry sediment, and normalised to the organic carbon contents of the sediments (mg/ 100mg Corg, Corg will be used for sediment organic carbon weight percentage). The percentage that each monosaccharide contributed to total yields was calculated on a mass basis.

Inventories of reactive and total monosaccharides were calculated after fitting a smooth curve to the data. Reactive monosaccharides were those in excess of the downcore asymptote (unreactive) concentration. Total inventories also included the

monosaccharides that comprised this unreactive background concentration. Reactive inventories were calculated at the depth where the reactive concentration became zero (where the curve reached the asymptote). Total inventories were calculated at 20 cm depth for all cores. The model curves were the best-fit results of the exponential decay model:

$$[carbos]_x = [carbos]_0 \, e^{-(k/Db)\,x} \qquad (1)$$

where [carbos] is the concentration of carbohydrate at the sediment surface (o) or at depth x, k is the carbohydrate decay constant (day^{-1}), and Db is the diffusive mixing rate (day^{-1}).

Both biodiffusion and decay rates were adjusted to optimise curve fit, therefore neither parameter was accurately modelled. Unreactive background concentrations were subtracted from the data before curve fitting, and added back in to modelled concentrations.

Correlation coefficients (ρ) of pairs of data sets were calculated using the Microsoft Excel data analysis add-in. They represent the covariance, divided by the product of the standard deviations of the two data populations in question. The 5% significance level for this data set (12 cores = 10 degrees of freedom) was 0.576.

Principle component analysis was carried out on aldose weight percentage data. In order to set the Pakistan margin in context, this analysis included not only all Pakistan margin surface and downcore data, but also additional data from phytoplankton, sediment trap materials and sediments from other marine environments (Appendix E). The analysis provided a score for each sample on each of the first and second principle component axes (PC1 and PC2), together with the percentage of the total variation accounted for by each axis, and a factor coefficient for each aldose, indicating to what extent it influenced the sample scores.

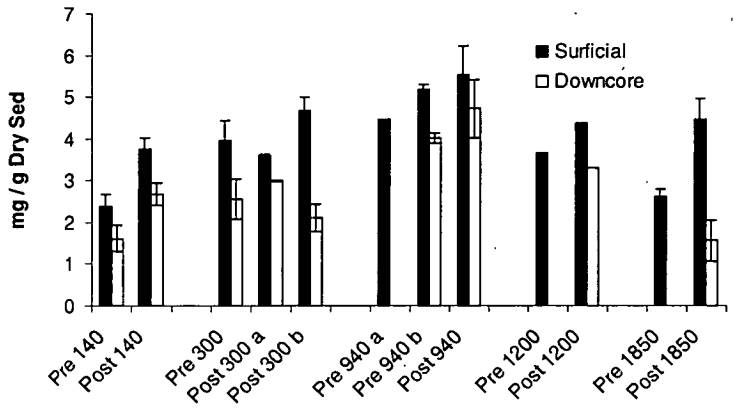
Where surface sediments are discussed, the data are averages over the surface 1cm of sediments, except for DI values, which are averages over the top 3cm.

7.3 Results

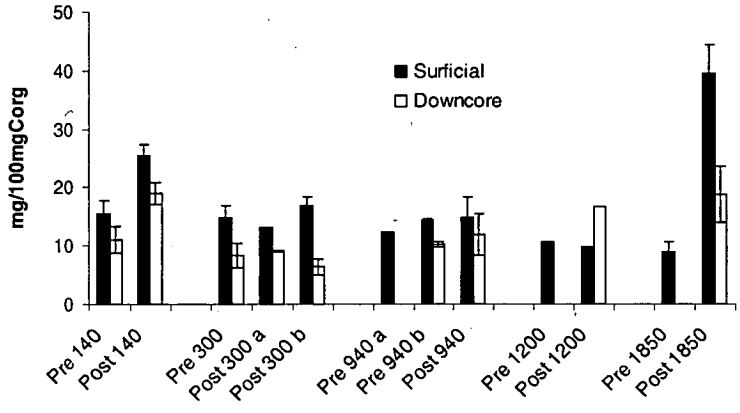
Aldose concentrations in the post-monsoon core from the 1850m site were anomalously high, and caused aspects of the whole data set to be self-contradictory. The downcore trends and aldose suites in this core were however consistent with the

other samples. The anomalously high values may be the result of a calibration error, or may be the result of an OM quality hotspot having been sampled. Such intense within site heterogeneity was also shown by the analysis of 1850m site sediments for their lipid contents. Downcore trends and the aldose composition of this core are described and discussed, but it is excluded from cross-margin trends and correlations.

7.3.1 Distribution of Total Sugars In Surface Sediments



A

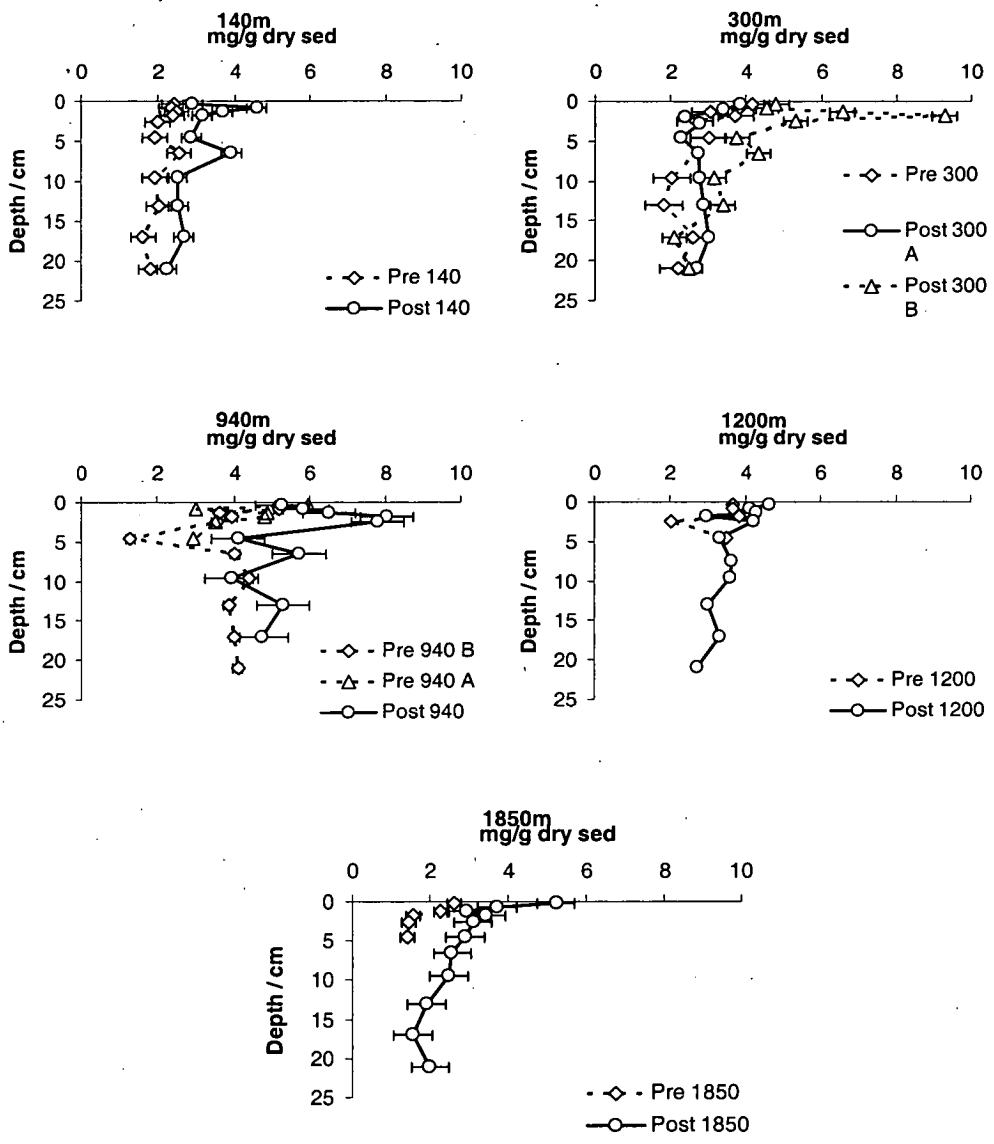


B

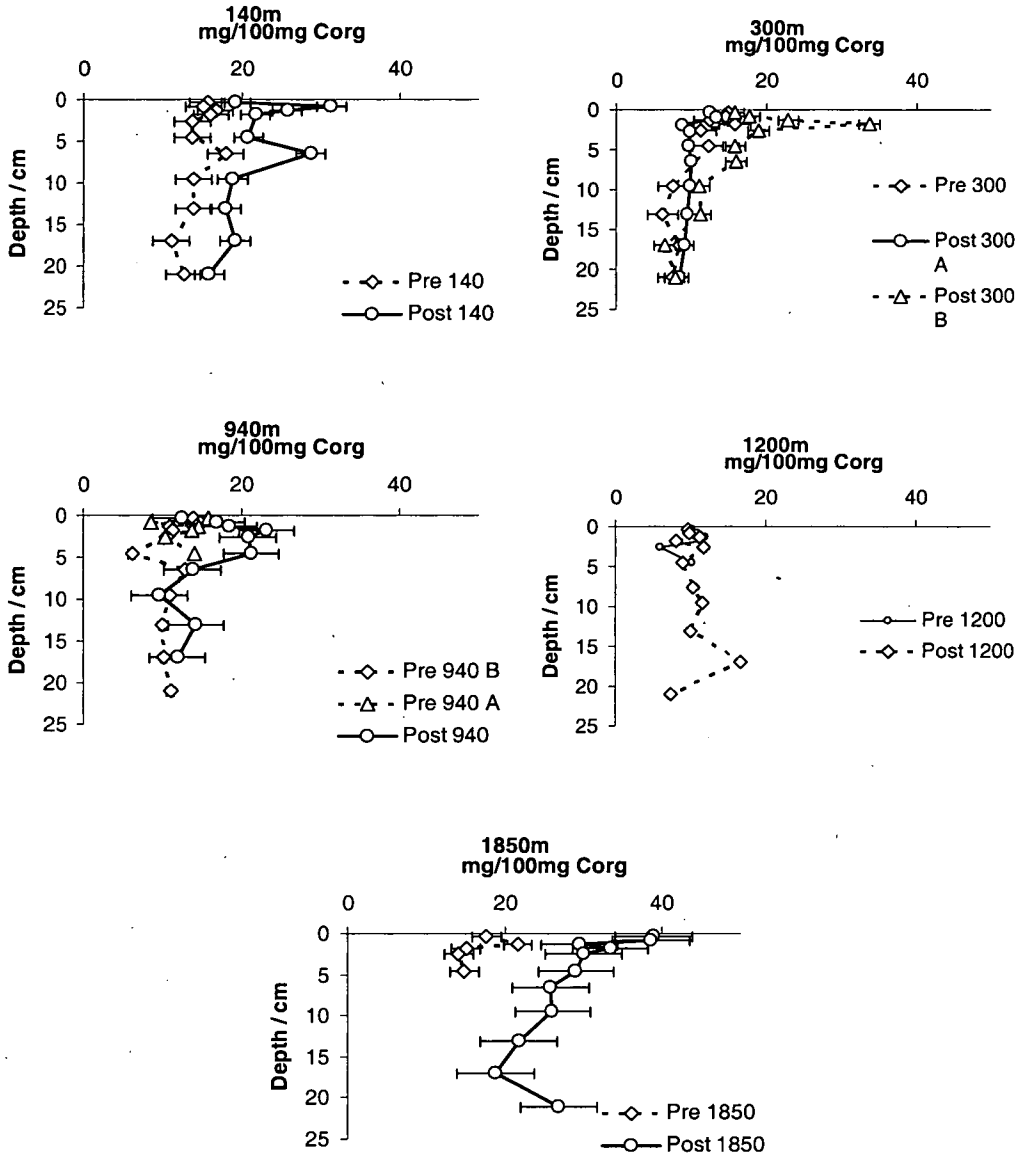
Figure 7.1. Surface (0-1cm) and downcore (17 cm) concentrations of total aldoses as A) μg/g of dry sediment, and B) mg/100mg of organic carbon. Error bars are 1 standard deviation.

Variation across the Pakistan margin in surficial sediment total aldose yields was relatively subtle, with the lowest values found at the 140m and 1850m sites (minimum value 2.4 ± 0.3 mg/g dry sediment), and the highest values at the 300m and 940m sites (maximum 5.5 ± 0.7 mg/g dry sediment) (Fig. 7.1A).

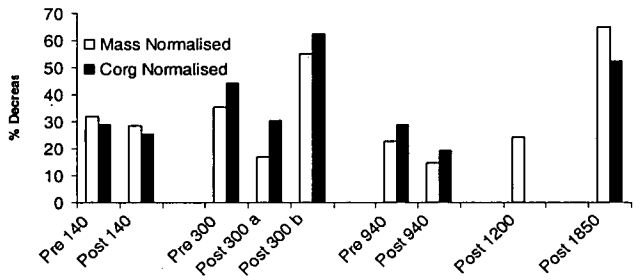
Total Corg-normalised aldose yields ranged between 9 and 26 mg/100mgCorg, and showed a gradual decrease with increasing water depth (Fig 7.1B). The average percentage of organic C identified as carbohydrates for surface sediments over all sites and seasons was 6 %.



A



B



C

Figure 7.2. Downcore trends in total aldoses as A) µg/g dry sediment, and B) mg/100mg Corg. C) The percentage decrease in these parameters between the surface and 17cm depth.

7.3.2 Seasonal Changes

There were slight increases in surficial total carbohydrate concentrations between pre- and post-monsoon seasons at all sites, and these were larger than analytical error for all except the 300m and 940m sites (Fig. 1A). This seasonal increase was also seen in Corg-normalised carbohydrate yields at the 140m site (Fig. 7.1B).

7.3.3 Downcore Trends

At all sites, the abundances of total aldoses as mg/g dry sediment and Corg-normalised yields decreased downcore (Fig. 7.2). These decreases varied between 14% and 65% of the surface concentration, and were more pronounced at the 140m, 300m and 1850m sites than at the 940m and 1200m sites (Fig. 7.2).

As with surface data, differences in downcore concentrations between sites were subtle, and the patterns were similar. Mass normalised yields were highest at the 940m site, then at the 300m and 1200m sites, and lowest at the 140m and 1850m sites. The Corg-normalised total aldose downcore concentrations did not vary outside analytical error among sites, but there was a suggestion that yields were slightly higher at the 140m site (Fig 7.1).

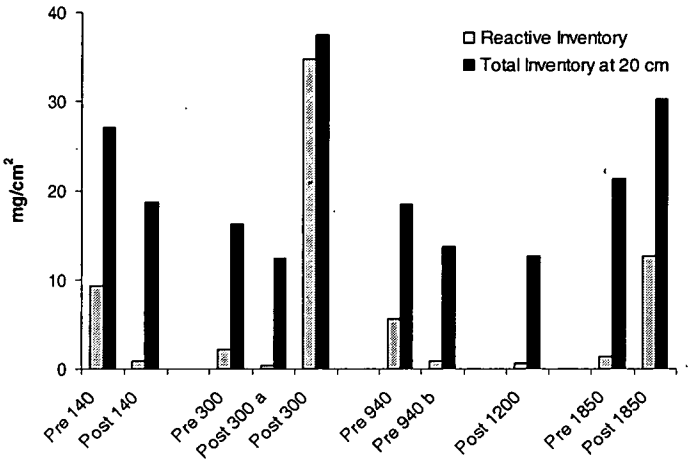
7.3.4 Inventories

Reactive inventories of total aldoses showed a range of 0.5-35 mg cm^{-2}. Total inventories and un-reactive background concentrations showed less variation between stations (than reactive inventories), with ranges of 12-37 mg cm^{-2}, and 0.8-6 mg/g dry sediment respectively (Fig. 7.3, Table 7.2). Modelling of all inventories was complicated by the spiky nature of some downcore profiles. Reactive inventory calculations, which depend only on above-background

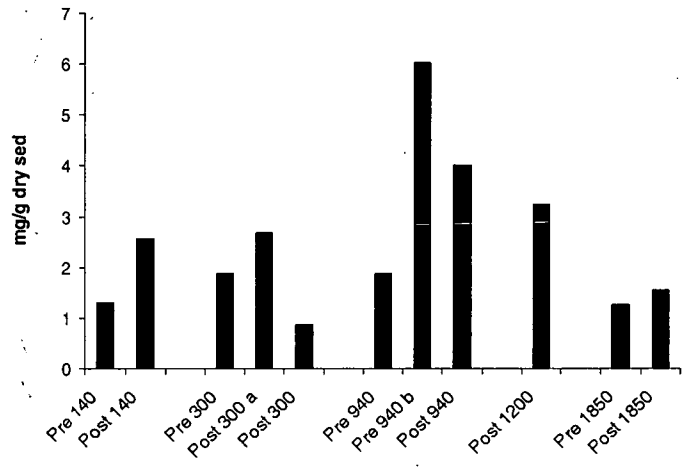
Season	Site	Reactive Inventory / mg cm^{-2}	Total Inventory (at 20 cm depth) / mg cm^{-2}	Unreactive Background / mg g^{-1} dry sed
Pre-Monsoon	140	9.3	27.1	1.3
	300	2.2	16.3	1.9
	940	5.6	18.5	1.9
	940 B	0.8	13.7	6.0
	1850	1.4	21.4	1.3
Post-Monsoon	140	0.9	18.6	2.6
	300 A	0.3	12.4	2.7
	300	34.8	37.4	0.9
	1200	0.6	12.6	3.2
	1850	12.6	30.3	1.6

Table 7.2. Reactive and total inventories, and unreactive background concentrations of total aldoses.

concentrations, suffered the greatest impact from this feature, which produced an estimated error of 100-140%. The error for total inventories and unreactive background concentrations was ~ 70%.



A



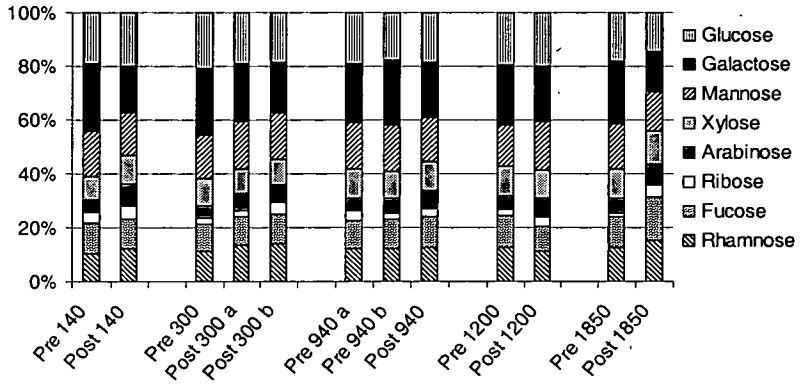
B

Figure 7.3. Inventories of A) reactive aldoses and total aldoses at 20 cm depth in mg cm^{-2}, (data from the post-monsoon 940m core have been excluded), and B) un-reactive background concentrations in mg/g dry sediment.

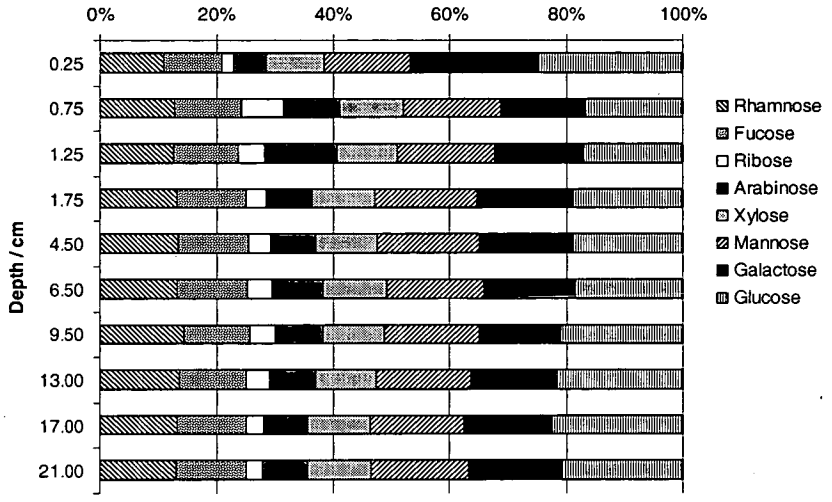
7.3.5 Aldose Suites

The suites of aldoses in Pakistan Margin sediments were dominated by galactose, glucose and mannose, in roughly equal proportions (average 21%, 18% and 16% respectively). Rhamnose, fucose and xylose were nearly as significant (on average 13%, 12% and 11% respectively), and the other sugars (ribose, 3% and arabinose,

6%) were relatively minor constituents (Fig. 7.4). By visual inspection alone, the suite did not vary systematically either across the margin or downcore.



A



B

Figure 7.4. Carbohydrate suites in A) the surface 1 cm of sediments across the Pakistan Margin, and B) downcore at the 140m site in the post-monsoon season.

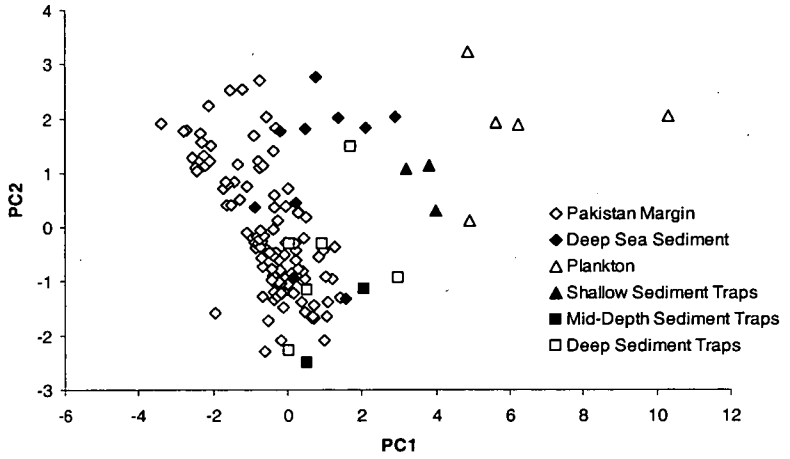
7.3.6 Principle Component Analysis

Principal component analysis was carried out on the Pakistan margin carbohydrate data, together with additional data from samples representing a wide range of stages of decay, including plankton, sediment traps and seafloor sediments from a wide range of locations (Appendix E). This was intended to set the Pakistan margin in

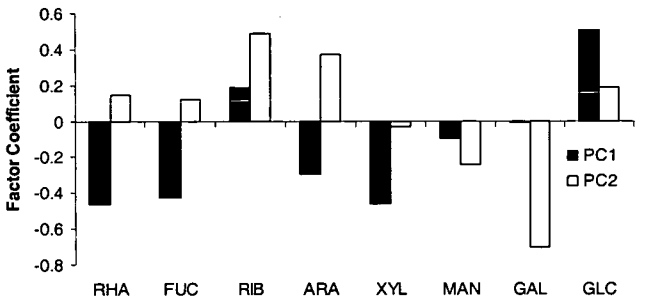
context, and test whether there is a consistent and continuous alteration of carbohydrate suites during decay.

7.3.6.1 Comparison of Sample Types

Principle component analysis separated samples along the PC1 axis (which accounted for 40.1% of the total variance).



A



B

Figure 7.5. A) Principle component 1 and 2 scores for Pakistan margin sediments, and plankton, sediment trap and sediment samples from other marine environments (no trap data was available for the Pakistan margin). B) Aldose PC1 and PC2 factor

The freshest, most organic-rich sample types had the highest scores (Fig. 7.5A). Shallow sediment trap materials (all from within the photic zone or at its base) showed slightly lower scores than fresh plankton. Mid-depth (~100-1000m) and deep (>2000m) sediment trap materials, and deep-sea sediments, had lower scores than surface traps and plankton, but were nearly indistinguishable from each other.

The mid-depth trap materials showed slightly higher PC1 scores than the deep traps, but both groups were indistinguishable from Pakistan margin sediments.

High PC1 scores were associated with fresh materials. Degradation was marked by a loss of glucose and ribose, and increased significance of rhamnose, fucose, arabinose and xylose, and this was reflected in aldose factor coefficients (Fig. 7.5B).

Principle component 2 scores showed a wide range, and the PC2 axis accounted for 20.1 % of the total variance.

7.3.6.2 Cross-Margin Trends

Principle component analysis scores produced a separation of Pakistan margin sites (within each season) along the PC1 axis, roughly in order of site water depth (Fig. 7.6). Shallow sites showed higher PC1 scores, indicative of relatively fresher OM, than deeper sites within the same season.

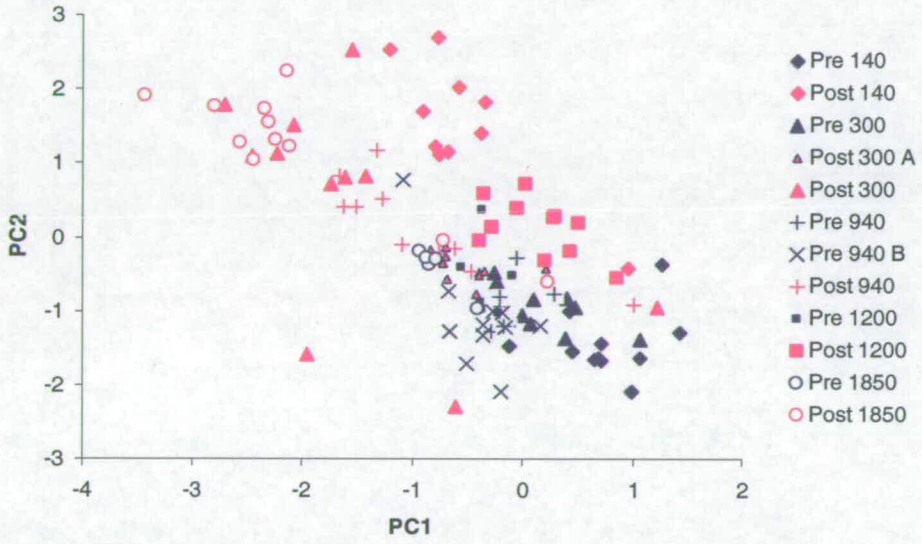


Figure 7.6. Principle component 1 and 2 scores for all Pakistan margin samples, showing separation by site and season. All surface and downcore data are plotted.

Pre-monsoon samples showed slightly higher PC1 scores than post-monsoon samples, and thus appeared somewhat fresher, however the reason for this was unclear (Fig. 7.6). It is unlikely to be an analytical artefact, as such an effect would be expected to disrupt the observed cross-margin trends, but there is no obvious mechanism that could cause a shift to less fresh OM in the post-monsoon season, not only at the sediment surface, but in downcore samples as well.

7.3.6.3Downcore Trends

In 140m, 300m and 940m site cores, PC1 scores decreased downcore. This decrease tended to be concentrated in the surface 2-3 cm of sediment, after which scores evened out at background values, with occasional sub-surface peaks (Fig. 7.7). The decreases indicated relative losses of glucose and ribose, and accumulation of rhamnose and fucose, during compound-selective decay in the sediments. Principle component 2 scores decreased downcore in some cores, but were constant downcore in others, with no consistent trends between sites. The PC2 decreases were associated with the loss of ribose and arabinose, and the accumulation of galactose, typically reactive and recalcitrant compounds respectively.

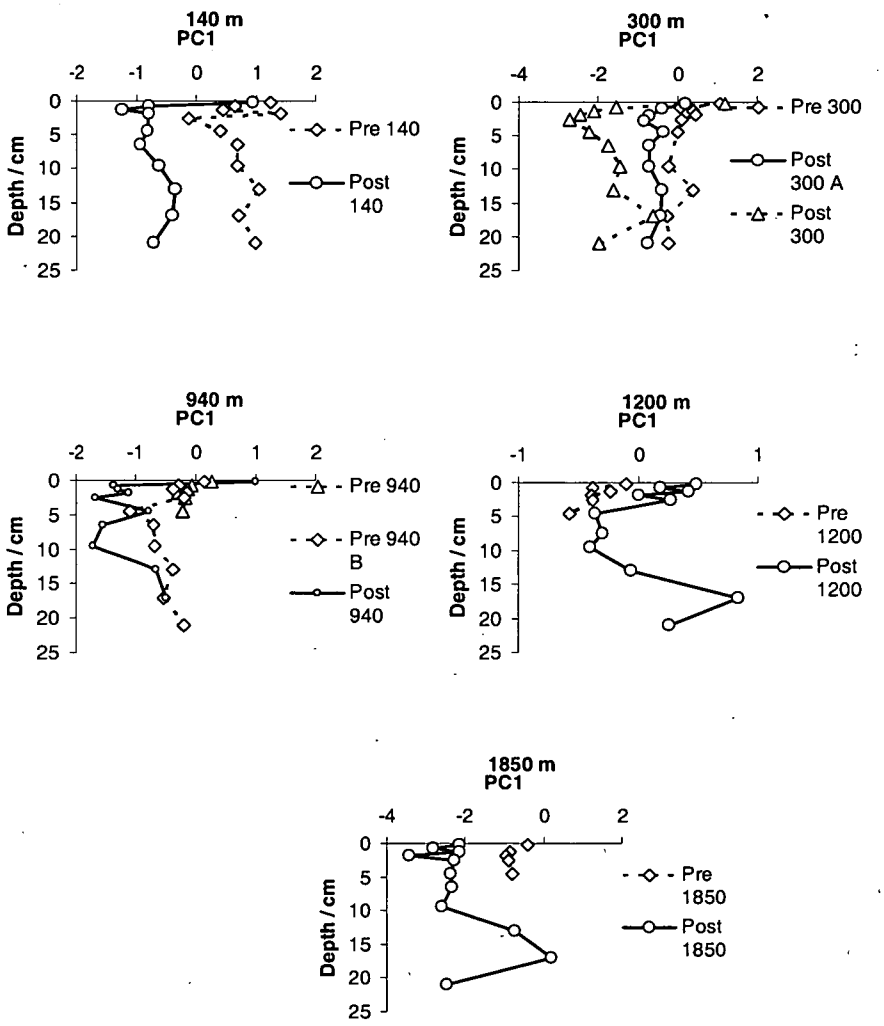


Figure 7.7. Downcore trends in PC1 scores for all Pakistan margin cores studied.

7.4 Discussion

7.4.1 Organic Matter Sources

Monosaccharide-derived parameters have been shown to be potentially diagnostic of organic matter source (Cowie and Hedges, 1984a). These include the glucose/ribose ratio, the Corg-normalised total carbohydrate yield, and the weight percentage of aldoses accounted for by ribose plus fucose. Relatively low values of the first two of these, and high values of the latter are indicative of a predominantly marine, as opposed to a terrestrial higher plant, OM source. High weight percentages of ribose are sometimes used to indicate a bacterial OM source (Hedges et al., 1994), and arabinose:fucose ratios greater than 1 have been suggested to indicate calcareous as opposed to siliceous phytoplankton (Ittekkot et al., 1984 a, b).

The average value of glucose/ribose for all Pakistan margin samples was 7.5 ± 3.3, which is firmly within the range of marine derived OM (values of <~20 indicate marine OM, Cowie and Hedges, 1984a). Both the average total aldose yield (15.9 ± 6.9 mg/100mg Corg) and the average weight percentage of total monosaccharides represented by ribose plus fucose (on a glucose free basis) (16.9 ± 3.5 %) of all samples analysed also fell within the ranges of marine OM (≤ 17 mg/100mg OC and 12-33% respectively, Cowie and Hedges, 1984a). Further, the average arabinose:fucose ratio of Pakistan margin sediments was 0.55 ± 0.15, indicating predominantly siliceous plankton as the source of OM (Ittekkot et al., 1984 a, b). This is consistent with studies of the plankton community in this region, which was found to be dominated by diatoms (Barlow et al., 1999).

The observed Corg-normalised total sugars yield could conceivably be produced by selective degradation of terrestrial OM, but given that two other compositional indices support the yield-based source indication, it is reasonable to conclude that the carbohydrate suite in Pakistan margin sediments indicates an almost exclusively marine source. This is unsurprising given the offshore location of the sampling sites, but does indicate that terrigenous discharge from the nearby Indus River did not have a measurable impact on the organic content of sediments on this part of the margin. This is possibly due to long-shore currents, or the channelling of terrigenous sediments to the continental rise via the Indus canyon system (Cowie, 2005, and

Station (Depth (m)	Temperature (°C)	Dissolved Oxygen ml L^{-1}	Sediment %Corg	OM Quality (DI)	Macrofauna Biomass g(wet) m^{-2} / Diversity	Foraminifera Density / Diversity
Pre Monsoon						
140m	22.5	2.05	1.46 ± 0.08		9 / 51 (± 5)	593 / 19
300m	15.5	0.10	2.36 ± 0.09		0.020 (± 0.022) / 2 (± 0.5)	549 / 18
940m	9.0	0.13	3.31 ± 0.12		62 (± 45) / 12 (± 1)	80 / 13
1200m	7.2	0.34	3.27 ± 0.26		0.4 (± 45) / 13 (± 2.6)	77 / 16
1850m	3.5	1.78	1.40 ± 0.10		9 (± 15) / 53 (± 6)	24 / 8
Post Monsoon						
140m	18.2	0.11	1.43 ± 0.07	-0.99 ± 0.06	5 (± 2) / 45 (± 3)	1163 / 20
300m	14.8	0.11	2.56 ± 0.29	-0.40 ± 0.12	0.013 (± 0.019) / 1	839 / 14
940m	9.3	0.17	3.40 ± 0.13	-0.48 ± 0.03	45.7 (± 0.02) / 13 (± 1)	
1200m	7.3	0.27	3.27 ± 0.26	-0.49 ± 0.18	7 (± 11) / 14 (± 0.7)	
1850m	3.7	1.7	1.20 ± 0.25	-1.17 ± 0.14	2 (± 0.9) / 44 (± 4)	

Table7. 3. Site conditions in pre- and post-monsoon seasons. Oxygen concentrations are from CTD casts, %Corg values are for the surface 0-0.5 cm, DI values are averaged over the surface 3 cm. Macrofauna diversity is species number per megacore averaged from 5 cores (Peter Lamont, pers.comm.), foraminifera density data are total number of calcareous individuals in 15 cm^2 of the surface 1 cm of sediment, and diversity data are species number for the same sample volume (Stefanie Schumacher, pers. comm.).

references therein). The indication of a marine source for aldoses on the Pakistan margin is consistent with previous studies of the same location, which also showed a predominantly marine source for OM, based on combinations of δ^{13}Corg, C/N ratios, lignin yields (Cowie et al., 1999), and δ^{13}C and odd/even carbon preference index of *n*-alkanes (Schulte et al., 2000). Monosaccharide yields and compositions do not readily permit distinctions between plankton and bacterial carbon sources.

7.4.2 Relationship of Carbohydrate Concentrations and Compositions With Site Conditions

In this section the variation in conditions among sites will be briefly described, and these will then be related to cross-margin changes in carbohydrate concentration and composition, in order to highlight the factors that influence carbohydrate abundance and decay.

Bottom water oxygen availability, and sediment %Corg and OM quality varied across the Pakistan margin (Table 7.3). Bottom waters at the 1200m and 1850m sites were well oxygenated (max 1.8 ml L^{-1}), while the 940m and 300m sites were intensely hypoxic (and 0.15 and 0.10 ml L^{-1} respectively). The 140m site showed oxygen levels similar to those at the 1850m site before the summer monsoon, but oxygen consumption associated with monsoon-induced productivity caused a shoaling of the OMZ upper boundary, and oxygen concentrations after the monsoon were similar to those at the 300m site. Surface sediment %Corg outside the OMZ (at the 140m and 1850m sites) was ~1.2-1.5%. Within the OMZ, this rose to ~ 2.5%, but maximal values (~ 3.3%) were found within and slightly below the OMZ lower boundary, at the 940m and 1200m sites. Organic matter quality, as indicated by the amino-acid-based degradation index (DI, Dauwe and Middelburg, 1998) also varied across the margin (Sandra Vandewiele, pers. comm.). This index showed the highest quality OM (most positive DI values) to be coincident with the lowest oxygen values, at the 300m site. The next highest quality OM was found at the 940m and 1200m sites, followed then (after a considerable gap) by the 140m and finally the 1850m sites (Table 7.3). Pigment analysis also showed higher absolute concentrations at lower oxygen sites, again indicative of fresher OM within the OMZ (Chapter 6).

The 940m site hosted a considerably larger macrofaunal biomass than the140m and 1850m sites. Sediments at the 1200m site were bioturbated, but very little macrofauna was recovered, while at the 300m site, macrofauna were almost absent, and the faunal community was dominated by foraminifera.

Together, these results suggest that bulk sediment OM content and OM quality were influenced by bottom-water oxygen availability, but in slightly different ways. Thus oxygen exposure is thought to be one of a number of factors influencing OM distribution across the Pakistan margin (Cowie et al., 1999, Calvert et al., 1995).

At first glance, carbohydrate abundances seemed to be comparatively invariant across the Pakistan margin (+/- ~ 70 % of the average), and to follow more closely cross-margin trends in OM quality (as indicated by DI values) than in OM quantity. None the less, the general maximum in carbohydrate yields that was roughly coincident with the OMZ indicates a similar influence of oxygen availability on carbohydrate concentrations to that on bulk OM quantity. It is worth noting however, that correlations between total aldose yields and site conditions were much weaker than those between %Corg, DI and oxygen concentration (ρ = 0.7-0.9), suggesting that carbohydrates were neither particularly labile, nor a major determinant of OM quality in Pakistan margin sediments.

7.4.2.1 Organic Carbon

The Corg contents of sediments on this margin are influenced by multiple factors, including supply as well as preservation effects (e.g. Calvert et al). Mass-normalised total aldose yields (in surficial sediments) showed a positive relationship with %Corg (ρ = 0.77), with maximal values within and just below the OMZ. This positive relationship between aldose yield and Corg, of which aldoses form a significant part, is to be expected.

Total Corg-normalised carbohydrate yields showed subtler differences between sites than mass-normalised yields (Fig.7.1), which suggests that the factors controlling bulk OM distributions are essentially the same as those controlling carbohydrate yields.

7.4.2.2 Oxygen

Total mass-normalised aldose yields displayed a negative correlation with bottom-water oxygen concentrations ($\rho = 0.78$). This suggests that carbohydrate distributions may be partly controlled by redox-related differences in preservation. In line with this, Hedges et al., (1999) found the carbohydrate contents of sediments to be dependent on oxygen exposure time (OET), up to a threshold at around 200 years, where decay ceased to be selective in favour of carbohydrates, and became indiscriminate.

Unreactive background aldose concentrations also showed a weak negative correlation with bottom water oxygen concentrations ($\rho = 0.53$, excluding the post-monsoon 300m site, which is questionable due to downcore scatter), which was not shown by reactive aldose inventories. Thus, it is possible that to some extent oxygen influences the abundance of the more refractory fraction of sedimentary carbohydrates. This is potentially in accordance with previous findings that the decay of refractory OM is more oxygen-dependent than that of fresh OM (Hulthe et al., 1998, Kristensen, 2000). The lack of correlation between reactive inventories and oxygen concentration may also indicate that the reactive fraction of sedimentary carbohydrates is ultimately removed regardless of depositional redox conditions. The negative relationship with oxygen availability was more pronounced for glucose ($\rho = 0.81$) than for total aldoses, suggesting that redox-related preservation is compound selective.

Total sugar yields normalised to %Corg however showed no correlation with oxygen availability. Thus, the absolute abundance of aldoses in Pakistan margin sediments was dependent on oxygen availability in the same way as was %Corg, however low oxygen conditions did not favour the preservation of carbohydrates over bulk OM, and other factors must control the fraction of OM comprised by aldoses.

7.4.2.3 Organic Matter Quality

Mass-normalised total aldose yields showed a positive relationship ($\rho = 0.70$) with OM quality (DI) (Sandra Vandewiele, pers. comm.), and this relationship was stronger for galactose and glucose ($\rho = 0.72$ and 0.73 respectively). Oxygen availability showed a negative correlation with DI ($\rho = 0.82$). Together, these relationships suggest that higher quality OM is associated with high absolute

abundances of aldoses, and preservation of this high quality OM may be partly controlled by redox-related preservation.

Total Corg-normalised aldose yields showed only a weak relationship with DI (ρ = 0.52), suggesting that to some extent high quality OM was enriched in carbohydrates. Although this relationship was weak, it is consistent with Cowie and Hedges (1994), who, in a sample set representing a wide range of marine environments, found that the percentage of Corg consisting of amino acids plus aldoses consistently decreased with progressive decay.

Neither reactive nor total carbohydrate inventories correlated with DI, but the un-reactive background concentration of aldoses showed a weak positive correlation (ρ = 0.48). Thus, it is the un-reactive pool of carbohydrates preserved at depth that co-varies with surface-sediment OM quality. Correspondingly, the conditions that led to the presence of higher quality OM (high DI values) at the sediment surface (such as low oxygen concentration/exposure) appear also to partition a greater part of sedimentary carbohydrates into the refractory pool. The lack of correlation between reactive inventories and surface-sediment OM quality could be due to errors resulting from spiky downcore profiles.

7.4.2.4 Benthic Community

Total mass-normalised aldose yields showed a positive correlation with macrofaunal biomass (ρ = 0.59, Table 7.3). This is a stronger relationship than macrofaunal biomass showed with either sediment %Corg (ρ = 0.52) or OM quality (DI, ρ = 0.26) and potentially suggests that carbohydrate yields are a better indication of food availability (the dominant control on benthic faunal communities) than either DI or %Corg. In ocean floor sediment, only 10% of extractable carbohydrates have been found to be bioavailable, and these are thought to be an important food source to organisms feeding below the sediment surface, where amino acids have already decayed (Danovaro et al., 2001).

The correlation between aldose abundance and macrofaunal biomass, however, may not be directly mechanistic, and the relative invariance of aldose yields across the margin would suggest that they are probably not a good indicator of OM food quality. The lack of correlation between macrofaunal biomass and DI is probably an indirect effect of DI being highest under low oxygen conditions, where macrofauna

could not survive. Total aldose yields may correlate better with macrofaunal biomass by default, through not being markedly enriched under low oxygen conditions. This suggestion is supported by the weak nature of the correlation between aldose reactive inventory (which might be expected to be a better indication of food availability) and macrofaunal biomass ($\rho = 0.34$).

The degree of biological mixing, as determined by visual inspection of sediment x-radiographs, was much lower at the 300m site than at the 140m site (see chapter 2). Sites below the OMZ, showed the greatest degree of bioturbation, to the point where, in x-radiographs of 1200m and 1850m site sediments, individual biological structures could not be distinguished or quantified (Chapter 2, Fig. 2.3). The 140m and 1850m sites exhibited minimal surficial mass-normalised total aldose yields (Fig. 7.1), thus, data from these sites suggests the actions of benthic fauna may be linked to overall lower abundances of carbohydrates. The 1200m site did not however fit this pattern, as it showed both intense burrowing, and relatively high aldose yields. The post-monsoon sample from the 1850m site also produced such an anomaly, but in this case lipid data suggests that relatively rare OM rich patches are a feature of this site (Rachel Jeffreys, pers. comm.).

Downcore decreases (between the surface and 17 cm) in mass and Corg normalised aldose yields ranged from 14-65 % of the values in surficial sediments. The greatest downcore decreases were seen at the 140m, 300m and 1850m sites, with more constant profiles at the 940m and 1200m sites (Fig. 7.2C). Thus the relative magnitudes of downcore decreases in aldose abundance did not appear to be related to bioturbation, or mixed versus laminated sediment. Similarly, PCA of carbohydrate compositional data did not separate the sites with macrofauna from those without; therefore, the actions of burrowing fauna did not appear to cause any clear selective decay among the aldoses.

In general the links between aldose distribution and macrofaunal biomass and activity were relatively weak. Macrofaunal biomass was higher at sites with higher mass normalised aldose abundance, but this relationship may not be mechanistic. There was a suggestion that sites with the greatest degree of bioturbation had minimal mass-normalised yields, but not all sites followed this trend. The relative amount of downcore aldose decay appeared unrelated to bioturbation.

7.4.2.5 Site Depth

Aldose yields normalised to %Corg showed a negative correlation with water depth ($\rho = 0.74$, Fig. 7.1), suggesting that preferential decay of carbohydrates occurs during sinking, and therefore water column length influences the carbohydrate content of sedimentary OM.

Principle component analysis of aldose weight percentages produced a separation of sites in order of site water depth (Fig. 7.6), such that shallower sites had higher PC1 scores, indicative of fresher aldose suites (enriched in glucose and ribose). This lends support to the suggestion that the majority of compound specific carbohydrate decay occurs in the water column, thus the length of the water column influences the aldose composition of the underlying sediments.

No aldose inventory parameters correlated significantly with water depth. Thus, while decay during sinking appeared to influence the aldose composition of OM delivered to the sediment, aldose mass-normalised concentrations and inventories were subsequently influenced by redox and bioturbation related preservation factors.

In summary, the correlations between carbohydrate parameters and site conditions indicated that the distribution of aldoses was controlled by similar factors to those that control bulk OM quantity and quality. High absolute aldose yields were associated with low oxygen conditions and higher quality OM, and oxygen concentration seemed to influence carbohydrate preservation by partitioning it into an un-reactive pool. Carbohydrate abundances were reduced at sites that exhibited intense biomixing. Water depth appeared to influence the %Corg-normalised aldose content and composition of OM delivered to the sediment, through selective decay during passage through the water column.

7.4.3 Selective Decay Of Bulk Carbohydrates

Evidence exists in the literature both in favour of and against the selective decay of carbohydrates over bulk OM, over a wide range of environments.

In decay studies of fresh marine OM, at early stages of alteration, aldoses have been generally observed to be more reactive than bulk OM (e.g. Liebezeit, 1987, Opsahl and Benner, 1999). Aldoses have also been observed to be as, or more reactive than bulk OM in zooplankton guts (Ittekkot et al., 1984 a, Cowie and Hedges 1996),

through the water column, across the sediment-water interface, and down through the sediments in coastal inlets (Cowie et al., 1992, Hedges et al., 1988, Hamilton and Hedges, 1988).

Other studies have observed constant %Corg-normalised aldose yields and suites downcore through marine sediments (e.g. Vichkovitten and Holmer, 2005, Cowie and Hedges, 1984, Cowie et al., 1995, Hedges et al., 1999). In addition, %Corg-normalised aldose yields and suites have been observed to change through the water column, but to be constant through the underlying sediments of the same sites (Cowie et al., 1992, Ogier et al., 2001). Furthermore, in some settings, the preferential preservation of carbohydrates over bulk OM has been suggested (Hernes et al., 1996, Danovaro et al., 2001).

Lack of significant differences in sediment %Corg-normalised carbohydrate yields has been observed between quite different environments. Burdige et al (2000) found similar %Corg-normalised carbohydrate concentrations in estuarine and continental margin sediments. In addition, despite the fact that OM reactivity (determined by the downcore decrease in %Corg) varied over 3-4 orders of magnitude between these sites, the percentage of C oxidation accounted for by sugars remained roughly constant, and was also found to be similar to that in reduced and oxidised portions of the Madeira Abyssal Plain (MAP) turbidite (Cowie et al., 1995).

Together, these results suggest that selective decay of carbohydrates over bulk OM occurs primarily early, and within the water column, leaving relatively refractory and compositionally uniform OM to be deposited (Vichkovitten and Holmer, 2005). Also that, in the sediment, continued OM decay is accompanied by much less distinct aldose compositional alteration.

Given the above discussion, carbohydrates (associated with marine OM) may fall into two pools with different reactivities, the origins of which are possibly related to cell structure. The more readily hydrolysed and reactive pool, which may be largely derived from storage polymers associated with algal cell contents, suffers selective decay among aldoses and with respect to bulk Corg. This decay seems to be rapid enough that, generally, it all occurs within the water column and across the benthic boundary layer, occasionally persisting into the surface layer of the sediment (e.g.

Vichkovitten and Holmer, 2005). The refractory pool, which may be predominantly derived from cell structural components, decays at roughly the same rate as bulk OM. This is consistent with the findings of Hernes et al. (1996), who deduced the existence of three pools of carbohydrates (from water column and sediment studies). The third pool was of intermediate reactivity, and rich in galactose and arabinose, the persistence and subsequent degradation of which caused mid-water galactose and arabinose maxima in sinking particles.

On the Pakistan margin, %Corg-normalised sediment carbohydrate concentrations were found to correlate with water depth, indicating that selective degradation of carbohydrates occurs in the water column. Both mass and %Corg-normalised yields also decreased downcore, the latter suggesting that carbohydrates are also more reactive than bulk OM in the sediments. The relative subtlety of cross margin and downcore variations in yields however suggests that the difference in reactivity between carbohydrates and bulk OM was slight, compared to that between for example pigments and bulk OM (see chapter 6). Thus, the carbohydrates on the Pakistan margin were most similar to the second, refractory pool, described above.

7.4.4 Selective Decay Among The Aldoses

Previous studies of fresh OM degradation (in incubation, water column particulate and coastal sediment studies) have reported selective decay among the aldoses during early OM alteration, during which monosaccharides from intracellular polymers were preferentially lost compared to those from structural polysaccharides (e.g. Ittekkot et al., 1984 b, Ittekkot and Degens, 1982). Specifically, glucose and ribose have been observed to be particularly reactive, while rhamnose, fucose, xylose and mannose typically increased in weight percentage during decay, due either to microbial production or to chemical mechanisms causing selective preservation (e.g. Opsahl and Benner, 1999; Hedges et al., 1988; Hedges et al., 1999; D'souza et al., 2003; Hernes et al., 1996). These suite alterations have been observed during descent through the water column (e.g. Hamanaka et al., 2002), across the sediment-water interface, and downcore through the sediments in a terrestrially influenced fjord and other settings (e.g. Cowie and Hedges, 1984a; Hamilton and Hedges, 1988, Vichkovitten and Holmer, 2005). In a slight variation of the reactivity pattern,

Cowie et al. (1992) observed ribose arabinose and glucose to be preferentially lost across the sediment-water interface, and other aldoses, including xylose, to be near conservative. These compositional trends have been attributed to the selective loss of soluble cell contents polymers such as starch, and the possible production of the deoxysugars rhamnose and fucose during microbial decay.

Visual inspection of Pakistan margin carbohydrate data did not reveal any obvious variations in aldose suites, either across the margin, or with depth in the sediment. Weight percentages of glucose and (rhamnose + fucose) did not decrease or increase (respectively) downcore, and nor did these indices show a negative correlation, as might be expected. Therefore, the evidence suggests that decay of Pakistan margin carbohydrates, where it occurs is not compound-selective.

The weight percentage of (rhamnose + fucose) in surface sediments did, however, show weak negative correlations with sediment %Corg, and with DI ($\rho = 0.41$ and 0.39 respectively), suggesting that these monosaccharides were more abundant in OM-poor, degraded sediments.

More detailed analysis of the data, together with data from a wide range of other studies, reveals further systematic alteration of the aldose suite with progressive decay.

Principle component analysis of aldose weight percentage data from a range of samples, revealed that plankton samples had the highest PC1 scores, and these decreased in the order shallow, mid-depth, and deep sediment trap materials, and finally deep sea sediments (Fig. 7.5). Surficial sediments from shallow Pakistan margin sites had higher PC1 scores than those at deeper sites (Fig. 7.6). High PC1 scores were the result of relatively high proportions of glucose and ribose, which have been shown to be enriched in intracellular algal polymers (starch-like materials for example), and low proportions of rhamnose, fucose, arabinose and xylose, common in structural polymers associated with the cell wall (Fig. 7.5) (e.g. Cowie and Hedges 1996, and references therein). Thus, PCA revealed the occurrence of compound-selective degradation, and showed that the observed suite changes were in line with previously established decay-related alteration.

The observed separation between plankton and shallow (photic zone) trap PC1 scores, indicates that even during sinking over <100m, and recycling within the

photic zone, a significant degree of aldose suite alteration occurs. In contrast, the lack of separation of mid-depth and deep sediment trap PC1 scores from those of Pakistan margin sediments serves to confirm suggestions in this and other studies that compound-selective alteration among the aldoses is a feature of early decay, and that by the time OM reaches mid-to-deep parts of the water column, and into the sediment, carbohydrate decay is largely non-selective.

This suggestion is consistent with other observations made in this study, that %Corg-normalised aldose yields correlated negatively with water depth, and that PC1 scores separated Pakistan margin sites by depth, implying that most compound selective decay occurs in the water column (i.e. early). In addition, the only sites at which PC1 score changed downcore were the shallower 140m, 300m and 940m sites (Fig. 7.7), where water column decay had been minimal. The suggestion is also consistent with previous studies that have found the vast majority of carbohydrate loss to occur between surface and mid-depth sediment traps (Hamilton and Hedges, 1988, Ittekkot et al., 1984 b, Hernes et al., 1996, Unger et al., 2005, Wakeham et al., 1997), and also with Hedges et al. (1999), who found that preferential glucose decay was only apparent over the inshore (and thus shallow) section of a Washington margin transect.

Thus, this collection of a wide range of sample types conforms to previous findings that compound-specific loss tends to occur during early carbohydrate decay, and is associated with preferential loss of glucose and ribose, and preservation, or even production, of rhamnose and fucose.

7.4.5 Aldose Suites And State of Decay

Previous work has found the intracellular aldose composition of algae to be variable among taxa, but to be generally dominated by glucose, with arabinose, xylose, galactose and fucose representing similar proportions to glucose for some species (Biersmith and Benner, 1998). The structural parts of the cell were also dominated by glucose (28-71%), followed by mannose and galactose (5-21% and 5-11% respectively), with others compounds being relatively minor (Biersmith and Benner, 1998, Cowie and Hedges, 1996, and references therein). Glucose has also been observed to be the major aldose (54-70%), followed by xylose, arabinose, rhamnose,

mannose and galactose, in a selection of vascular plants (Vitchkovitten and Holmer, 2004). Decay of these tissues has generally been found to be marked by relative losses of glucose and xylose, and relative increases in rhamnose and fucose, prompting the observation that decay tends to generate a relatively uniform suite of aldoses (Opsahl and Benner, 1999).

The suites of aldoses observed in Pakistan Margin sediments were relatively uniform (Fig. 7.4). Glucose, galactose and mannose all contributed similar proportions (15-20 %), and these were closely followed by rhamnose, fucose, xylose and arabinose (10-15%) with ribose being a minor contributor. Thus, the aldose compositions in surficial and downcore sediments, based on relatively uniform suites and the lack of glucose dominance, suggest that the OM in Pakistan margin sediments is comparatively degraded.

The aldose suites found on the Pakistan margin were remarkably uniform, even compared to other marine sediments. In Dabob Bay, a coastal fjord, the suite was dominated by glucose (~30%), followed by mannose and galactose (~14-19%), rhamnose, fucose, xylose and arabinose (~4-10%), with ribose and lyxose as minor components (<4%) (Cowie and Hedges, 1984a). The glucose dominance there may however partly have been a function of a significant terrestrial organic carbon input, which tends to contain higher proportions of (cellulose-associated) glucose than marine OM. Hernes et al. (1996) noted that the aldose compositions of Equatorial Pacific sediments were remarkably similar to those in Dabob bay and Saanich inlet (Hamilton and Hedges, 1988), and this implies that all of these suites were relatively degraded, and perhaps not susceptible to compositional alteration with continued decay.

Sediments from the Danish coast showed aldose suites similar to those found on the Pakistan margin, in that galactose, glucose and (mannose + xylose) (analysed as one peak) were equally significant (all ~20%), followed by fucose (15%), rhamnose (13%) and arabinose (8%) (Vichkovitten and Holmer, 2004). Kerhervé et al. (2002) also found remarkably uniform aldose compositions in Mediterranean Sea sediments, despite having sampled sites with widely varying physical characteristics. Thus, all Pakistan margin sediments appeared to host aldose suites indicative of comparatively extensive OM decay.

Glucose has often been observed to be the most readily lost aldose during carbohydrate decay from fresh OM into the sediment (e.g. Opsahl and Benner, 1999, Hedges et al., 1988, Hedges et al., 1999). This had led to the suggestion that the weight percentage of glucose might be the best carbohydrate-derived indicator of OM quality (Hernes et al., 1996)

In MAP turbidite sediments, which were ca. 140,000 y old before emplacement on the deep ocean floor, glucose was found to still dominate the aldose composition, comprising ~35-45%, with galactose, mannose, fucose, rhamnose and xylose each comprising ~10-15%, and ribose, arabinose and lyxose comprising ~1-10% of neutral sugars. This glucose dominance was unchanged across the boundary from reducing to re-oxidised sediments (despite an 80% loss of Corg due to oxidation), implying that the aldose suite was unchanged, even during extensive decay (Cowie et al., 1995). Multiple indices (%Corg, DI) showed that these sediments were extremely degraded compared to Pakistan margin sediments, and thus their relatively high percentages of glucose compared to Pakistan margin sediments were likely to be related to differences in OM source (possibly significantly terrestrially influenced), and/or to the persistence of extremely recalcitrant polymers. This example calls into question the use of the weight percentage of glucose as an indicator of state of decay over wide ranges of OM alteration, and among different regions, as it suggests that even after extreme decay, the aldose suite of sediments can still be influenced by OM source.

Site	Total Sugars (mg/100mg OC)	Source
Chesapeake Bay*	8.8 (surface sediment average)	Burdige et al, 2000
Atlantic Slope Break*	5.8 (surface sediment average)	Burdige et al, 2000
Dabob Bay	11-15	Cowie and Hedges, 1984
Saanich Inlet (sed trap)	26	Cowie et al., 1992
Madeira Abyassal Plain Turbidite	11-20	Cowie et al., 1995
Pakistan Margin	16 (average of all samples)	This Study

Table 7.4. Organic carbon-normalised total aldose concentrations from a range of locations. All data concerns sums of individual aldoses, except where * indicates data from total carbohydrate assessment by a spectroflurometric method.

This is a particular problem in settings with significant inputs of terrestrial OM, which tends to be particularly glucose-rich (Unger et al., 2005, Cowie and Hedges, 1984a).

Compared to many of the locations mentioned above, Pakistan Margin sediments contained relatively high %Corg-normalised aldose concentrations (Table 7.4), despite the fact that their aldose suites appeared to be the results of more extensive decay. It has been suggested that the proportion of organic carbon present as total aldoses (observed to decrease as decay progresses) is the best carbohydrate-derived indication of state of decay, either alone (Opsahl and Benner, 1999) or together with the C contribution from total amino acids (Cowie and Hedges 1994). However, in the comparison provided in Table 7.4, this would suggest that relatively fresh OM in coastal Chesapeake Bay sediments was more degraded than turbidite OM on the ocean floor, which is ca. 140,000 years old. Differences in analytical techniques and OM sources may partly account for these inconsistencies; however some studies (e.g. Vichkovitten and Holmer, 2005) have suggested that in some settings carbohydrates are preferentially preserved compared to bulk OM. Thus the percentage of Corg in aldoses cannot be used as an indicator of OM decay in all circumstances.

Principle component 1 scores (derived from PCA of a wide range of samples) may also be indicative of OM degradation state. Being derived from the full aldose data set could help to make PC1 scores a more robust proxy, as has been the case for the degradation index (DI), derived from PCA applied to amino acid compositional data (Dauwe and Middelburg, 1998).

The PC1 scores generated in this study separated plankton, shallow, mid-depth and deep sediment trap materials and deep-sea sediments in that order, and thus appeared to be a good proxy for degradation state. The PC1 scores of Pakistan margin surficial sediments however showed only a weak positive correlation with the amino acid derived degradation index (DI) ($\rho = 0.44$) (Dauwe and Middelburg, 1998). This, and the fact that surface-sediment PC1 scores showed a significant relationship only with depth, whereas DI values were maximal within and around the OMZ (Table 4), clearly indicates that amino acid and carbohydrate decay are de-coupled. Selective compositional change appeared to occur at different stages of marine OM alteration for these two classes of biochemical; early, usually within the water column, for aldoses, and over a wider range of degradation states (continuing into the sediments) for the amino acids.

Unger et al. (2005) performed a similar analysis on carbohydrate data from samples from the Bay of Bengal. Their results also indicated similar suite alterations during decay, and PC1 scores correlated with DI. They noted, however, that the aldose-based index could not consistently differentiate between samples at similar stages of decay, and that the amino-acid-based indicator was more capable of resolving subtle differences in OM quality.

Principle component 1 scores, it should be noted, are susceptible to producing potentially confusing results when other factors are in play. This is illustrated by the fact that MAP turbidite samples (from above and below the oxidation front) had higher (fresher) scores than Pakistan margin sediments, despite evidence from DI and %Corg, to suggest they were much more degraded (Cowie et al., 1995; Dauwe and Middelburg 1998). It is has been noted already that these sediments showed a relatively high glucose content, and that this may have been source related. This glucose content was responsible for the surprisingly high PC1 scores of the MAP turbidite sediments, and thus PC1 scores, as an indicator of degradation state, are vulnerable to interference from source effects, even after extensive decay.

In summary, the uniformity of the aldose suite on the Pakistan margin indicated that the carbohydrates (and therefore the bulk OM) found there were relatively degraded. Principle component 1 scores derived from PCA of aldose weight percentage data were more consistently indicative of OM degradation state than the weight percentage of glucose, or the %Corg-normalised carbohydrate yield. However even PC1 scores were subject to interference from source effects, and could not resolve differences in degradation state as well as the amino acid based DI.

7.4.6 Carbohydrates And The Wider Geochemistry of Pakistan Margin Sediments

The relatively invariant nature of carbohydrate distribution and composition across the Pakistan margin stands in stark contrast to cross margin patterns of %Corg, pigments, amino acids and lipids. These last four parameters showed distinct maxima within, and in the case of %Corg, slightly below the OMZ, and were clearly related to OM quality. In contrast, the carbohydrates showed only weak relationships with bottom water oxygen concentration and faunal activity. The

aldoses showed a relatively uniform suite, which changed slightly but systematically both across the margin and, in some instances, downcore (at the 140m, 300m and 940m sites), supporting the (perhaps surprising) conclusion that they form a relatively refractory fraction of the bulk OM.

7.5 Conclusions

- The carbohydrate yields and compositions of Pakistan margin sediments were rather invariant, despite the presence of an OMZ.
- Sediment mass-normalised yields co-varied with bulk OM and OM quality, and seemed to be influenced to some extent by oxygen availability.
- Links between the distribution of carbohydrates and the size and activities of benthic communities were weak, however intense biomixing was associated with reduced mass-normalised carbohydrate yields.
- Organic-carbon-normalised carbohydrate yields in surficial sediments varied only slightly across the margin, but correlated negatively with water depth; thus decay during sinking appeared to influence the carbohydrate yields of surficial sediments.
- Consistent with the previous conclusion, PCA showed that the aldose composition of surficial sediments became more degraded with increasing water depth. The same analysis showed that downcore compositional alteration was only present at the shallower (140m, 300m and 940m) sites, where surface OM was of higher quality.
- At all sites Corg-normalised aldose yields decreased downcore, thus carbohydrates decayed in preference to bulk OM.
- Principle component analysis of Pakistan margin samples and a wide range of other sample types showed systematic aldose composition changes between plankton, sediment trap materials and ocean floor sediment. These were characterised by relative losses of glucose and ribose, and preservation or production of rhamnose and fucose. They agree with previously published studies of the way aldose suites alter during decay.

- Comparisons of Pakistan margin carbohydrate data with that from other marine settings, suggests that neither Corg-normalised aldose yields, nor glucose weight percentages are consistent measures of OM quality. Principle component analysis sample scores were shown to be a more robust measure, as they are based on the variation of a whole suite of compounds. Such PC1 scores did not however correlate well with the amino acid derived degradation index, suggesting that the locations and mechanisms for amino acid and aldose selective decay were de-coupled.

CHAPTER 8

Synthesis

8.1 Summary of Achievements

8.1.1 Practical

The Arabian Sea project, and my contribution to it have broken new ground on several fronts in the study of continental margin biogeochemistry. The series of four research cruises that were completed allowed a cross-margin study at high spatial resolution of an unprecedented range of parameters, which bridged the divide between benthic geochemistry and ecology. This study was duplicated either side of a monsoon, producing a detailed inter-season comparison.

Several methodological developments were made for this study. Whole-community ^{13}C tracer experiments were conducted both aboard ship and on the seafloor, using a specially adapted benthic lander and a purpose-built shipboard incubation system. These both featured oxystat systems capable of maintaining ambient oxygen concentrations in chamber water, which was vital, given the very low initial oxygen availabilities at several of the study sites. The shipboard system was entirely new, and the protocols for its successful use were devised during cruise CD 146. The simultaneous use of the two systems not only provided backup in case of lander failure, but also allowed comparisons to be made between them. The shipboard system was found to replicate *in situ* results very well (Schwartz et al., in prep.).

These experiments were conducted at sites exhibiting markedly different environmental conditions and faunal communities, and allow the first direct comparisons between such settings. In addition, a method was developed for quantitatively tracing ^{13}C at the molecular level in the form of amino acids. This was not only the first demonstration of the method, but was also the first time C had been traced at the molecular level in whole community, and (in some cases) using *in situ* tracer experiments. Significant progress was also made in the development of a similar method for tracing ^{13}C-labelled carbohydrates, but sample quantities in this study did not permit its use.

8.1.2 Scientific

Isotope labelling experiments were conducted at sites displaying a wide range of oxygen availability and OM quality. This allowed conclusions to be drawn regarding

the short-term fate of OM in marine sediments, the fauna responsible for OM processing, and the environmental conditions that influence these, and bring about variations among sites. Taxon-specific effects were observed, and several keystone taxa were identified. The label was also traced into individual amino acids. Selective assimilation patterns were identified, and these were also observed to be taxon specific. The pigment and carbohydrate contents of Pakistan margin sediments were characterised. These revealed the sources and relatively degraded nature of the OM present on the Pakistan margin, and allowed further conclusions to be drawn regarding the impact of faunal activity on sediment geochemistry.

8.2 Summary Of Conclusions

- The proportion of freshly deposited OM rapidly taken up by the benthos was enhanced where macrofauna dominated the community, higher quality OM was naturally available, oxygen was sufficiently abundant, and temperatures were relatively high.
- The availability of oxygen controlled the faunal groups responsible for early OM uptake and cycling in a threshold manner. Above the threshold (which is probably species-specific), the macrofauna out-competed the foraminifera, but below the threshold only the foraminifera were able to function effectively.
- The propensity to ingest fresh OM varied among species, and observations in this study were consistent with previously established feeding modes, food preferences, and gut architectures.
- Two species in particular were observed to dominate OM processing in their environments, and on this basis they were posited as keystone taxa at certain sites on the Pakistan margin. These were the polychaete *Linopherus sp.*, present at the 850m and 940m sites, and the foraminiferan *Uvigerina sp.*, which dominated OM processing at the 300m site, and at the 140m site during periods of hypoxia.
- A seasonal pulse of OM to the sediments enhanced faunal OM uptake in longer (5-day) experiments at the 940m and 300m sites, but the effect was subtle, and was not seen at other sites.

- A new, quantitative method for tracing ^{13}C-labelled amino acids was developed and successfully tested. This molecular-level tracing was then applied for the first time to faunal samples from whole-community and, in one case *in situ*, isotope tracing experiments.
- Tracing of isotopically labelled amino acids revealed selective assimilation and egestion of individual compounds.
- Metazoan macrofauna appeared to both selectively assimilate glycine, and also to generate it from other freshly assimilated amino acids. They assimilated alanine in lower proportions to that in which it was present in the food source.
- Selective assimilation patterns were taxon-dependent, as was the extent to which biochemical alteration progressed during digestion. The polychaete *Linopherus sp.* appeared to cause a greater degree of alteration than did those from the family Cirratulidae.
- The alteration effected by the foraminiferan *Uvigerina sp.* was distinctly different from that effected by the metazoan macrofauna. They did not become enriched in glycine (in fact they were depleted in labelled glycine), and they were instead enriched in labelled alanine.
- Pigment concentrations were maximal within the OMZ, and were therefore thought to be at least partly controlled by redox-related preservation mechanisms.
- The pigment suite was dominated by pheopigments, and modelled pigment decay half-lives fell in the range of 300-5000 days. Both of these results indicated that decay in the water column had rendered the deposited OM relatively refractory.
- Carbohydrate yields varied only subtly across the margin, implying that carbohydrates were a relatively refractory constituent of the residual OM.
- Mass-normalised carbohydrate yields were maximal at low-oxygen sites, suggesting that their distribution was influenced by redox-related preservation.

- Organic-carbon-normalised yields decreased with increasing water depth, suggesting that carbohydrates decayed more rapidly than bulk OM during sinking. These yields also decreased downcore; thus, selective decay continued in the sediment.
- Overall, the aldose suites of surficial and downcore sediments were quite uniform, again highlighting that OM on the Pakistan margin was relatively degraded.
- Decay in the water column, and downcore at the 140m, 300m and 940m sites, was associated with reductions in the weight percentages of glucose and ribose, and increases in the weight percentages of other aldoses (particularly rhamnose and fucose). These suite alterations were consistent with those seen in previous studies, and with those shown by a larger sample set, including plankton and sediment trap materials.

8.3 Factors Controlling The Organic Geochemistry of Arabian Sea Margin Sediments

The OM contents of Arabian Sea margin sediments were among those that initiated the debate over which factors exert the dominant controls on sedimentary OM abundance and burial efficiency. In 1992, Paropkari and colleagues presented a compilation of Arabian Sea surface sediment % organic carbon (%Corg) data, and used it to address the question of whether surface water productivity or redox-related preservation held the greatest influence over sediment %Corg in the whole Arabian Sea. They concluded that while the former was a factor, the latter exerted the dominant control, and that other factors such as sediment texture, sedimentation rate, winnowing and dilution by terrigenous input were also influential. Conversely, on the Oman margin, Pedersen et al. (1992) failed to find significant correlations between oxygen availability and sediment %Corg and OM quality (measured using the hydrogen index). They suggested instead that high OM abundances were a function of high supply rates from the surface waters, and a lack of winnowing at mid-depths on the margin. In particular, they found that OM within the OMZ was no fresher than that above or below it, and thus was not preferentially preserved, and suggested that all Oman margin OM was relatively degraded due to sediment re-

working. This disagreement became a matter of continued debate (Paropkari et al., 1993a; Pedersen et al., 1993).

A point worth noting is that Paropkari et al. (1992) initially failed to acknowledge a mis-match between the spatial extent of low oxygen conditions and that of high OM abundances. They later registered this feature, but suggested it was of little consequence, and most likely a factor of where the OMZ lower boundary was considered to be, and maintained that redox-related preservation was the principal factor controlling OM abundance and quality (Parpokari et al., 1993b).

More recent studies on the Pakistan margin have continued this debate, using new molecular level data. Organic matter abundance and quality (measured using the DI), and the abundances of some lipids have shown maxima roughly coincident with the OMZ, supporting the case that redox-related preservation controls these parameters to some degree (Cowie et al., 1999; Schulte et al., 2000; Suthhof et al., 2000). However, while a δ^{13}Corg minimum (possibly a mark of lack of decay) has been found to be exactly coincident with the OMZ, OM and amino acid abundances have shown maximal values slightly below the OMZ, and the hydogen index of surficial sediments (another measure of OM quality) has been observed to be invariant across the margin (Cowie et al., 1999; Suthhof et al., 2000). Such mismatches have leant support to the suggestion that oxygen availability is certainly not the only control on the OM contents of Arabian Sea margin sediments, and may be secondary to other factors. Calvert et al. (1995) suggested that OM distribution was instead controlled by variations in supply, dilution by other sediment types, and sorptive protection (Keil and Cowie, 1999).

Notably, all of these past studies have been limited by the fact that tests of oxygen effects on OM preservation were based on correlations between bottom-water oxygen concentration and measures of OM content and quality. The limitation of this approach is that measured oxygen concentrations do not reflect the degree to which organic matter is exposed to oxygen, and OM concentrations alone do not reveal the degree of OM preservation. The key relationship that needs to be tested is between C burial efficiency and total oxygen exposure time (or other potential controls). At present, these terms are not available from Arabian Sea margin

sediments, although they will eventually be generated by the present study of the Pakistan margin.

None the less, the biochemical molecular data collected in this study provide further insights on the causes for observed changes in OM content and composition across the Pakistan margin, and the possible importance of oxygen to these. Total carbohydrate and pigment yields (normalised to sample weight) showed maximal values at sites where oxygen was least abundant (see chapter 6 Figure 6.2 and chapter 7 Figure 7.1). In this sense they were broadly similar to bulk OM, which showed elevated abundance within the OMZ (Cowie et al, 1998), but maximal values at and slightly below its lower boundary. The amino-acid-based degradation index of OM quality also showed this broad trend (Vandewiele, pers. comm., chapter 2, Table 2.1). Low-oxygen sites also showed minimal δ^{13}Corg values, which (through preferential loss of ^{12}C during remineralisation) could be due to less decay in the absence of oxygen, but which could also be due to the occurrence of chemosynthesis within the OMZ (Cowie pers. comm., Cowie et al., 1999).

Hence, maximal pigment and carbohydrate mass-normalised yields, and maximal DI and %Corg values, were generally associated with low oxygen availability, suggesting that redox-related preservation plays a role in determining the organic geochemistry of Pakistan margin sediments. While maximal pigment and DI values were associated with the lowest oxygen concentrations however, the fact that the maxima in carbohydrate yields and %Corg occurred at and slightly below the lower OMZ boundary suggests that other factors also exert an influence. Some of these factors are discussed below.

Organic carbon-normalised lipid (all classes) (Jeffreys pers. comm.) and carbohydrate yields in surficial sediments (surface 0.5 and 1cm respectively) did not display maximal values within the OMZ, but instead showed general decreases with increasing water depth (chapter 7, Fig. 7.1). This is in contrast with the findings of Schulte et al. (2000), who observed enrichments in Corg-normalised fatty acid and sterol yields within the OMZ at some locations on the Pakistan margin, but decreasing fatty acid concentrations with increasing water depth in others. Thus, the carbohydrate and lipid richness of sedimentary OM in this study was influenced by selective decay processes in the water column, and hence by water column length,

rather than by oxygen availability. Maximal absolute (mass normalised) concentrations of carbohydrates and lipids in the OMZ were therefore associated with OM enrichment in general, rather than of carbohydrates and lipids in particular, as might be expected of a strong oxygen effect on OM preservation. This was supported by principle component analysis of aldose weight percentage data, which showed that the carbohydrate composition of both surficial and deeper sedimentary OM was also dependent on water column length and not clearly on local redox conditions (chapter 7, Fig. 7.6). This is in contrast to the pigments, which showed maximal Corg-normalised (as well as mass-normalised) concentrations at low-oxygen sites (data not shown), and which were therefore enriched in the OMZ, apparently due to redox-related preservation of pigments in particular, as well as of OM in general.

Sediment grain size (or surface area) and sorptive protection have been shown not to be dominant controls on the OM contents of Pakistan margin sediments (Keil and Cowie, 1999, Vandewiele pers. comm.), as has been seen in other environments (Mayer, 1994). Only sediments from the 140m site showed a "monolayer equivalent" abundance of OM (ca. 0.5-1.0 mgC/m^2), while those from the 1850m site had less, and those from the 300m, 940m and 1200m sites had more (Vandewiele, pers. comm.). The "excess" relative to available surface area at the oxygen-depleted sites is in line with previous observations of low-oxygen settings, and again suggests that oxygen has a contributing effect on sedimentary OM distributions across the Pakistan margin. The "supermonolayer" C loadings are generated at low oxygen exposure times, disrupting the typical near-constant relationship between C content and surface area observed across most continental shelves and upper slopes. In contrast, increasing oxygen exposure time with increasing depth and oxygen concentration below the OMZ may be the cause of decreasing %Corg values (and submonolayer coverage) at the 1850m site, a feature that is usually found only on the continental rise and abyssal plain.

The feature of OM distribution on all Arabian Sea margins which most evades explanation is the fact that maximal values of OM abundance and quality have consistently been observed to occur at and below the lower boundaries of the OMZs, rather than within them (e.g. Pedersen et al., 1992; Paropkari et al., 1993a, b; Calvert

et al., 1995; Cowie et al., 1999; this study). In the present study, this feature was manifest as high %Corg and DI values at the 1200m site (chapter 2, Table 2.1), and was also slightly apparent in 1200m site carbohydrate abundances, but not in those of pigments. It has been suggested that such sites should not in fact be considered as being outside the OMZ (Paropkari et al., 1993b). A common definition for an OMZ is a region where the concentration of dissolved oxygen in bottom water is below 0.5 mlL^{-1} (e.g. Helley and Levin, 2004), and in this sense the 1200m site was indeed within the OMZ (chapter 2, Table 2.1). The fact remains, however, that the lowest bottom-water oxygen concentrations (at ~300m) were spatially removed from maximal sedimentary %Corg values. Alternatively, the OMZ could be defined by the absence of preserved laminae in the sediment. On this basis the well-mixed nature of the 1200m site sediments (chapter 2, Fig. 2.3) clearly positions it outside the OMZ. This is supported by I/Corg and Mn/Al values (both of which are elevated in the presence of oxygen), which have been found to be considerably elevated in surface and downcore sediments at the 1200m site compared to OMZ sites (Cowie et al., 1999). Sediment structure seems the least arbitrary way of defining the OMZ boundary, and has the benefit of being process- based. However its use means that the anomaly of the 1200m site remains.

Thus, the presence of maximal OM abundances slightly below the OMZ (i.e. at the 1200m site) is yet to be thoroughly explained. Previous studies have proposed that winnowing, rather than redox-related preservation is responsible for OM distributions (Pedersen et al., 1992). If winnowing and other OM supply factors were the only controls on OM distribution, and oxygen was assumed not to have any influence at all, the OM contents of the 1200m site sediments may cease to appear anomalous. It has also been suggested that winnowing of OM from shelf and shelf-break localities, followed by cross-margin transport and deposition at greater depth (apparently focused near 1200m), may at least in part be responsible for the general C maximum and particularly for the elevated concentrations at 1200m (Schulz et al., 1996).

Further light may be shed on this issue in the near future by data from the Arabian Sea project. Determinations will be made of sediment accumulation and mixing rates, as well as of fluxes of OM to the sediment, and thus of OETs. The abundance

of OM at the 1200m site could still be influenced by oxygen, if cross-margin transport of OM, and rapid accumulation lead to a short OET, despite the presence of relatively oxygenated bottom water and sediment biomixing. Only by considering OM flux rates, OETs and measured bioturbation rates (the exact nature of bioturbation cannot be judged from X-radiographs alone), and comparing them with measured Corg burial efficiencies, can the principal factors influencing OM cycling and burial be determined.

In summary, this study has provided new material for the debate regarding the factors that influence OM distribution and composition in continental margin sediments. Pigment and carbohydrate data suggest that oxygen exposure (thus far measured only in the form of concentrations) and redox-related preservation plays a significant role, but that other factors including water-column decay (and thus depth) and sediment texture are also influential. From previous studies it seems likely that hydrodynamics, winnowing and cross-margin transport of OM also exert some control over OM distributions. Further contributions will be possible from the data presented here once oxygen exposure times and burial efficiencies have been determined.

8.4 Organic Matter Quality on the Pakistan Margin

The suites of pigments and carbohydrates found on the Pakistan margin were indicative of relatively low quality (degraded) OM. The pigment suite was dominated by the chlorophyll-a decay products pheophytin and pheophorbide, with only very low concentrations of intact pigments. The carbohydrate suite was very uniform, having lost much of the high weight percentage of glucose often found in fresh OM (plankton and shallow sediment trap materials, e.g. Cowie and Hedges, 1984a; Hernes et al., 1996; Hamanaka et al., 2002). In addition, when DI was plotted against non-protein amino acid abundance, and the resulting line was extrapolated to zero on the non-protein amino acid axis (assumed to indicate zero decay, which is only approximately true, as fresh plankton contain small amounts of non-protein amino acids), the intercept on the DI axis (indicative of the DI values for fresh OM) was at ~ +0.65 (Vandewiele, pers. comm.). The range of surficial DI values on the Pakistan margin was ~ -0.4 to –1.2. Therefore, these data are also indicative of

relatively degraded OM. This is consistent with previous studies, which have shown that hydrogen index values for the Pakistan and Oman margins are considerably lower than those for the comparable Peru margin (Pedersen et al., 1992; Cowie et al., 1999). Previously published hydrogen index values do not show a distinct difference in OM quality between the Pakistan and Oman margins (Pedersen et al., 1992, Paropkari et al., 1993b), except that the range for the Pakistan margin reaches slightly higher values. The detection of chlorophyll-a in Pakistan margin sediments in this study, compared with its observed absence from Oman margin sediments (Shankle et al., 2002) also suggests that the OM on the Pakistan margin is slightly fresher than that on the Oman margin. This is despite the fact that the %Corg values for Oman margin sediments can be much higher (max 7.5%, compared to ~4% off Pakistan, Paropkari et al., 1992).

8.5 Evidence For A Seasonal Pulse of Organic Matter

Studies of particle fluxes in the Arabian Sea in general, including the eastern basin and the Pakistan margin, have shown increases in total mass and OM flux to the sediments in response to the dominant SW monsoon (e.g. Haake et al., 1993a). Seasonality in primary productivity is seen in most oceans, and is often observed to lead to pulsed inputs of OM to the seafloor. Thus, a pulse of fresh, relatively reactive OM to Pakistan margin sediments was expected in response to the SW monsoon. Comparisons of the organic contents and compositions of surficial sediments from the same sites before and after the SW monsoon generally did not show clear evidence of a major OM deposition event during the intervening months. Despite clear evidence of increased productivity in the water column during the SW monsoon, and an intensification and broadening of the water-column OMZ, (Bett et al., 2004a, b, Cowie et al., 2005a, b), there was no consistent seasonal change in the %Corg values, C/N ratios or δ^{13}Corg values of surficial sediments (Cowie pers. comm.), nor in pigment abundances (except at the 1200m site). Slight increases in carbohydrate and lipid yields (Jeffreys pers. comm.) were observed, but these changes were subtle. Similarly, pigment and carbohydrate unreactive inventories / background concentrations showed slight increases at some sites in the post-

monsoon season, however these could not be linked to a mechanism, and may be the result of profile spikiness.

Evidence of a seasonal pulse of OM to the sediment may be lacking for a number of reasons. Firstly, the pulse may not have occurred, or may have been reduced in scale in 2003. It has previously been observed that the intensity of the sedimentation peak varies between years (Haake et al., 1993a).

Secondly, the OM pulse may always be relatively small, and may be largely removed within the water column, and so may never impart a particularly marked imprint on Pakistan margin sediment organic content or composition. The seasonal variation in sediment flux rates have indeed been observed to be reduced on the eastern side of the Arabian Sea compared to off Oman, but the SW monsoon still dominates, and a pulse of OM would therefore still be expected (e.g. Haake et al., 1993a).

Any sinking OM would have been subject to re-cycling process within the water column, and its impact would thus be expected to reduce with station depth. A relatively small OM pulse falling on relatively OM-rich sediments would have only a small impact on sediment geochemistry. This would however lead to the slightly different expectation of greater seasonal impacts at less OM-rich sites. Although a particularly carbohydrate-rich post-monsoon sample was analysed from the 1850m site, by analogy with lipid data, this is thought to be largely due to within-site heterogeneity (Jeffreys, pers. comm.).

Finally, the seasonal signal may have been partially or wholly missed by this study. If the pulse of OM was particularly reactive, its impact on sediment geochemistry may have been severely reduced by the time sampling took place. Evidence for this was shown by chlorophyll-a concentrations which were lower in post monsoon samples taken in September-October, than in samples taken from the same sites a few weeks earlier (chapter 6, Fig. 6.2). It seems surprising, however, that OM which survives sinking through these relatively long water columns would be sufficiently reactive to be almost completely decayed within a few weeks in the sediments. In addition, early post-monsoon sampling (cruise CD 150) was conducted while monsoon winds were still blowing, so it is unlikely that the OM pulse was missed, unless it occurred much earlier in the monsoon. It is possible, however, that the

benthic communities may have actively scavenged a monsoon-driven pulse of OM over short time scales.

The faunal response to labelled-OM addition appeared to be affected by a monsoon-induced pulse of OM. Label uptake was greater in the post monsoon-season in 5-day experiments at the 300m and 940m sites (chapter 4, Fig. 4.2). This suggests that the faunal communities at these sites were primed by a natural input of fresh OM, and shifted into a more efficient and active feeding mode. However, the 300m 2-day experiments and the 1850m experiments displayed no significant trends between seasons. In the latter case this may have been due to decay of the OM pulse in the water column, as discussed above, but in the case of the 300m site, the reason remains unclear.

In summary, the evidence suggests that a monsoon-induced pulse of OM was delivered to the Pakistan margin during the SW monsoon, but also that it was relatively small or had already been significantly degraded, in the water column or in the sediments, prior to the late post-monsoon (CD 151) sampling period.

8.6 The Relationship Between Organic Matter, Bioturbation, and The Benthic Community

Previous studies have observed that the presence of burrowing macrofauna enhances the rates of decay of bulk OM and of specific compound classes (e.g. Wheatcroft, 1992; Sun et al., 1999, 2002; Sun and Dai, 2005).

In this study, no significant correlations were observed between macrofaunal biomass and the abundance of carbohydrates, pigments or bulk OM (chapters 6 and 7). Generally, it could be said that the oxygenated 140m and 1850m sites, where significant numbers of macrofauna were present, displayed the lowest OM and biochemical contents, while sediment %Corg and pigment and carbohydrate concentrations were high, and macrofaunal abundance was low, at the 300 m site (at the OMZ core). However, sediments at the 940m site showed the highest macrofaunal abundance, as well as some of the highest OM and biochemical concentrations. Also, although abundant macrofauna were not recovered from the 1200m site, sediment X-radiographs suggested that this OM-rich site was home to relatively large and deep-burrowing deposit feeders.

In short, the relationship between OM content and macrofaunal abundance was not straightforward. This may partly be due to the fact that fauna tend to be abundant where there is plentiful, higher-quality food (e.g. Smith et al., 2000, Levin et al., 2000, Cook et al., 2000). Macrofauna were not, however, present at all sites where higher quality OM was observed, due to the absence of oxygen. Thus, the presence of the OMZ obscured and complicated the commonly observed relationship between macrofaunal biomass and sediment OM content.

Pigment concentrations were minimal at sites with maximal bioturbation (the 140m, 1200m and 1850m sites), and this relationship was also shown by the carbohydrates (chapter 6, Fig. 6.2; chapter 7, Fig. 7.1). Downcore profiles showed slightly deeper penetrations of reactive carbohydrates and pigments, and lower unreactive background concentrations at sites that were bioturbated (chapter 6, Fig, 6.3; chapter 7, Fig. 7.2), however downcore carbohydrate and pigment compositions did not appear to be affected by bioturbation. Thus, bioturbation seemed to reduce the buried concentrations of pigments and carbohydrates, however, the effect was overprinted by an oxygen effect at the 940m site, where macrofauna and bioturbation were present, but surficial and downcore biochemical concentrations remained high.

The exact nature of the faunal processes occurring at a site, and thus the composition of the benthic community, may be a more important influence on OM preservation than overall faunal abundance. Bioturbation and related process probably varied among sites in several ways. The maximum depth of burrowing, for example, was observed to be shallower at the 940m site than at the 140m site (chapter 4, Fig. 4.5), while burrows appeared to exhibit maximal diameters at the 1200m and 1850m sites (chapter 2, Fig 2.3). Burrows at the 940m site co-existed with laminae, and thus were probably re-formed only rarely compared to those at the 140m, 1200m and 1850m sites, and the extent, regularity and timing of sediment ventilation is very likely to have varied between sites.

Isotopic labelling experiments highlighted the control that the natural availability of higher-quality OM exerts on macrofaunal biomass and activity. Experiments at the 940m site showed that the fauna there processed a much larger proportion of the introduced label than the fauna at the 140m site. Thus, the abundance of OM at the 940m site led to the existence there of a large faunal community that was adapted to

rapid processing of OM, despite living at oxygen levels that were just above the minimum required. In addition, the seasonal pulse of OM appeared to be sufficient to prime the communities at the 300m and 940m sites to be more efficient at OM uptake and cycling after the monsoon.

Overall, the effects of low oxygen availability overprinted the effects of faunal activity and OM abundance on one another, and these were difficult to deconvolve. The variation in faunal communities across the Pakistan margin was a further complicating factor. However, in general, where oxygen levels were just sufficient, higher-quality OM supported large macrofaunal communities, and where oxygen and macrofauna were relatively abundant, the resultant OM abundances were relatively low. Where oxygen was almost absent, so were the macrofauna, and the OM contents of the sediments was consequently high (and of higher quality). This study also demonstrated that different taxa take up, process, and transport OM in very different ways (chapters 4 and 5), and this is likely to produce considerable differences in the effects of fauna on sediment organic geochemistry among sites. This highlights the need for more detailed studies of the OM uptake, bioturbation, OM alteration and sediment ventilation effects of various key fauna.

8.7 Links Between Faunal Digestion and Sediment Geochemistry

Tracing of isotopically labelled amino acids revealed that metazoan macrofauna selectively assimilated, and also generated, relatively large amounts of glycine. Thomas and Blair (2002) also observed this glycine enrichment not only in faunal tissues, but also in faecal pellets. Downcore sediment samples from ^{13}C labelling experiments in this study showed that it also occurred in the bulk sediment (chapter 5, Fig. 5.15-5.17). Naturally occurring sediments have been observed to become progressively enriched in glycine during decay (e.g. Horsfall and Wolff, 1997; Dauwe and Middelburg, 1998), and this has previously been attributed to the preferential preservation of cell-wall protein. This study has shown that while the previous explanation may indeed be a contributing factor, the enrichment of degraded sediments with glycine may now be at least partially attributed to the

processing of OM by macrofauna. Thus, a link has been established between macrofaunal digestion and sediment geochemistry.

A link between macrofaunal abundance and sediment geochemistry was also suggested by downcore increases in pheophytin and pheophorbide:chlorophyll-a ratios across the margin. The formation of pheopigments can be the result of grazing of phytoplankton by herbivorous zooplankton, or digestion of OM by burrowing fauna in the water column or in the sediment (Shuman and Lorenzen, 1975, Bianchi et al., 1996, Sun and Dai, 2005). The abundances of these compounds compared to chlorophyll-a increased most markedly downcore at the 140m and 940m sites, where macrofaunal populations were greatest (chapter 6, Figs. 6.11, 6.12), suggesting that pheopigment production during macrofaunal digestion had an impact on downcore pigment compositions. Less marked increases at the 1200m site may have been due to the combined effects of less abundant macrofauna, and differences in the feeding or burrowing modes of the taxa present, and were also probably partly due to the initially low concentration of chlorophyll-a in surficial sediments. These ratios could not be examined at the 1850m site, where chlorophyll-a was not detected.

Thus, macrofaunal digestion appeared to influence both the amino acid and pigment contents of Pakistan margin sediments. These results may be representative of wider faunal impacts on sediment geochemistry. However, the effects of macrofaunal digestion on sediment geochemistry are likely to be highly dependent on the types and feeding modes of the fauna present, as well as their abundance. This complexity is yet to be revealed, because the necessary experimentation and molecular-level tracing has yet to be conducted.

8.8 Future studies

This study involved a combination of organic geochemical investigation with novel whole-community tracer studies, and has generated several clear conclusions regarding the role of fauna in early OM cycling and ultimate burial in seafloor sediments. However, inevitably, the limitations of the study were also highlighted and, in some areas, as many questions were generated as were answers. The need for a range of additional studies has been clearly shown.

Specific to this study and the Arabian Sea project, the processing of radioisotope and oxygen data sets will allow sediment accumulation rates, bioturbation rates and oxygen exposure times to be calculated for all sites studied. These in turn will allow thorough modelling of true pigment degradation rates, and determinations to be made of the flux rate of OM to the sediments and of OM burial efficiency. Only once this has been done, can the question of to what extent oxygen controls OM burial efficiency, and how this is related to faunal processes, be properly addressed.

The C tracing experiments conducted in this study showed that on average 16% of an OM pulse was processed by the benthic community in just a few days, and that up to half of that could be due to uptake, rather than respiration. The following are suggestions of studies which are required so that the role of fauna in seafloor C-cycling and burial, and in determining parameters such as oxygen exposure and sediment OM quality, in both the short and long terms, can be better described and quantified.

- Detailed studies are required of the biochemical alterations that occur during faunal gut passage. These should be conducted on fauna that are thought to be keystone taxa in their environments, in that they process large proportions of sedimenting OM. They should include the fullest ranges of major biochemical classes possible (amino acids, carbohydrates, lipids, pigments), and biochemicals should also be traced into gut contents, faecal pellets and the surrounding sediments. This could lead to quantitative biochemical budgets for digestion.
- The above experiments should be supported by whole-community feeding studies (in the relevant environments), to characterise and quantify the oxygen dynamics of, and role of the chosen species in, C-cycling in the natural setting.
- Further whole-community feeding studies should be conducted, *in situ* where possible, in a wide range of benthic environments, from estuaries to the deep-sea, and from clay- and organic rich, to sandy and organic-poor sediment types. This will allow these systems to be compared and contrasted, and the results of these studies to be collectively applied on a global scale.

- All of the experiments described above should be of extended duration (4 weeks and longer), to allow connections to be made between the short-term processing of OM by fauna, and the long-term fate of OM in terms of remineralisation or burial. Extended biochemical tracing experiments will also reveal the biochemical pathways by which fresh OM is transformed into uncharacterisable, buried OM, which may provide clues as to the biochemical fractions of sedimentary OM that tend to be preserved, and the roles that fauna play in this.
- Isotope tracing experiments should be conducted on whole and de-constructed faunal communities in microcosm. This will provide a direct means of assigning responsibility for measured respiration among the faunal classes, which to this point has remained a limitation of whole-community labelling experiments. These studies can also be used to reveal the extents to which various faunal groups inhibit or enhance the functioning in C-cycling of others when they are present as part of a whole community.
- The isotope tracing experiments that have been performed to date have all used fresh algal detritus as the labelled food source, which is not necessarily representative of the OM typically processed by benthic communities. Further experiments should be conducted using previously aged OM (faecal pellets, for example). This will show whether previous results can be replicated using a more realistic food source, and whether certain faunal classes (e.g. the foraminifera and bacteria) are adapted for, or more efficient at, processing relatively degraded OM.
- Finally, the difficulties experienced here and elsewhere in extrapolating the results of faunal process studies up to the regional scale, and into the medium and long term, have been at least partially due to our lack of detailed knowledge of how individual taxa behave. Further characterisation, in microcosm, and then in whole communities, of gut/vacuole turnover times, feeding rates, digestion efficiencies, burrowing rates and ventilation patterns of a wide range of taxa, will be of great value in overcoming the challenge presented to studies of sediment processes by the diversity of benthic communities.

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