The Influence of Physical Dynamics on Trophic and Biogeochemical Processes in Celtic Seas

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BY

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Abstract

Shelf seas are small in size (< 10% surface area of the oceans) but are highly productive, where up to 40% of oceanic carbon sequestration takes place. They also have great economic and social importance as they are widely used for fishing, waste disposal and fossil fuel extraction. Fishing resources are economically important and it is estimated that the UK industry is worth £800-1200 million. As a result there is a greater need to understand the dynamics of these systems. This study focuses on the Celtic and Irish Seas of the North West European Continental Shelf and assesses biological and biogeochemical processes across four distinct physical regions. A multidisciplinary approach has been employed to study biological communities across the shelf sea. In this study it has been observed that benthic, pelagic and particulate organic matter (POM) communities appear to adapt in response to different physical environments. Small scale adaptations of organisms were observed between regions of different water column structure. Hence, statistically significant differences in nitrogen isotope values for benthic suspension feeders indicate that these invertebrates are flexible in their feeding, depending on the environment and food availability. Changes in the pelagic community were also observed across the shelf sea. At the shelf break large typically oceanic zooplankton species and larvae were present coinciding with a change in phytoplankton community structure towards larger cells in surface waters when compared to the rest of the shelf sea. The structure of the water column, particularly with respect to mixing and turbulence seems to influence the lability of material sinking through the water column and in turn, the response of heterotrophic communities, which apparently follow the food resource. A change in hydrodynamic setting of a seasonally stratified water column, which led to the partial erosion of the subsurface chlorophyll max (SCM), enabled the impact of a storm-driven mixing event to be determined. The apparent loss of chlorophyll *a* from the SCM led to the material being redistributed vertically through the water column, as illustrated by the distributions of biological markers (lipids) through the water column before and after the storm. Zooplankton communities also showed evidence of vertical redistribution; prior to the wind event, 80% of the community was in the upper water column, whereas their distribution was even after the wind event. As a result, the storm apparently increased the complexity of the food web dynamic and this change drew more similarities to vertically mixed regions of the shelf than stratified regions.

I certify that the work described in this thesis is my own except where otherwise stated and has not previously been submitted for any degree at this or any other university.

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Chapter 1

Introduction

1. Introduction

1.1 Background

Shelf seas are commonly described as the region of sea between a landmass and the edge of the continental shelf. They are small in size, accounting for less than 10% surface area of the oceans, but are highly productive and are responsible for up to 30% of oceanic primary production (Muller-Kager et al 2005). They are typically shallow, usually up to ~200m in depth, but experience high levels of energy inputs from tidal forces and wind mixing events and can be strongly influenced by freshwater (Simpson and Hunter 1974; Sharples et al 2007). The variation of physical forces across the continental shelf results in different water column structures. Areas close to landmasses are most strongly influenced by tidal forces often resulting in mixed water columns, where physical parameters are uniform (Simpson and Sharples 2012). In mid-shelf regions, seasonal stratification occurs as tidal forces are weaker and stratification is induced through solar heating (Pingree et al 1976; Sharples and Holligan 2006). At the edge of the shelf the dynamics change and enhanced mixing and pulses of nutrients onto the shelf from the deep ocean are observed (Sharples et al 2007).

1.2 Economic and social importance

As shelf seas have a close proximity to landmasses, they are consequently a key interface between the terrestrial and open ocean environments. Dumping of industrial waste, fossil fuel exploration and fishing pressures are some examples of the varied exploitation these regions experience. One of the largest pressures on shelf-sea ecosystems and habitats is fishing activity. It has previously been estimated that ~40% of the world's population lives within 100 km of shelf-sea coastal regions therefore, up to 2.8 billion people (of a global population of ~ 7 billion) rely on marine resources for protein (Simpson and Sharples 2012).

In 2002 the United Nations Fisheries and Aquaculture Department reported that the fish catch landed by the UK fleet had a value of £546 million and the total value of the fishing industry (including aquaculture) was £800-1200 million. It has been estimated that in the North Sea (part of the NW European Continental Shelf Sea), 25% of total fish biomass is removed every year (McGlade 2002). The continual demand for fish is having damaging impacts on fragile marine ecosystems through destructive methods which impact benthic communities directly. A 10 year study showed that significant habitat and benthic

community changes occur where fishing activities were intense (Kaiser et al 2000). As fishing methods have improved the yield of fish caught has greatly increased contributing to several well documented collapses of fish stocks. These include the collapse of the Peruvian anchovy (*Engraulis ringens*) and the Atlantic cod (*Gadus morhua*) (Idyll 1973; Myers et al 1996). As a result of species crashes and evidence of habitat damage through quantitative studies, the development of fisheries management strategies have become commonplace in trying to control and protect fisheries resources for future generations (Auster et al 1996; Jennings and Kaiser 1998).

1.3 Multidisciplinary studies – physics, biology and biogeochemistry

As shelf seas are important for economic and social resources, there is a need for further understanding of the dynamics and ecosystem functionality. In the present study, the focus is on the NW European Continental Shelf Sea, specifically the Celtic and Irish Seas. This region has been widely studied with respect to physical dynamics and models have been developed to further understand stratification, fronts and tidal mixing and progress has been made in the understanding of these complex regions (e.g. Simpson and Bowers 1981; Elliott and Clarke 1991; Simpson et al 2009). However, links with biological and biogeochemical processes were made. More recently, multidisciplinary studies that incorporate physics, biology and biogeochemistry have been developed in an effort to better understand the world's oceans, particularly their response to climate and environmental change (e.g. Thomas et al 2004; Coyle et al 2008; Sharples et al 2009). Here, such a multidisciplinary approach has been employed in studying biological communities across the NW European Continental Shelf Sea.

1.4 Examining biological communities in shelf seas

Up to 40% of global carbon sequestration takes place in shelf sea regions (Chen et al 2009). The processing of organic matter (OM) via the food web is known as the biological pump and this is critical for the eventual export to and burial of OM in sediments (Gattuso et al 1998; Thomas et al 2004). However, only a small fraction of OM reaches the sea floor as the majority of it (~90%) is remineralised by microbes and grazers while biological repackaging (often into larger aggregates) distribute OM through the water column (Wakeham 1988; Wollast 1998; Sheridan et al 2002).

In biological communities, composition analysis is frequently employed to numerate species through identification and calculation of abundances and diversity indices. Zooplankton are known to reprocess OM within the water column, which affects the quality and lability of material that ultimately reaches the benthos (Wakeham 1988; Sheridan et al 2002). Benthic communities further utilise particulate OM (POM) that reaches the sediment and many species rely on sinking material for nutrition. However, in shelf sea communities where tidal forcing, wind-driven mixing and variable currents are frequently observed, pelagic and benthic communities are considered to be more closely coupled than open ocean environments (Raffaelli et al 2003). This implies that there is a two-way exchange between the benthos and overlying water as a result, these benthic communities respond to the quantity and quality of POM falling through the water column (Raffaelli et al 2003). It has been observed that migrations of organisms from the pelagic to benthic and benthic to pelagic environments occur. In the former case, larval release from molluscs and amphipods has been observed and in the latter squid and fish eggs have been shown to sink to the benthos as part of their developmental cycle (Dybas 2001; Herring 2002). By studying the biological communities, food web linkages and resource use can be assessed.

In order to assess biological and biogeochemical dynamics in a shelf sea several different techniques can be employed. These include:

1. Benthic and pelagic community structure analysis through species identification and numeration from benthic grab and vertical pelagic net hauls.
2. Total lipid analysis and pigment analysis from particulate organic matter samples collected *in situ*.
3. Stable isotope analysis conducted on individual benthic and pelagic species and POM to assess food web linkages, organic matter quality and dynamics.

In later chapters specific descriptions of these techniques are provided.

1.5 Aims of this research

The different physical regimes across the NW European Continental Shelf and their dynamics have been thoroughly studied. However, the biological community, trophic

dynamics and POM composition in these different regions are poorly documented. Examination of organic matter cycling through the water column has been conducted in deep-sea and some continental margin environments, but rarely is the analysis paired with community analysis of mesozooplankton and macrobenthic species. The aim of the present study is to examine how sources of OM, trophic interactions and biological community structure are affected by different physical dynamics across the shelf. The investigation was conducted across the continental shelf targeting three distinct types of water column structure; permanently mixed, seasonally stratified and the permanently stratified waters at the edge of the continental shelf. In each of the following three data chapters, broad overarching themes are assessed. In each of the chapters specific hypotheses have been presented. The over arching themes and hypotheses for the chapters are as follows:

Chapter 2

This chapter focuses on the distinct physical regions of the Irish and Celtic Seas and how this impacts on POM transport, reprocessing and cycling. The following hypotheses have been posed:

1) Variation between physically distinct regions of the shelf sea lead to differences in the composition and lability of suspended POM. These differences are reflected in lipid, pigment, isotope and phytoplankton distributions within the water column.
2) Compositional variation in suspended POM is influenced by the distribution of zooplankton within the water column, which is reflected in the biomarker composition.

Chapter 3

This chapter focuses on observable differences in benthic and pelagic community structure between the distinct physical regions of the Irish and Celtic Seas. The following hypotheses have been posed:

1) Pelagic and benthic communities are influenced by water column structure and organisms are able to change feeding strategies to cope with different physical conditions in these environments. In regions with increased turbidity and mixing

evidence of coupling is stronger and evidence of resuspension of benthic larval species is observed.

2) Changing feeding strategies in different physical conditions leads to individual species displaying different trophic behaviour due to varying carbon sources and specialisation.
3) Dynamics at the edge of the continental shelf supports different biological communities. Evidence of production at higher trophic levels may be apparent through larval and egg populations.

Chapter 4

This chapter focuses on a change in hydrodynamic setting of the water column (i.e. a wind induced mixing event), and how this impacts the biological communities and processing of POM. The following hypotheses have been posed:

1) In the event of mixing within a stratified water column (even if there is only minimal erosion of the DCM and the thermocline remains intact) the distribution of POM becomes dispersed differently through the water column. This is apparent through the composition of POM and biomarker evidence.
2) During a mixing event within the stratified region of the shelf sea, chlorophyll is distributed more evenly thorough the water column. As a result of this chlorophyll homogenisation through the water column pelagic organisms are also observed to orient themselves differently optimising food intake and ultimately growth and reproduction.

Several sampling strategies and analytical techniques were employed during the scientific cruise RRS Discovery 352 across the NW Continental Shelf Sea from sites in Liverpool Bay, the Irish and Celtic Seas and at the shelf break. The main scientific objective was to investigate the effect of wind stress and the impact this has on mixing across the thermocline. Additionally, biological and biogeochemical sampling was carried out in order to assess changes in trophic structure, from suspended POM to pelagic and benthic communities, in regions of different physical dynamics.

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Chapter 2

Examining the fate of particulate organic matter in shelf seas – lipids and stable isotopes provide evidence of heterotrophic re-working

2.1 Introduction

2.1.1 Background

Continental shelf seas are small in size, accounting for less than 10% surface area of the oceans. However, they are responsible for up to 30% of air-to-sea carbon flux through primary production. Additionally, it has been estimated that up to 40% of particulate carbon sequestration also occurs in these regions (Muller-Kager et al 2005; Chen et al 2009). The enhanced biological production in these regions supports near-coast communities by providing up to 90% of global fisheries production (Pauly et al 2002). Carbon fixation, through primary production, supports shelf seas and is a key interface in the global carbon cycle as well as acting as an export pathway for terrestrially derived material. In the region under study, the North West European Continental Shelf, different physical water column features are observed due to the interaction of tides, mixing events, warming of the water column in the summer months leading to seasonal stratification, and the topographic features of the shelf break. This chapter focuses on four study sites across the North West European Continental Shelf; these are IS-M, CS1-SS, CS2-SS and SB-S (Figure 2.1).

The four study sites examined here have contrasting water depth, water column structures and dynamics these were (Figure 2.1; Table 2.1);

a) Vertically mixed – permanently and periodically (IS-M)
b) Mid-shelf seasonally stratified (summer) (CS1-SS, CS2-SS)
c) Shelf-break stratified (SB-S)

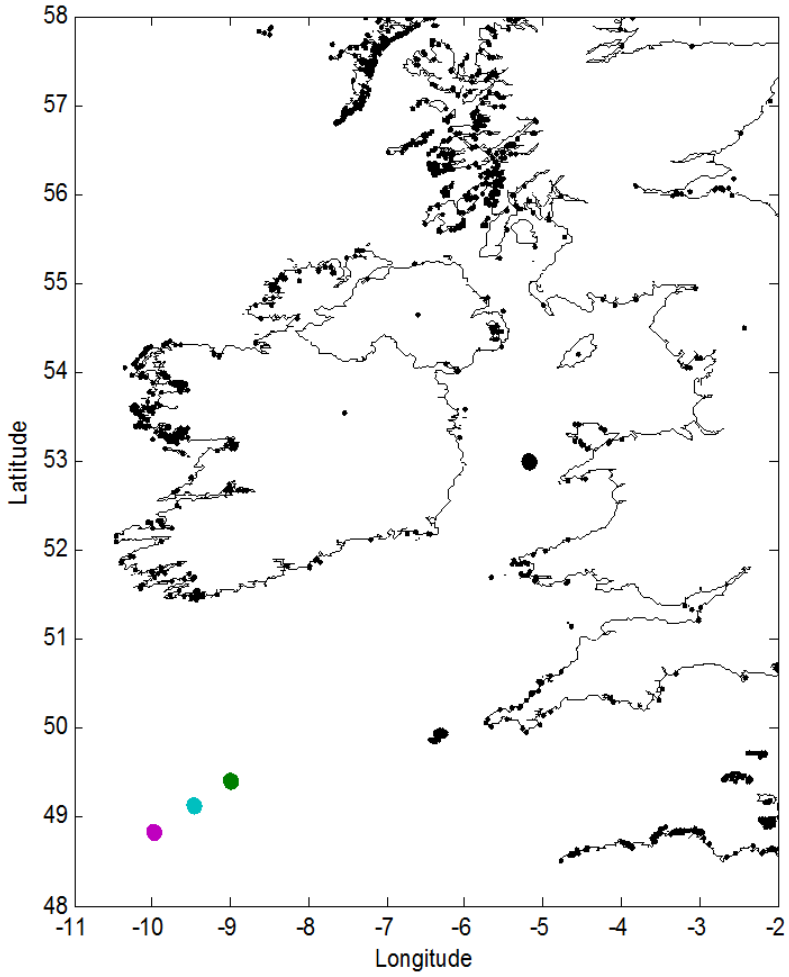


Figure 2.1 Map showing all five survey sites; ●= IS-M, ●= CS1-SS, ●= CS2-SS and ●= SB-S.

The Irish Sea study site (IS-M) is a site where tidal mixing dominates and its close proximity to anthropogenic activity leads to concerns about nutrient enrichment, pollution and potential eutrophication (Gowen and Stewart 2005). At present, despite elevated nutrient levels and some areas of oxygen depletion, the Irish Sea is not considered eutrophic but the impact of anthropogenic nutrients and inputs into the coastal system requires continuous monitoring (Gowen et al 2008). Study sites at the mid-shelf region of the Celtic Sea (CS1-SS and CS2-SS) display characteristic seasonal thermal stratification, the onset of which drives the spring phytoplankton bloom (Sharples and Holligan 2006). The study site at the edge of the continental margin (SB-S), is away from anthropogenic and freshwater inputs while

enhanced mixing, by an internal tide, driving nutrient pulses from the deeper ocean lead to elevated phytoplankton production (Sharples et al 2007).

Table 2.1 Date of sampling, nominal depth (m), latitude and longitude at each sampling site. (Cruise report, Sharples 2011)

	Date	Nominal depth (m)	Latitude	Longitude
IS-M	24 June 2010	104	52 59.96N	005 10.96W
CS1-SS	5 June 2010	137	49 25.11N	008 59.47W
CS2-SS	13 June 2010	159	49 06.94N	009 27.42W
SB-S	20 June 2010	191	48 46.82N	009 58.58W

2.1.2 Shelf sea physics

Shelf seas are dynamic regions of the world's oceans and due to their shallow depth (0-200m) are strongly influenced by frictional boundary layers at the surface and bottom of the water column (Simpson and Sharples 2012). Tidal forces, wind stress, solar heating and freshwater inputs can cause density gradients within the water column, creating regions with varying water column structure (Simpson and Hunter 1974; Sharples et al 2007; Simpson and Sharples 2012).

Some regions of the shelf sea are strongly vertically mixed so that stratification is either periodic, rare or absent. These areas are influenced by tidal forcing and wind stress, creating a mixed water column where physical parameters remain constant throughout (Simpson and Sharples 2012). This can be observed between 53^{o}N and 52^{o}N, where the temperature (Figure 2.2; upper plot) and chlorophyll concentrations (Figure 2.2; lower plot) are homogenous through the water column. Regions of freshwater influence (ROFI), an example of this on the NW European Continental Shelf is Liverpool Bay, incur periodic (and large) freshwater inputs leading to episodes of stratification, temporarily interrupting the usual mixed state.

In the mid-region of shelf seas, during summer months, seasonal stratification develops due to relatively weak tidal mixing and increased solar heating. The onset of stratification in

spring causes a phytoplankton bloom which depletes the surface waters of nitrate, light penetrates further into the water column and a subsurface chlorophyll max (SCM) is formed (Pingree et al 1976; Fasham et al 1983; Sharples and Holligan 2006). Stratification of the water column is clearly observed between 51^{o}N and 50^{o}N at the mid shelf region where temperature (Figure 2.2; upper plot) is highest at the surface, with a clear thermocline and a chlorophyll maximum (Figure 2.2; lower plot) at ~40m below the surface.

At the shelf edge, the boundary between continental shelf and slope, the hydrodynamic setting is different. Aided by onshore winds and enhanced internal mixing, pulses of nutrients onto the shelf further fuel primary production and alter the community structure of phytoplankton and zooplankton (Sharples et al 2007). These dynamics are apparent in the temperature and chlorophyll profiles, where temperature (Figure 2.2; upper plot) is observed to be greater in the surface waters but mixing at the base of the thermocline is apparent. Chlorophyll (Figure 2.2; lower plot) is distributed throughout the surface layer and not restricted to subsurface as at the mid-shelf region. High levels of chlorophyll are apparent in satellite data at the edge of the shelf across the NW European Continental Shelf (Figure 2.3).

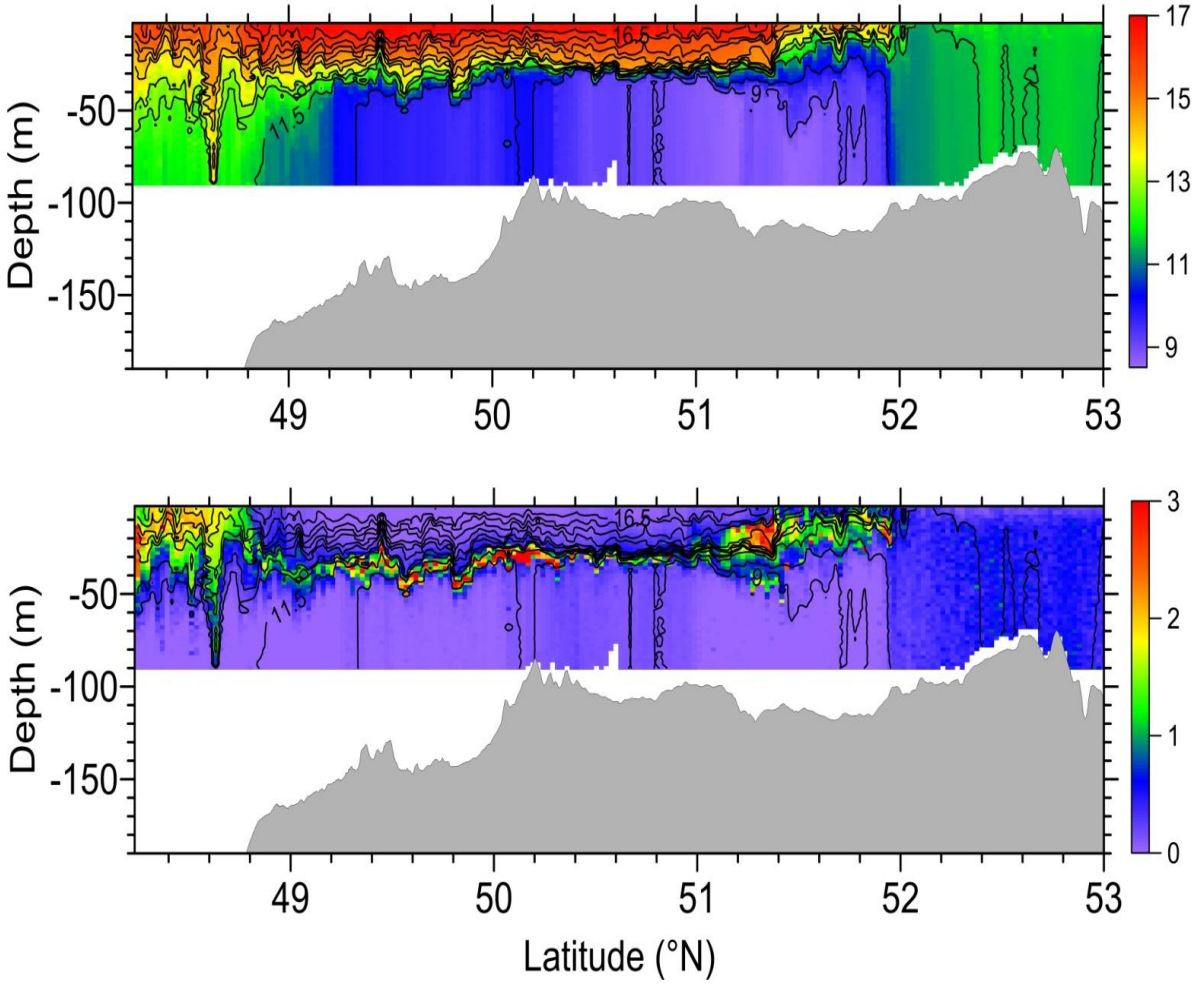


Figure 2.2 Profile across the NW European Continental Shelf from the coastal region 53°N to the shelf edge. The upper profile is of temperature (°C) and the lower plot is of chlorophyll (mg m^{-3}). These profiles were taken during the research cruise in June 2010.

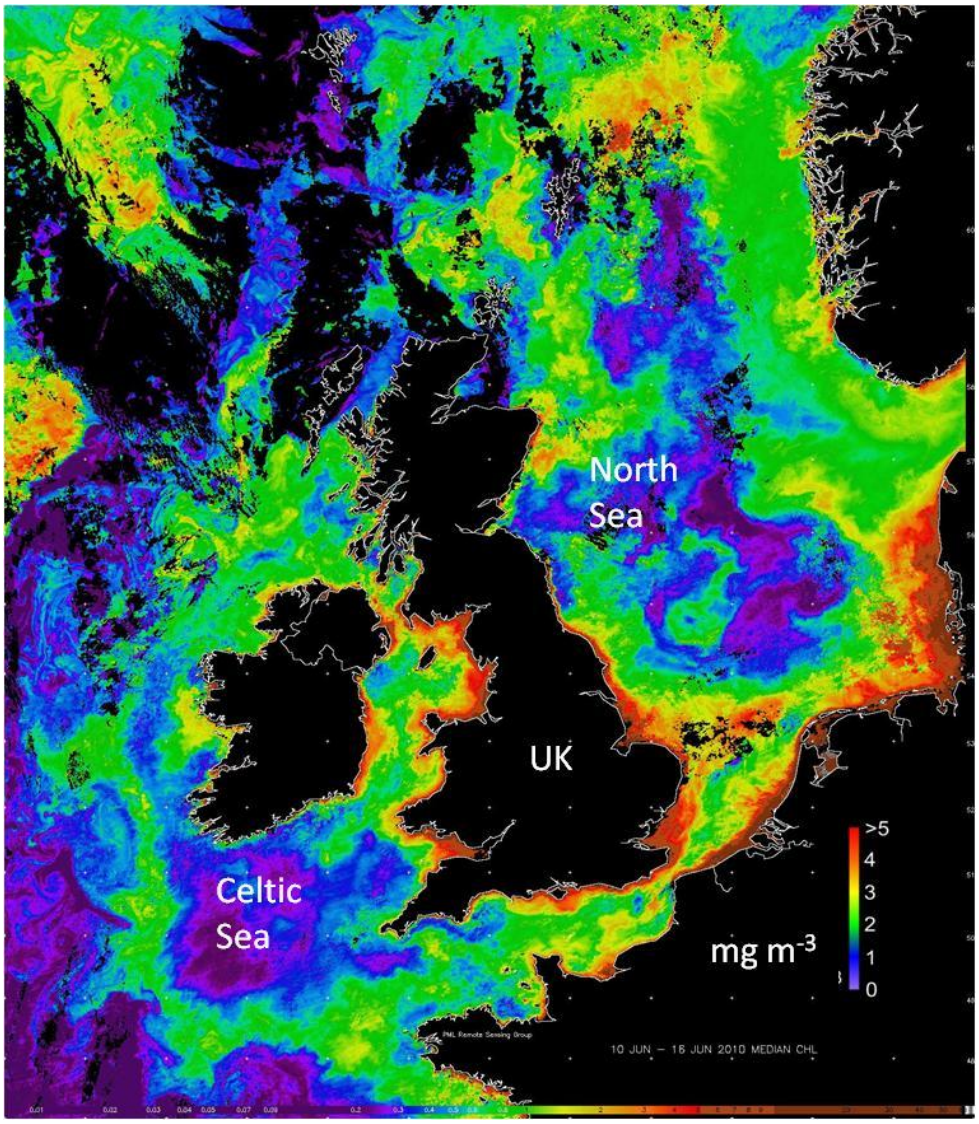


Figure 2.3 Sea surface chlorophyll (mg Chl m^{-3}) image from satellite data over the NW European Continental Shelf seas (10 – 16th June 2010). The image is a MODIS composite chlorophyll image courtesy of J Sharples (NOC and University of Liverpool).

2.1.3 Particulate organic matter in shelf seas

Particulate organic matter (POM) plays an important role in the export of organic carbon from surface water primary production. High levels of biological activity in shelf sea environments stimulate the efficient use of nutrients leading to enhanced drawdown of CO_2 from the atmosphere; subsequently this is exported from surface waters by "the biological pump" (Gattuso et al 1998; Thomas et al 2004). Primary production in surface waters (fixing atmospheric CO_2) and the resulting phytoplankton-produced organic matter is remineralised by microbes and grazers and the process of biological repackaging (often into larger aggregates) distributes POM vertically through the water column, which ultimately sinks out

to the benthos (Wakeham and Canuel 1988; Sheridan et al 2002). Pools of suspended POM within the water column undergo further degradation through zooplankton consumption and microbial reprocessing; this further alters the organic matter content and nutrients that are available to higher trophic levels (Cho and Azam 1988). POM varies both spatially and temporally in deep-sea and shallow-water environments as a result of changing primary production dynamics (Cushing 1959; Bjornsen et al 1993; Schaal et al 2010). The understanding of these processes has also proven to be vital in determining variability in food sources in different systems across the world's oceans (Iken et al 2010). There are a number of biogeochemical techniques that can be employed to investigate the fate of POM within the oceans. Relevant to this thesis include the analyses of lipids and phytopigments and the determination of the isotopic composition of carbon and nitrogen-containing compounds.

Lipids have been used widely as molecular biomarkers in deep-sea environments to assess the characteristics of POM, namely the source, how it is transformed through the water column and ultimately its fate as it is transported from the surface waters to the benthos (e.g. Wakeham 1982; Wakeham and Canuel 1988; Santos et al 1994; Kiriakoulakis et al 2001; Hedges et al 2001; Wakeham 2002; Jeffreys et al 2009). The characterisation of POM through the use of lipids aids identification of heterotrophic processes and biological repackaging through the water column. It provides clues as to the efficiency of the biological pump and how the pelagic and benthic ecosystems function. Identification of temporal variability is useful in assessing changing POM composition as particles sink to the sea floor from the suspended organic matter pool (e.g. Canuel and Martens 1993; Kiriakoulakis et al 2001). Few such studies have been conducted in shelf-sea environments but in principle, biomarker tools could be applied in a similar way.

Photosynthetic pigments are useful in characterising phytoplankton communities as they can be used as taxanomic biomarkers (e.g. Jeffrey 1997; Breton et al 2000; Ansotegui et al 2001; Gibb et al 2001; Wright and Jeffrey 2006). The development of high-performance liquid chromatography (HPLC) has allowed the accurate estimation of chlorophyll *a* and up to fifty other chloropigments (Jeffrey 1997). Pigments are ubiquitous in all photosynthetic algae, but some are limited to specific taxa and allow the composition of the phytoplankton

to be determined (Wright and Jeffrey 2006). As a result phytopigments have become a widely used and important tool in estimating phytoplankton composition particularly as conventional microscopic community analysis is time consuming and frequently impractical. Developments have also been made in the assessment of HPLC-derived data and through the development of a computer program, CHEMTAX (Wright et al 1996), which estimates contributions of relevant pigments to phytoplankton classes. Under certain circumstances, the overall phytoplankton community structure can be accurately assessed. However, this process relies on diagnostic input ratios from phytoplankton species, which can vary depending on the oceanic setting; these data are not yet available for many parts of the world's oceans.

Natural stable isotopes have proven to be a useful tool in tracing carbon and nitrogen compounds through food webs; this has frequently been carried out in benthic environments (Hobson and Welch 1992; Iken et al 2001). Many marine organisms (especially fish and zooplankton) excrete ammonium (NH_4^+) and isotopic discrimination during biological processing in the digestive system produces NH_4^+ which is depleted in ^{15}N relative to the source material (Macko et al 1986). This leads to an enrichment of the residual pool of ^{15}N within the organism's body (Montoya et al 2002). The likely sources of nitrogen to phytoplankton within the water column and their isotopic composition are shown in Table 2.2. The eventual isotopic signature of the suspended POM (phytoplankton) is dependent on isotopic signature of the nitrogen source and the biological fractionation processes that occur during uptake and assimilation (Mahaffey et al 2004).

Table 2.2 Nitrogen sources and their associated δ15N values from literature sources. (* deep ocean source references were from the North Atlantic basin from ~ 1500m).

Nitrogen source	$\delta^{15}N$ associated with the source	Literature reference
Atmospheric nitrogen (N_2 fixation)	-0.6 – 0.9‰	Minagawa and Wada 1986
Nitrate (NO^-_3) - deep ocean*	6 - 7.5‰	Liu and Kaplan 1989
Ammonium (NH_4^+)	-2 - 4‰	Checkley and Miller 1989

Nitrogen isotopes in particular are useful in assessing trophic relationships between organisms and their trophic levels. From prey to consumer there is an estimated enrichment of $\delta^{15}N$ 3-4‰ (van der Zanden and Rasmussen, 2001; Post, 2002) while for $\delta^{13}C$ fractionation is significantly less between trophic levels (0-1‰, (Post, 2002). More recently, other studies based on whole organisms from benthic food webs suggests that a more reasonable estimate of enrichment in ^{15}N is 2.2‰ per trophic level (McCutchan et al 2003; Equation 2.1).

Equation 2.1 $$TL = 1 + \frac{D_{org} - D_{POM}}{2.2}$$

Stable isotopes have also been found to be useful in assessing environmental degradation within a system as anthropogenic activities have been observed to alter fractionation processes (particularly waste water inputs) (Heikoop et al 2000). Waste water nutrients are enriched in ^{15}N, which impacts dissolved inorganic nitrogen pools. Additionally, it is possible to disseminate marine POM from sewage POM as the isotopic signature of the latter is often low when compared to background organic matter (Sweeny and Kaplan 1980).

2.1.4 Aims and hypotheses

The aim of this study was to characterise suspended POM across the changing physical environment of the NW European Continental Shelf Sea. Much work has been conducted at the continental margins and in the deep sea on this topic, however, there have been few studies investigating biogeochemical processes across a shelf sea region encompassing different physical environments. Hence, the study focused on four sites with distinct water column structures;

a) Vertically mixed (permanently).
b) Mid-shelf seasonally stratified (summer).
c) Shelf break stratified

Two hypotheses were tested:

1) Variation between physically distinct regions of the shelf sea lead to differences in the composition and lability of suspended POM. These differences are reflected in lipid, pigment, isotope and phytoplankton distributions within the water column.

2) Compositional variation in suspended POM is influenced by the distribution of zooplankton within the water column, which is reflected in the biomarker composition.

2.2 Methods

2.2.1 *Analysis of water column structure*

A Seabird CTD instrument profiled temperature (^{o}C), salinity (pss), transmission (%) and calibrated chlorophyll (mg m^{-2}). The flourometer was calibrated in two ways from bulk chlorophyll measurements; one for the shelf edge and one for the Celtic Sea. Samples were taken from all depths of the CTD profile and casts were conducted pre-dawn. Calibration was further verified through calibration of chlorophyll *a* on return to the laboratory (Hopkins and Hickman, NOC). All CTD data were processed using SeaBird Software Processing, Version 7.18c (SBE Data Processing-Win32) (Hopkins, NOC). From the CTD profiles, features of the water column were identified (mixed surface layer (MSL), subsurface chlorophyll max (SCM) and bottom mixed layer (BML)) and the positioning of other instruments (e.g. *in situ* stand alone pumps, SAPs) was informed from these data (Table 2.3).

Table 2.3 Depths and horizons of suspended POM samples at each station and subsequent analyses performed on filters, total nitrogen (TN) and total organic carbon (TOC), total lipids (TL), stable isotope analysis (SIA), micro-zooplankton and phytoplankton community structure and high pressure liquid chromatography analysis (HPLC).

	Nominal water column depth (m)	sPOM – depth horizon sampled (m)			TN and TOC	TL	SIA	Micro-zoo and phyto. community	HPLC
		Surface	SCM	Bottom					
IS-M	104	3	-	90	X	X	X	X	X
CS1-SS	137	-	40	100	X	X	X	X	X
CS2-SS	159	5	38	100	X	X	X	X	X
SB-S	191	3.5	25	120	X	X	X	X	X

2.2.2 Micro-zooplankton and phytoplankton community analysis (the analysis of these samples was conducted by A. Panton, UOL).

Duplicate water samples were collected from each depth horizon directly from Niskin bottles on the CTD rosette (Table 2.3). Glass amber jars were filled with 2 mL of 2% (final

concentration) acidic Lugol's iodine and 125 mL of seawater sample were added to each. Samples were stored at 4^{o}C in dark conditions. Microscopy of samples was conducted using the settling method described by Utermohl (1931) and a WILD inverted microscope. IS-M samples were settled for a minimum of 10 h (10 mL settled) and CS1-SS, CS2-SS and SB-S samples were settled for a minimum of 50 h (50 mL settled). Small flagellates were numerated with five field of view (FOV) examinations at x 400 magnification. Small dinoflagellates and cryptomonads were numerated through a full transect of the chamber at x 400 magnification. Larger dinoflagellates, diatoms, ciliates and mesozooplankton were numerated for the whole chamber at x 200 magnification. Species identification was carried out according to Tomas (1997) and counts were converted to cells L^{-1}.

2.2.3 Collection of suspended particulate organic matter

Suspended particulate organic matter (suspended POM) was collected at each site using *in-situ* stand alone pumps (SAPs; Challenger Oceanic). The SAPs were deployed on a wire to a prescribed depth targeting specific features within the water column (e.g. surface mixed layer, subsurface chlorophyll max and bottom mixed layer). The pumps were programmed to filter water through two combusted GF/F filters (293mm diameter). Two filters were used to account for sorption of dissolved organic matter (DOM) onto the filter (Garner et al 2003; Liu et al 2005). It is commonly assumed that POM will not pass through a pore size of 0.7µm, therefore it will be retained on the upper filter. The lower filter is then used to determine the contribution of DOM; the organic carbon and nitrogen concentrations calculated for the bottom filter are subtracted from those of the top filter to give the POM concentration (Turneswitsch et al 2007). Here, mean values for sorbed DOM are displayed for each sampling site (Table 2.4) and are comparable to those quoted by Turneswitsch et al (2007) from a range of NE Atlantic sites with waters collected both from the surface and through the water column. On recovery, the filter housings were removed from the pumps and stored in a clean environment to ensure any remaining water had filtered through the filter bed. After draining for half an hour, the GF/F filters were removed and stored in clean foil at -80^{o}C until analysis.

Table 2.4 Amounts of organic carbon and nitrogen absorbed on the GF/F filters in this study. The data are displayed as mean values (with standard deviation) for each sampling location and displayed as per litre filtered (µmol/L).

	Sorbed DOC (± stdev) (µmol/L)	POC (± stdev) (µmol/L)	Sorbed DN (± stdev) (µmol/L)	PN (± stdev) (µmol/L)
LB-M	1.73	122.5	0.25	20.7
IS-M	1.33 (± 0.92)	73.4 (± 0.002)	0.71 (± 0.71)	20.6 (± 0)
CS1-SS	0.38 (± 0.01)	32 (± 0.02)	0.08 (± 0.01)	5.8 (± 0.005)
CS2-SS	0.55 (± 0.27)	72.8 (± 0.004)	0.13 (± 0.06)	13.6 (± 0)
SB-S	0.24 (± 0.05)	17.4 (± 0.002)	0.09 (± 0.71)	3.8 (± 0)

2.2.4 Pigment extraction and high pressure liquid chromatography (HPLC) analysis (HPLC analyses partly conducted by N. Carr, UOL)

Samples for analysis of chlorophyll *a* and secondary pigments were prepared using 3 mL 90% HPLC grade acetone and were spiked with an internal quantification standard, zinc-phthalocyanine (ZnPc; 50 µL; 29 ng μL^{-1}). Each sample was sonicated for two minutes then chilled overnight at 4^{o}C. Following this, samples were centrifuged (2800 r.p.m. 2 min; 15^{o}C). Extracts were then filtered (0.4 µm Phenex RC membrane filters, Phenomenex) into glass auto-sampler vials for analysis using an AGILENT 1100 Series LC. Pigment separation was carried out using a C18 column (Fortis; 5 µm; 100 mm X 4.6 mm i.d.; 25°C). Elution of pigments was carried out using 70:30 methanol/ammonium acetate and 60:40 methanol:acetone solutions (method adapted from Meyer-Harms et al 1999). Pigments were detected using a fluorescence detector (λ_{ex} 360 and 430 nm; λ_{em} 665 nm), which was calibrated using high purity chlorophyll *a* and mixed pigment standards (DHI, Denmark). Chromatogram data were processed using AGILENT Chemstation software (version A.10.02, SCOR spectrums, Jeffrey 1997).

The use of CHEMTAX, a programme for estimating contributions of relevant pigments to phytoplankton classes, was evaluated, but rejected due to the lack of ratio matrices for the study region. Therefore, to ascertain phytoplankton community structure and taxa at each

of the four sites sampled, a biomarker approach was used based on the literature (Table 2.5)

Table 2.5 Key identified pigments and their provenance from literature sources.

Pigment	Biomarker	Literature
19 – Butanoyloxyfucoxanthin	Some prymnesiophytes (e.g. coccoliths and *Phaeocystis*), marine chrysophytes (e.g. *Pelagococcus*)	Vesk and Jeffery 1987 Jeffrey and Wright 1994
19 – Hexanoyloxyfucoxanthin	Prymnesiophytes (e.g. coccoliths), some dinoflagellates	Arpin et al 1976 Jeffrey and Wright 1994
Alloxanthin	Cryptomonads (mostly photosynthetic but have an ability to be mixatrophic)	Chapman and Haxo 1966 Pennington et al 1985
Chlorophyll *a*	All photosynthetic algae and higher plants	Scheer 1991
Chlorophyll *b*	Higher plants, green algae, symbiotic prochlorophytes	Scheer 1991
Chlorophyll *c3*	Some prymnesiophytes (e.g. coccoliths), diatoms and chrysophytes	Vesk and Jeffery 1987 Jeffrey and Wright 1994
Fucoxanthin	Diatoms (major pigment), prymnesiophytes (e.g. coccoliths), brown seaweeds and some dinoflagellates	Jeffery and Wright 1994
Lutein	Red seaweed, green algae, higher plants	Hager and Stransky 1970
Peridinin	Autotrophic dinoflagellates	Jeffrey et al 1975

2.2.5 Total Lipids

For each analysis one ¼ of the GF/F SAP filters were cut up and spiked with 100 µL of two internal quantification standards, 5α(H)-cholestane and 5β(H)-cholanic acid. Particulate matter across the filters was homogeneous. Blank samples with internal quantification standards were extracted alongside SAP filters. Lipids from the filters were extracted by sonication with 9:1 solution of dichloromethane (DCM) and methanol (~15mL). The extracted solution was then transferred to a round-bottomed flask and the solvent evaporated before the residue was re-dissolved in methanol. The solution was then dried by passing through a column of anhydrous sodium sulphate. Methylation was carried out using 1 mL of a mixture of acetyl chloride and methanol (1:30; 0°C). The samples were then covered and left on a heating block (12 h; 45°C). The solvent was then removed under a stream of N_2 and the residue re-dissolved in DCM and neutralised by passing them through a column of potassium carbonate. The solvent was again removed under a stream of N_2 and derivitized using BSTFA (*bis*-trimethylsilytrifluoroacetamide) (~30 µL per sample; 1 h; 60°C). The derivitized samples were then placed under a stream of N_2 prior to storage or re-dissolution with DCM in preparation for analysis. Samples were analysed using Thermo Quest EC Instruments Trace 2000 series GC, coupled with Thermo Quest Finnigan TSQ7000 mass spectrometer. The column used was a J&W DB-5MS (60m length; 0.25mm ID; 0.1 µM film) with a cool on-column injector and helium as carrier gas (flow rate 1.2 mL min^{-1}). Typical operating conditions for the mass spectrometer were: Transfer line, 320°C; ionisation potential, 70eV; source temperature, 215°C; emission current, 350 µA.

2.2.5.1 Lipid quantification

Data were interpreted using X-Calibur (Finnigan Corporation, version 1.0) software. Individual compounds were identified through comparison of their mass spectra with those of authentic standards and from the literature (Table 2.6). 5α(H)-cholestane was used to calculate relative response factors (Equation 2.2). Comparison of the peak areas of the internal standards and compounds of interest allowed the concentrations of the analytes to be determined (Kiriakoulakis et al 2004). Full nomenclature and structures of compounds can be found in Appendix 2.6.

Equation 2.2 $RF – (C_{st}*A_{is})/(C_{is}*A_{st})$

Where: RF = response factor, A_{is} = area of the internal standard peak, A_{st} = area of compound being quantified, C_{is} = concentration of internal standard, C_{st} = concentration of compound being quantified.

Table 2.6 Source/indicator lipid biomarkers used in this stud and literature resources.

Lipid biomarker	Source/Indicator	Literature
Diatoms	$C_{16:1(n-7)}$ mono-unsaturated FA	Budge and Parrish (1998), Reuss and Poulson (2002)
	$C_{20:5(n-3)}$ (EPA) highly unsaturated FA	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006)
	$C_{16:1}/C_{16:0}$ mono-unsaturated FA	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006), Neto (2002)
Dinoflagellates	$C_{20:4(n-6)}$ highly unsaturated FA	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006)
	$C_{22:6}$ (DHA) highly unsaturated FA	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006)
Bacteria	$C_{18:1}$(n-7) mono-unsaturated FA	Budge and Parrish (1998)
	Branched *iso* and *anteiso* C_{15} and C_{17} FA	Gillan and Johns (1986), Kaneda (1991)
Zooplankton	$C_{20:1}$ mono-unsaturated FA	Sargent and Henderson (1986), Kattner et al (2003)
	$C_{22:1}$ mono-unsaturated FA	Sargent and Henderson (1986), Kattner et al (2003)
	$C_{20:1}$ and $C_{22:1}$ unsaturated alcohols	Sargent and Henderson (1986), Kattner et al (2003)
General biomarkers	C_{14}-C_{22} saturated fatty acid – derived from marine sources	Viso and Marty (1993), Dunstan et al (1994)
	C_{24}-$_{32}$ saturated fatty acids – derived from vascular plants	Harvey (1994), Santos et al (1994)
Labile diatom biomarker	$C_{16:1(n-7)}/C_{16:0}$	Najdek (1996), Neto (2002)
Invertebrate reworking of organic matter	C_{27}/C_{29} sterols ratio	Neto (2002)

Biomarkers detailed in Table 2.6 have been used as a tool to characterise suspended POM in the water column. It is important to note that while biomarkers provide important information on the makeup of POM, there are limitations as to their specificity . For example, here the ratio of $C_{16:1}/C_{16:0}$ has been used as an indicator of labile material in conjunction with the abundance/presence of more conventional biomarkers such as polyunsaturated fatty acids (PUFAs) (Budge and Parrish 1998). The $C_{16:1}/C_{16:0}$ ratio has mainly been utilised in deep-sea environments (Neto 2002) and caution has to be taken as the compounds degrade over time. However, used in conjunction with the PUFAs, they provide supporting evidence for the lability of POM. Ratios of C_{27} and C_{28} sterols have also been used in this study to investigate reworking of organic matter across the continental shelf. As for the biomarkers of labile POM, this ratio has been utilised mainly in deep-sea environments, so the sterol ratio has been assessed alongside other zooplankton biomarkers and not in isolation.

To assess how the grouping of biomarkers influenced the data interpretation multivariate statistical analyses were conducted using PERMANOVA and MDS analyses. However, statistically significant relationships between biomarker groups were absent.

2.2.6 Total organic carbon and nitrogen (analyses conducted by S. Blackbird, UOL)

Total nitrogen (TN) and total organic carbon (TOC) analyses of the SAP GF/F filters were carried in duplicate using a Carlo Erba Instruments NC2500 elemental analyser. For each analysis, two small punches (1 cm diameter) were taken from the outer edge and centre of the filter. For TN, a portion of filter was weighed using a Mettler Toledo UMX2 ultra-microbalance, placed into a tin capsule and stored in dry atmospheric conditions prior to analysis. For TOC analyses the glass fibre filters were pre-treated with acid to remove inorganic carbon fractions from each sample. This pre-treatment was conducted on samples overnight in a dessicator containing concentrated fuming HCl (11.4N; 250ml). Filters were weighed before and after treatment. Following acid treatment of the samples the portions of filter were placed into tin capsules and stored in dry atmospheric conditions prior to analysis. Quantitative and instantaneous combustion of the sample was carried out and this enabled gases (N_2 and CO_2) to be efficiently eluted through the Porapack PQS column (also known as the Flash Combustion method). Pure eluted combustion gases passed though a

thermoconductivity detector, which generates electrical signals proportional to the amount of eluted gas.

2.2.7 Stable isotope analysis

Suspended POM samples, collected on GF/F filters, were sub-sampled from the large filters using a 1cm diameter punch. Punches were taken from the edge and centre of the filter to ensure that representative samples were collected. Suspended POM samples were pre-treated with acid to remove inorganic carbon fractions prior to isotope analysis. De-carbonation was conducted using concentrated hydrochloric acid (11.4N; 250mL) in the base of a desiccator overnight. De-carbonation of samples is known to impact isotope values (Goering et al 1990; Bunn et al 1995). Therefore, samples were prepared and run in two batches, one for carbon analysis (de-carbonated) and the other for nitrogen analysis (untreated). Quantitative analysis in a previous study has shown that carbon isotope values can decrease by up to 1‰ following de-carbonation (Larrain et al 2003). Samples for isotope ratio mass spectrometry were wrapped in tin foil and folded into pellets prior to analysis.

Samples for isotope ratio mass spectrometry were transferred to tin capsules and loaded into an auto-sampler prior to combustion using Costech ECS 4010 CHNSO elemental analyser. The samples were combusted with oxygen (generating temperatures of 1700-1800^{o}C) breaking the sample into its elemental constituents. Excess oxygen is absorbed through copper wire prior to passing through a GC (NC 3m stainless steel column) and producing electrical signals proportional to the amount of element in the sample. Separated gases ($C0_2$, N_20) are then transferred via a Thermo Scientific Conflo IV to a Thermo Scientific Delta V Advantage continuous flow isotope ratio mass spectrometer where a report for each element, on a weight basis, is generated. All samples were run in triplicate so errors were calculated on this basis and analytical sensitivity is reported at 0.06‰ for carbon and nitrogen analysis. Sample isotopic ratios are reported according to Equation 2.3:

Equation 2.3
$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000\ ‰$$

Where X is ^{13}C or ^{15}N of the sample and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

2.2.8 Statistical and multivariate analysis

Statistical analyses were carried out using SigmaPlot (V 11) software. Statistical significance was tested between natural stable isotope abundances and the three depth horizons that samples were collected from using one-way ANOVA. Prior to the one-way ANOVA tests, Shapiro-Wilk normality tests were conducted.

Multivariate analyses were used to assess the relationships between community composition, stable isotope data and different water column dynamics. The multivariate statistical software package PRIMER 6 + PERMANOVA was used (Clarke and Gorely 2006). Due to overall small sample size, conventional analysis of similarity (ANOSIM) could not be carried out to assess the relationships between sample sites and water column structures, so PERMANOVA analyses were carried out instead. This analysis was better suited to the data set as the number of replicates and sample sites were limited; the PERMANOVA analysis does not assume normal distributions and Euclidian distance unlike ANOVA and ANOSIM tests. Data were analysed as a whole (main test) and then pair-wise interactions were calculated to establish where the significant interactions occur for each of the factors assessed “site”, “water column structure” and “water column depth”. These factors were used as they group the data differently in each case. The factor “site” tests the four sampling locations separately assessing any similarities between them. The factor “water column structure” groups the sampling locations with the same physical water column dynamics (IS-M was categorised as mixed water, CS1-SS and CS2-SS were grouped as seasonally stratified and SB-S was categorised as shelf break) assessing similarities between physical structure. The factor “water column depth” groups the data according to the depth of sampling (surface, SCM and bottom). Following PERMANOVA analysis, multidimensional scaling (MDS) was conducted in order to highlight similarities in the data through spatial representation. Data that are similar are represented by points that are close together and those that are dissimilar are further apart.

2.3 Results and discussion

2.3.1 *Organic matter variability through the water column*

In the deep sea and at continental margin environments, the importance of POM transport through the water column to the benthos as a food source has been highlighted (Bett and Rice 1993; Lampitt and Antia 1997). However, only a small proportion of primary productivity reaches the deep-sea benthic environment (1-3%) as much of this organic matter is utilised in the water column before reaching the seabed (Wakeham 1982; Fowler et al 1986; Santos et al 1994; Wakeham et al 2009). In shelf-sea environments, the relative amount of POM reaching the sea floor compared to that fixed in surface waters is greater than in the open ocean (Wollast 1998). The amount of POM reaching the sea floor is depth dependent, but in shelf seas, production is greater and the water column depth is much shallower. Here I assess the composition of suspended POM through the water column at four contrasting locations across the continental shelf sea. CTD profiles for each of the study sites across the continental shelf (Figure 2.4) showing chlorophyll (mg m^{-3}), salinity (pss), temperature (^{o}C) and transmission (%). IS-M shows a mixed water column structures with homogeneous physical parameters. CS1-SS and CS2-SS show seasonal stratification with a clear SCM and thermocline dynamics. SB-S also shows a slight SCM and shallow thermocline.

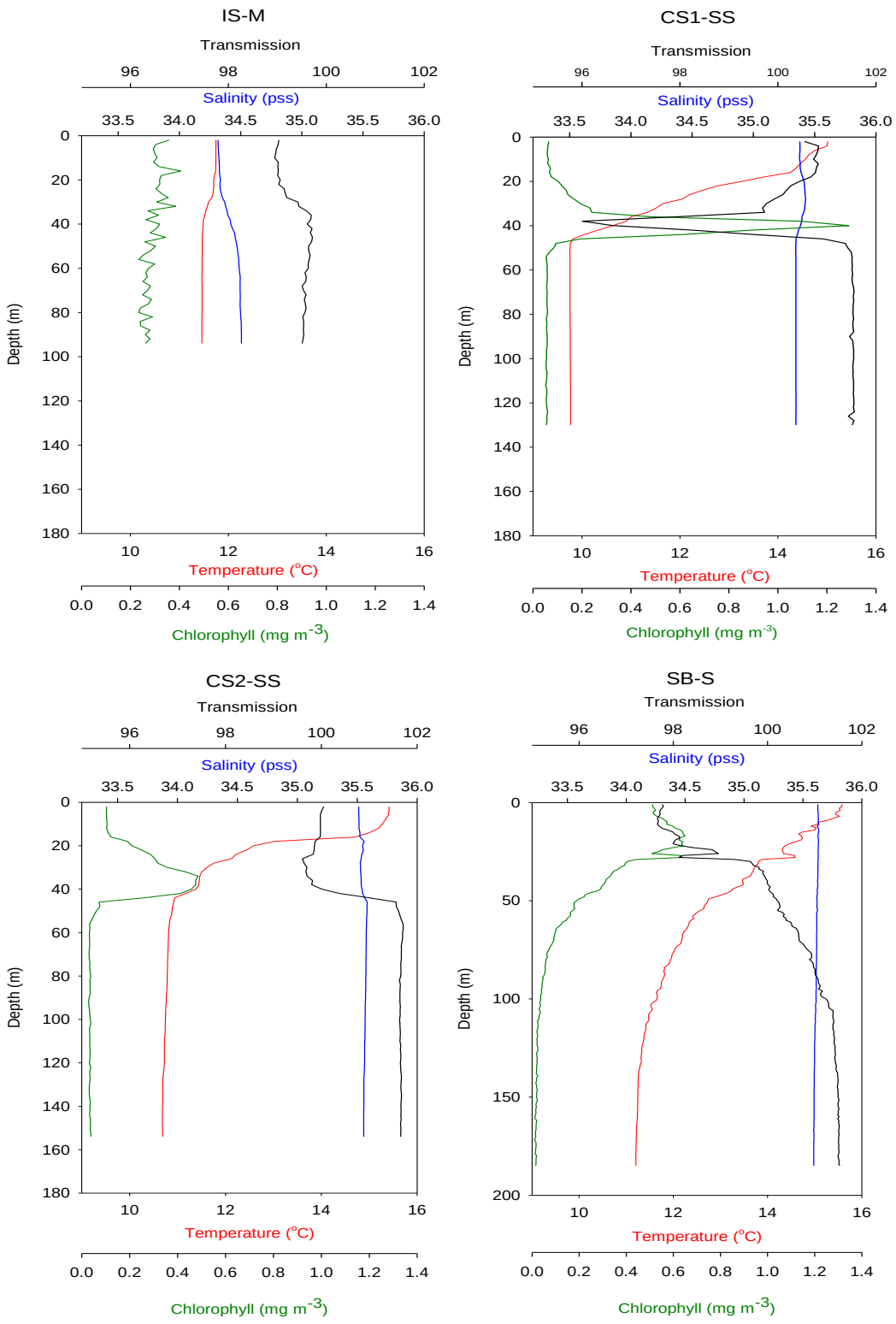


Figure 2.4 Chlorophyll, salinity and transmission measurements for CTD casts. IS-M is defined as a mixed water column and CS1-SS and CS2-SS are defined as stratified water columns due to the presence of a thermocline and subsurface chlorophyll max (SCM). SB-S shows typical stratification at the continental shelf edge.

2.3.2 The phytoplankton community - Pigment biomarkers and microscopy

The composition of phytoplankton species is controlled by a number of environmental and physical factors to which certain individuals are better adapted and therefore have greater competitive advantage (Hickman et al 2009). HPLC pigment analyses in conjunction with microscopy at discrete depth horizons were used to ascertain phytoplankton community structure (Ansotegui et al 2001).

Absolute concentrations of pigments from HPLC analysis at CS1-SS, CS2-SS and SB-S were greater at the SCM compared to the surface and bottom mixed layers of the water column (Table 2.7). As IS-M, higher concentrations were generally observed deeper in the water column when compared to the upper layers. The composition of pigments and plankton through microscopy counts were further examined through percentage composition analysis. A representative annotated chromatogram is shown with individual pigments identified (Figure 2.5; Table 2.8).

Table 2.7 Table showing the pigment components of each sampling site and at each depth, data are shown as μg L^{-1}.

	IS-M	CS1-SS	CS2-SS	SB-S
Total identifiable pigments				
Surface	4.432	n.d	2.026	1.202
SCM	n.d	1.137	10.545	0.614
BML	4.995	1.008	0.663	0.383
Chlorophyll C3				
Surface	0.174	n.d	0.000	0.129
SCM	n.d	0.226	1.278	0.054
BML	0.376	0.141	0.063	0.060
Peridinin				
Surface	0.137	n.d	0.000	0.000
SCM	n.d	0.000	0.213	0.000
BML	0.157	0.000	0.000	0.000
19-Butanoyloxyfucoxanthin				
Surface	0.000	n.d	0.131	0.183
SCM	n.d	0.125	1.325	0.098
BML	0.000	0.098	0.102	0.058
Fucoxanthin				
Surface	1.465	n.d	0.134	0.116
SCM	n.d	0.309	1.772	0.100
BML	2.071	0.255	0.204	0.049
19-Hexanoyloxyfucoxanthin				
Surface	0.160	n.d	0.000	0.000
SCM	n.d	0.000	0.000	0.000
BML	0.459	0.152	0.059	0.035
Alloxanthin				
Surface	0.368	n.d	0.000	0.069
SCM	n.d	0.063	0.809	0.090
	0.321	0.052	0.020	0.039
Lutein/Zeaxanthin				
Surface	0.129	n.d	1.127	0.268
SCM	n.d	0.000	1.784	0.045
BML	0.108	0.046	0.026	0.030
Chlorophyll b				
Surface	0.390	n.d	0.000	0.058
SCM	n.d	0.056	0.209	0.030
BML	0.306	0.015	0.019	0.018
Chlorophyll a				
Surface	1.610	n.d	0.635	0.378
SCM	n.d	0.358	3.156	0.198
BML	1.197	0.249	0.171	0.095

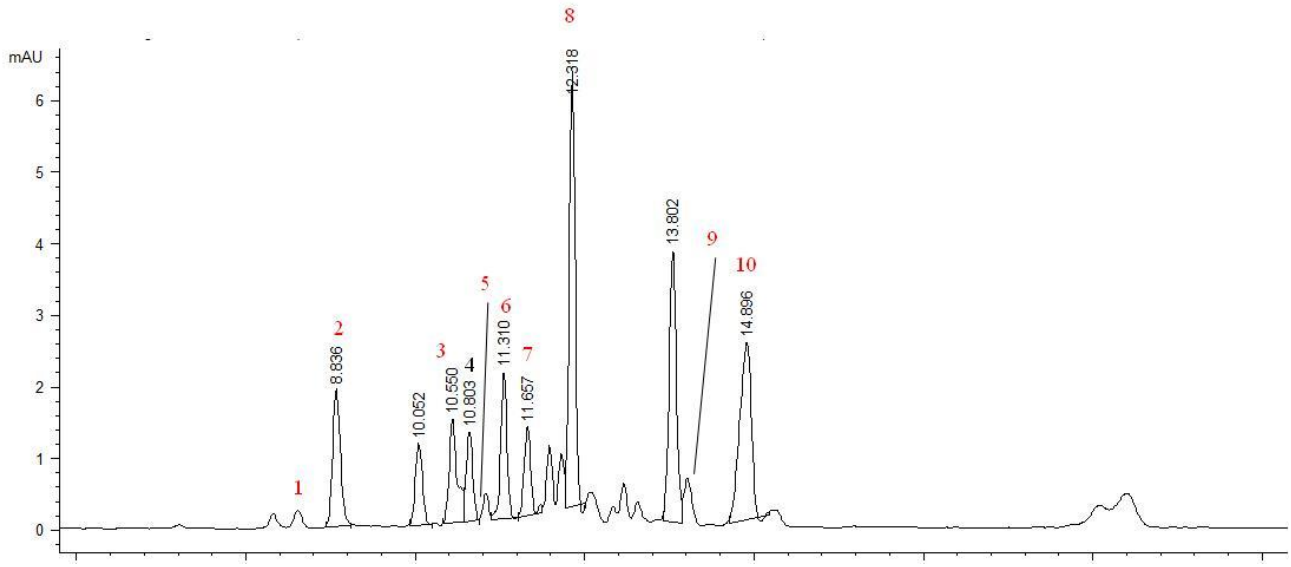


Figure 2.5 A representative annotated chromatogram showing all identified peaks and nomenclature for pigment analysis. This sample was a bottom sample at site CS1-SS.

Table 2.8Table showing all compounds observed in the sample. This sample was a bottom sample at site CS1-SS.

Peak No	Compound	Peak No	Compound
1	Chlorophyll C3	6	19-Hexanoyloxyfucoxanthin
2	Chlorophyll C2	7	Diadinoxanthin
3	19-Butanoyloxyfucoxanthin	8	Lutein/Zeaxanthin
4	Fucoxanthin	9	Chlorophyll b
5	Neoxanthin	10	Chlorophyll a

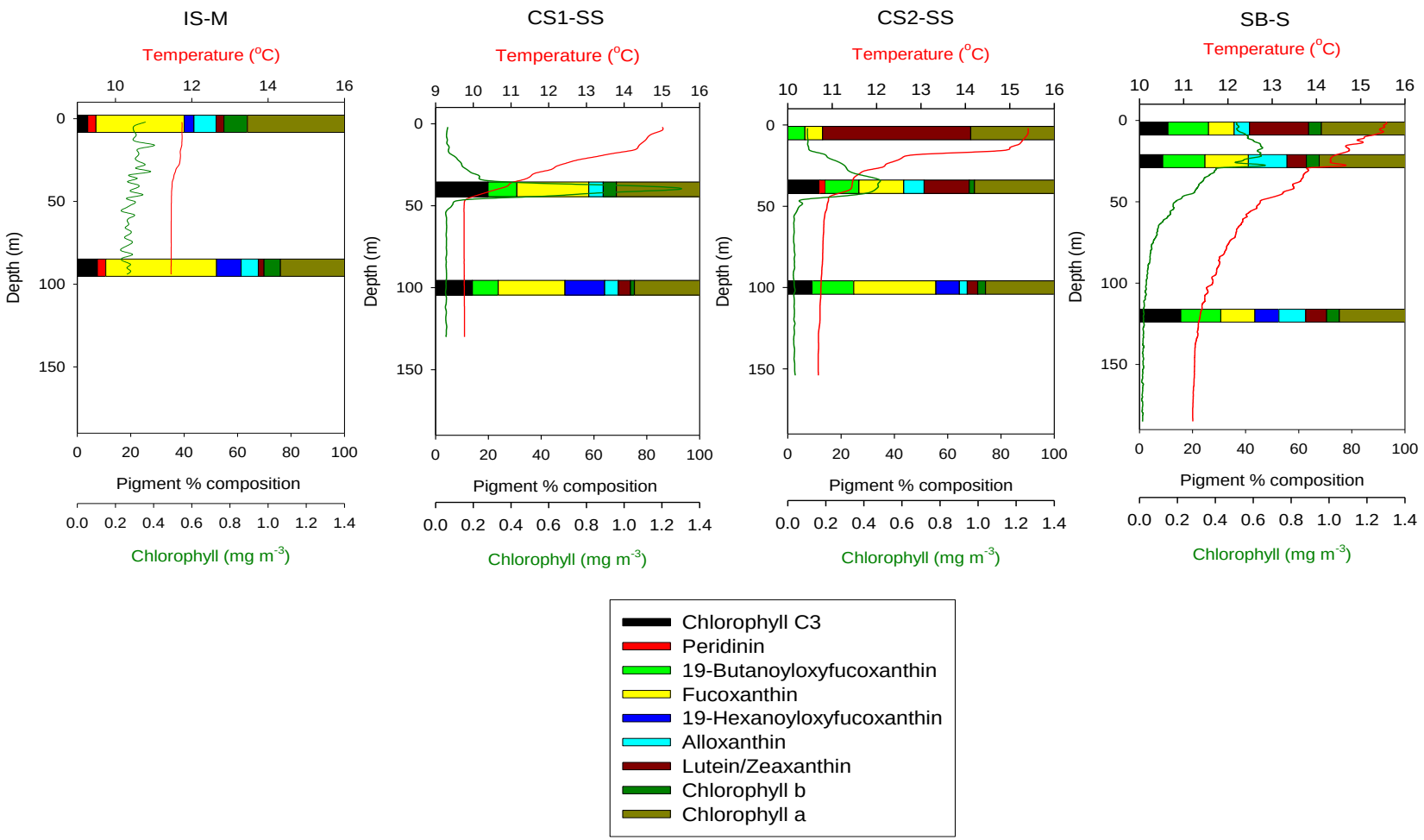


Figure 2.6 Pigment composition from the four survey sites as pigment % for each depth horizon sampled. Temperature (°C) and chlorophyll (mg m^{-3}) profiles are included for reference.

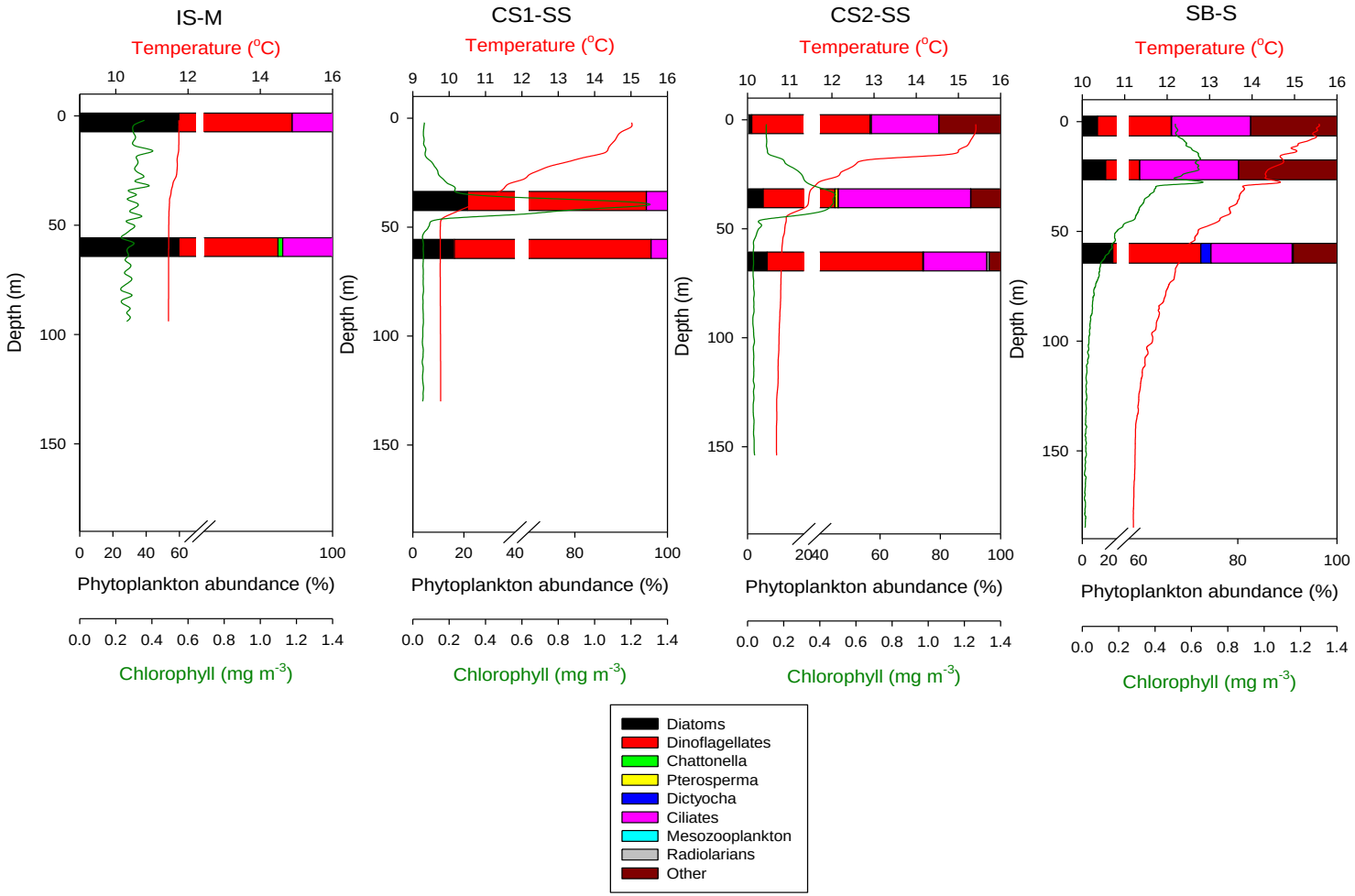


Figure 2.7 Plankton and phytoplankton count data from niskin bottle samples (analysed by A. Panton) the four survey sites shown as phytoplankton abundance (%) for each depth horizon sampled. Temperature (°C) and chlorophyll (mg m^{-3}) profiles are included for reference.

Mixed water column structure (IS-M). Absolute pigment values, from HPLC analysis, were observed to increase slightly with water column depth. This was observed for total identifiable pigments and most of the individual pigment groups (Table 2.7). Compositionally phytoplankton pigments (Figure 2.6) and microscopy counts (Figure 2.7) were observed to be fairly uniform in the surface and bottom waters. Pigments included fucoxanthin, a common diatom biomarker (Jeffery and Wright 1994), which increased in relative abundance with depth, as did the prymnesiophyte biomarker, 19 – butanoyloxyfucoxanthin (Vesk and Jeffery 1987; Jeffrey and Wright 1994). Microscopy counts also showed an increase of ciliates with depth. At depth, a genus associated with toxic algal blooms (*Chattonella* spp.) was identified by microscopy (Hallegraeff 1993). The uniformity of distribution of species observed in the water column is maintained due to the strength of tidal forcing and wind stress creating a mixed water column, where the physical parameters remain constant (Simpson and Sharples 2012). The slight increase of pigment abundance with depth may be due to turbulent conditions within the water column favouring the downward transport of labile material through the water column (Neto 2002; Huisman et al 2004; Simpson and Sharples 2012).

Seasonally stratified water column structure (CS1-SS and CS2-SS). Absolute pigment values at CS1-SS showed there to be greater abundance at the SCM than in the bottom mixed later of the water column (Table 2.7). Pigment analyses and microscopic counts, indicated that CS1-SS had a greater contribution from diatoms (pigment biomarker fucoxanthin; Jeffery and Wright 1994) at the SCM than at depth. There was also a greater contribution from coccolith and algal biomarkers (19 – butanoyloxyfucoxanthin and lutein; Vesk and Jeffery 1987; Jeffrey and Wright 1994) at depth compared to the SCM. Absolute pigment abundances support these observations. Microscopic counts showed the dinoflagellate population to increase with water column depth.

Similarly to the other seasonally stratified site, CS2-SS showed greater absolute pigment abundances at the SCM compared to surface and bottom mixed waters (Table 2.7). At CS2-SS both pigment analysis and microscopic counts showed the relative abundance of diatoms to increase with water column depth. Microscopy indicated that dinoflagellate species were present throughout the water column, but only at the SCM according to pigment analyses, where the pigment peridinin was present (Jeffrey et al 1975). As observed at CS1-SS, the

coccolith biomarker 19 – butanoyloxyfucoxanthin was present at the base of the water column but was absent elsewhere. Microscopic counts showed ciliate abundances to be greatest at the SCM. At this site diatoms were not as prominent in the surface waters as other sites, the growth of diatoms could have been limited by another factor in the surface mixed layer e.g. grazing. However, they were observed to increase in relative abundance with increasing depth. Diatoms are known to be good competitors under low nutrient and light conditions and this may explain these observations particularly at the SCM where nutrient fluxes occur and light levels are sufficient for photosynthesis (Chisholm 1992; Panton 2012).

In summary, at the two seasonally stratified sites, phytoplankton communities were observed to vary in composition with depth. Flagellate and small dinoflagellate communities dominated in the surface mixed layer with a more diverse assemblage and greater abundance (including diatoms and ciliates) at the SCM.

Stratified shelf break (SB-S). Absolute pigment abundances were observed to fairly similar between the surface and SCM layers with abundances decreasing below the SCM (Table 2.7). Some disparity between pigment biomarkers and microscopic counts were observed. The former showed diatom biomarkers (fucoxanthin) to be most abundant at the SCM, whereas microscopic counts indicated diatom populations to increase in relative abundance with water column depth. Microscopy data also showed increasing relative abundance of dinoflagellates with depth. As at other sampling stations across the shelf, the coccolith biomarker (19 – butanoyloxyfucoxanthin) was only present at the base of the water column. At the edge of the continental shelf, larger diatoms and dinoflagellates were present throughout the surface mixed layer; these are likely supported by strong vertical mixing in the water column, which is known to favour the growth of larger diatom cells (Joint et al 2001; Sharples et al 2007).

At each study site across the shelf, fucoxanthin and chlorophyll *a* were found to be dominant biomarkers. These compounds are generally short-lived due to their rapid degradation in phytoplankton cells (Ridout and Morris 1988; Abele-Oeschger 1991), which suggests that suspended POM was from a fresh source. Fucoxanthin has also been attributed to the presence of diatoms and this biomarker showed close correlation to

relative diatom abundances determined by microscopy. It has been suggested that a decreasing ratio of chlorophyll *a* : fucoxanthin is indicative of adaptation to light conditions (Ansotegui et al 2001). Here, this ratio was observed to decrease with water column depth, which is consistent with a shift to light limiting conditions at depth. Alloxanthin was observed at nearly all sampling depths; this pigment is commonly associated with cryptophytes. At stratified sites, the greatest relative abundance of this compound was observed at the SCM; cryptophytes at this depth horizon capitalise on sufficient light and nutrient supply (Camacho 2006). At each of the survey sites, prymnesiophyte biomarkers (19 – hexanoyloxyfucoxanthin, particularly ascribed to coccoliths) were present in greatest relative abundance in the deepest samples; this most likely reflects sinking processes and excretion of coccolith-derived products by heterotrophic re-working (Honjo 1976). The consumption of coccoliths by small zooplankton in the upper water column may result in faecal pellets containing coccolith material sinking through the water column (Honjo 1976). These sinking processes may lead to the increase in relative abundance of coccolith biomarkers with depth. At two of the sampling sites, there were disparities between pigment and microscopy data; these most likely reflect natural variation and patchiness within the water column at sampling sites, particularly at SB-S. Samples for pigment analysis and microscopy were not collected at the same time, so may have compounded the inferred differences in community structure between the techniques. An additional reason for these discrepancies between pigment and microscopy data may be due to the lack of specificity in pigment analysis. This is a limitation of this type of analysis, nevertheless pigment and microscopy analyses used together are powerful techniques in characterising phytoplankton communities.

2.3.3 Lipids

The nature of marine POM has previously been assessed through the use of lipids as molecular indicators, particularly when tracing the source, transformation and fate of organic matter (Hedges et al 2001; Wakeham 2002). Certain lipids, in particular poly-unsaturated fatty acids (PUFAs), are highly labile and have been used as markers of fresh organic matter (Sun et al 1997; Kiriakoulakis et al 2004). Highly-unsaturated fatty acids (HUFAs), in particular $C_{20:4}$, $C_{20:5}$ and $C_{22:6}$ have also been used as biomarkers, being indicative of dinoflagellates and diatoms, respectively (Budge and Parrish 1998; Reuss and

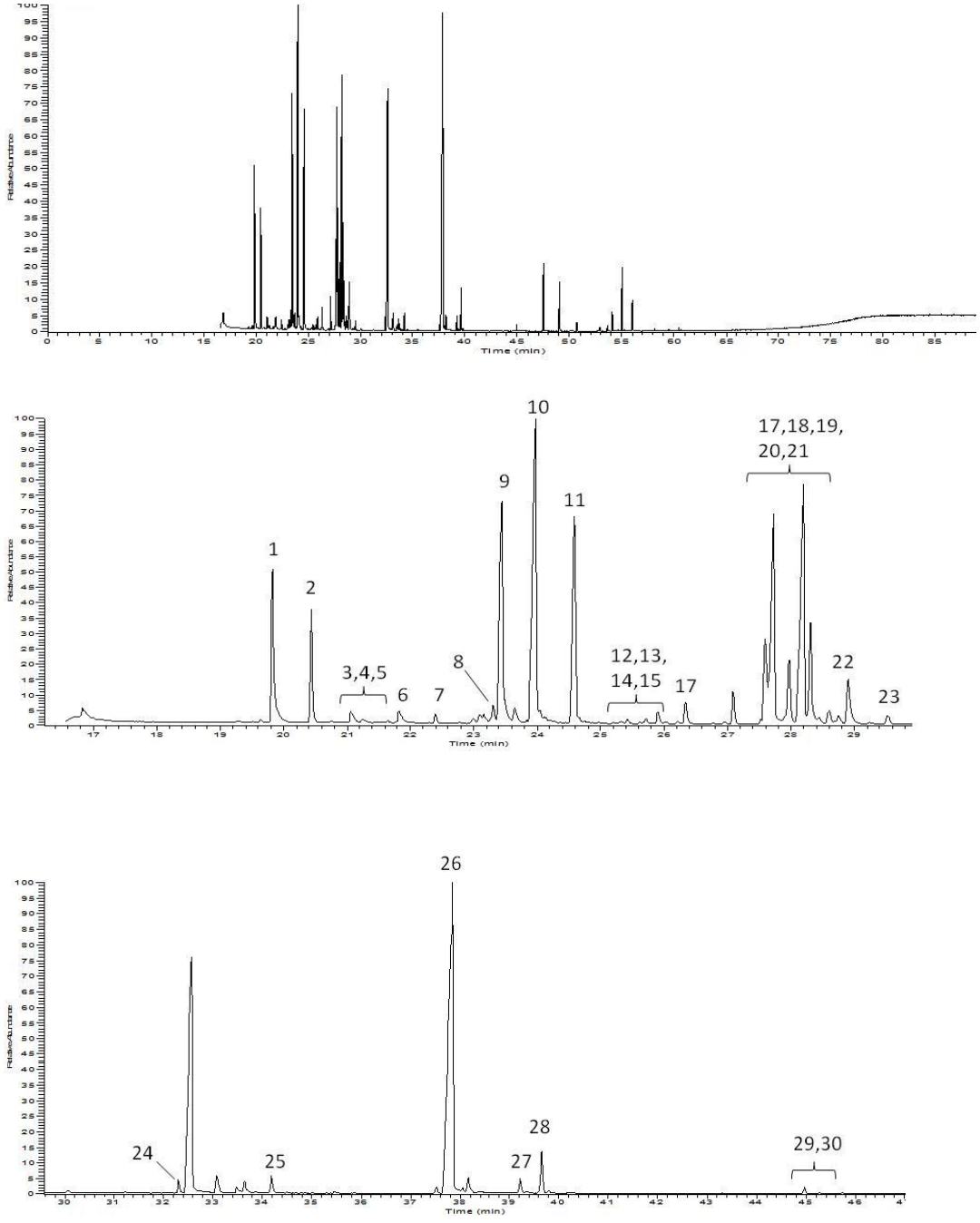
Poulson 2002; Fang et al 2006). In this study, the lipid composition of suspended POM across the four study sites has been examined at each of the sampled depth horizons.

Absolute concentrations of lipids at CS2-SS and SB-S a where higher at the SCM compared to surface and bottom mixed layers of the water column. At IS-M and CS1-SS, higher concentrations were generally observed deeper in the water column when compared to the upper layers. The composition of POM through the water column was further examined through percentage composition and groups of biomarkers which are indicative of specific taxa.

Table 2.9 Table showing the lipid components of each sampling site and at each depth, data are shown as ng L^{-1}.

	IS-M	CS1-SS	CS2-SS	SB-S
Total identifiable lipids				
Surface	14	nd	44582	189
SCM	nd	12839	99846	6103
BML	6647	37254	11879	3356
Fatty Acids				
Surface	7.12	nd	20538	79
SCM	nd	1583	67683	2374
BML	3160	2991	5828	1811
Mono-unsaturated Fatty Acids				
Surface	7	nd	17105	87
SCM	nd	1460	22452	1347
BML	3037	2461	5105	1291
Poly-unsaturated Fatty Acids				
Surface	0.15	nd	2144	9
SCM	nd	1216	7716	118
BML	291	3173	336	235
Alcohols				
Surface	0.34	nd	4588	2
SCM	nd	2597	1129	1680
BML	53	4834	307	15
Sterols				
Surface	0.17	nd	206	10
SCM	nd	5982	865	583
BML	105	23795	303	2

The abundances of lipids as percentage compositions have been assessed in order to assess and characterise the composition of POM at each of the depth horizons sampled across the shelf sea (Table 2.9). A representative annotated chromatogram is shown with individual lipids identified (Figure 2.8; Table 2.10; full chromatograms for all lipid samples Appendix 2.7).



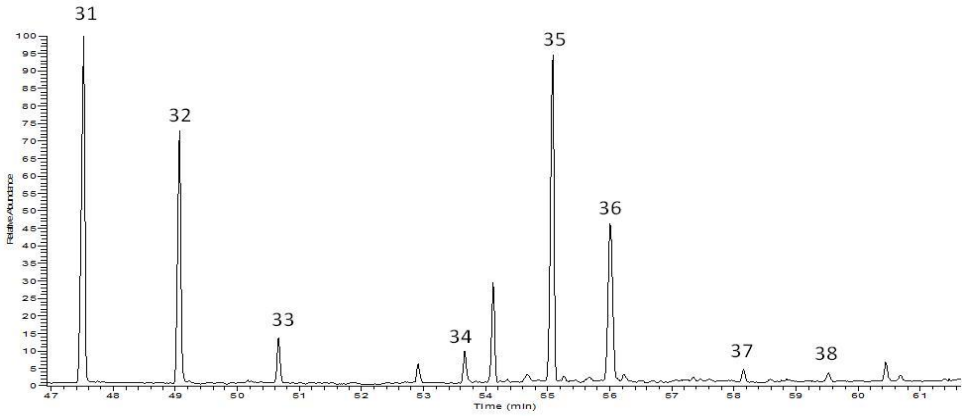


Figure 2.8 A representative annotated chromatogram showing all identified peaks and nomenclature for lipid analysis (see Appendix 2.6 for full structures of compounds). This sample was a surface sample at site CS1-SS. The top panel shows the whole sample and the three other panels show the sample in closer detail with labelled peaks.

Table 2.10 Nomenclature for all compounds observed in the sample, full structural diagrams can be found in Appendix 2.6.

Peak No	Compound	Peak No	Compound
1	$C_{14:0}$ acid: Tetradecanoic acid	20	Δ^{9}-$C_{18:1}$ acid: 9-Octadecenoic acid
2	$C_{14:0}$ alcohol: 1-Tetradecanol	21	Δ^{9}-$C_{18:1}$ acid: 9-Octadecenoic acid
3	*i*-$C_{15:0}$ acid: 13-Methyltetradecanoic acid	22	$C_{18:0}$ acid: Octadecanoic acid
4	*a*-$C_{15:0}$ acid: 12-Methyltetradecanoic acid	23	$C_{20:3}$ acid: 11, 14, 17-Eicosadienoate
5		24	$C_{18:0}$ alcohol: 1-Octadecanol
6	$C_{15:0}$ acid: Pentadecanoic acid	25	Δ^{11}-$C_{20:1}$ acid: 11-Eicosenoic acid
7	$C_{15:0}$ alcohol: 1-Pentadecanol	26	$C_{20:0}$ alcohol: 1-Eicosanol
8	Δ^{11}-$C_{16:1}$ acid: 11-Hexadecenoic acid	27	$C_{20:3}$ acid: 11, 14, 17-Eicosadienoate
9	Δ^{9}-$C_{16:1}$ acid: 9-Hexadecenoic acid	28	$C_{23:0}$ alcohol: 1-Tricosanol
10	$C_{16:0}$ acid: Hexadecanoic acid	29	$C_{24:1}$ acid: Tetracosenoic acid
11	$C_{16:0}$ alcohol: 1-Hexadecanol	30	$C_{24:0}$ acid: Tetracosanoic acid
12	*i*-$C_{17:0}$ acid: 15-Methylhexadecanoic acid	31	Cholestane – internal standard
13	*a*-$C_{17:0}$ acid: 14-Methylhexadecanoic acid	32	Cholanic acid – internal standard
14	Δ^{10}-$C_{17:1}$ acid: 10-Heptadecenoic acid	33	$C_{26}\Delta^{5,22}$ sterol: 26-norcholesta-5,22-dien-3β-ol
15	Δ^{10}-$C_{17:1}$ acid: 10-Heptadecenoic acid	34	$C_{27}\Delta^{5,22}$ sterol: cholesta-5,22-dien-3β-ol
16	$C_{17:0}$ acid: Heptadecanoic acid	35	Cholesterol ($C_{27}\Delta^{5}$ sterol): cholest-5-en-3β-ol
17	$C_{18:3}$ acid (*n-3*): 9,12,15-Octadecatrienoic acid	36	Brassicasterol ($C_{28}\Delta^{5,22}$ sterol): 24-methylcholesta-5,22-dien-3β-ol
18	$C_{18:2}$ acid (*n-6*): 9, 12-Octadienoic acid	37	Stigmasterol ($C_{29}\Delta^{5,22}$ sterol): 24-ethylcholesta-5,22-dien-3β-ol
19	Δ^{9}-$C_{18:1}$ acid: 9-Octadecenoic acid	38	$C_{25:0}$ acid: Pentacosanoic acid

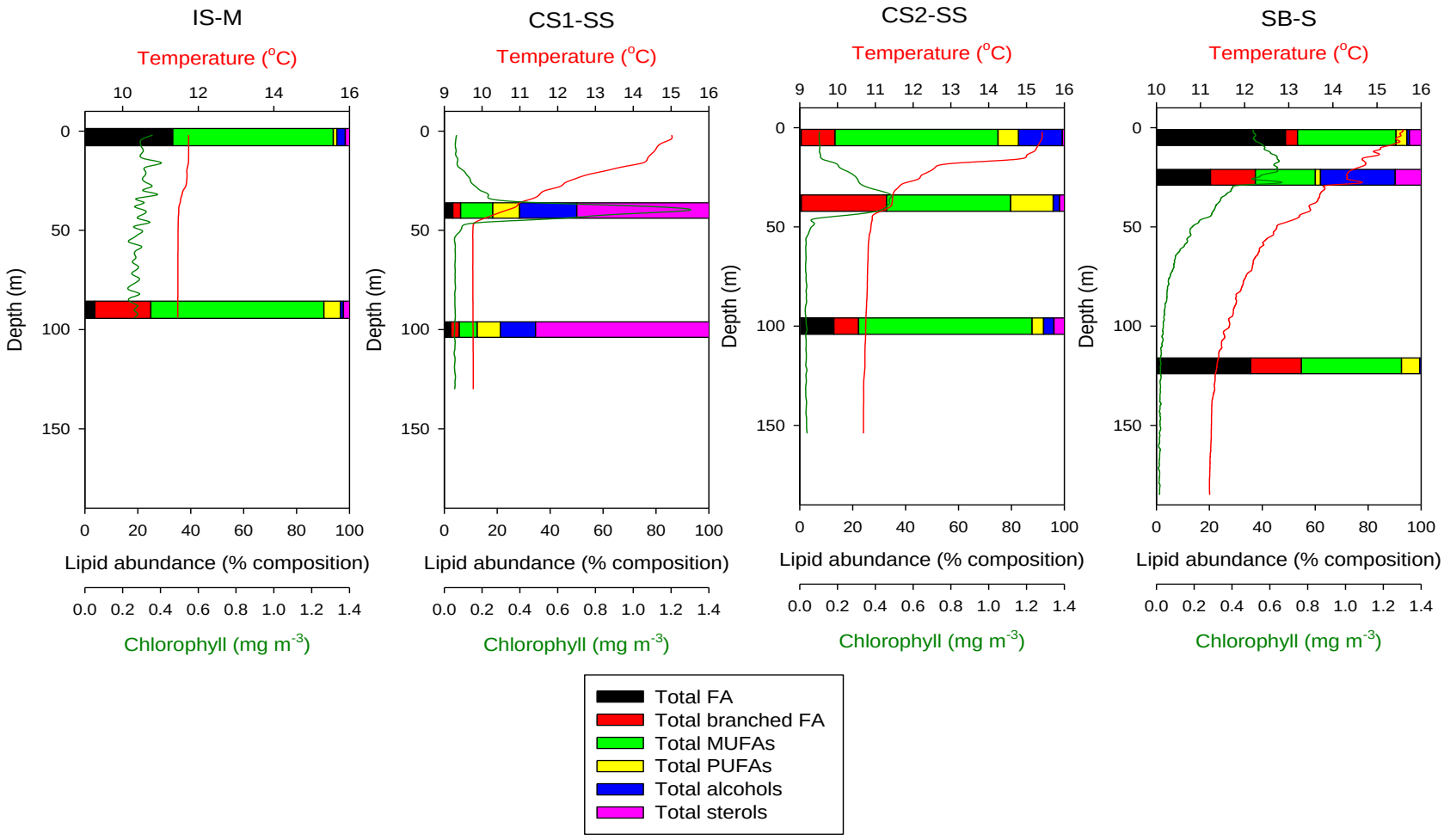


Figure 2.9 Percentage lipid abundance for each depth horizon sampled at the four survey sites. Temperature (°C) and chlorophyll (mg m^{-3}) profiles are shown for reference.

Furthermore, ratios of biomarkers have been used as indicators as supporting evidence for processes occurring within the water column at each of the sampling sites. Table 2.11 shows indicators of labile organic matter and invertebrate reworking for each sampling site and depth. As previously discussed (section 2.2.5.1), these ratios have been used in conjunction with other supporting biomarker data.

Table 2.11 Indicators of labile material and invertebrate reworking indicators at each sampling site and depth horizon sampled. Zooplankton biomarkers included $C_{20:1}$ FA and unsaturated alcohol and $C_{22:1}$ FA and unsaturated alcohol.

	IS-M		CS1-SS		CS2-SS			SB-S		
	S	B	SCM	B	S	SCM	B	S	SCM	B
Labile OM biomarker										
$C_{16:1}/C_{16:0}$	1.0	2.1	0.04	1.0	0	0	0.7	0.42	0.2	0.5
PUFAs (ng L^{-1})	0.15	291	1216	3173	2144	7716	336	9	118	235
Invertebrate reworking										
C_{27} /C_{29} sterol	0	9.4	0	0	0	0	16.6	0	0.9	0
Zooplankton biomarkers (ng L^{-1})	2	204	523	1898	4711	16761	345	64	2073	530

Mixed water column structure (IS-M). All groups of lipids analysed increased in absolute abundance with depth through the water column. This observation was also apparent from the relative abundance in the majority of the classification groups (FIGURE). In this region of mixed water column structure, indicators of labile material (PUFAs and HUFAs, REF) increased in relative abundance with depth. Furthermore, the ratio of $C_{16:1}/C_{16:0}$ also increased with water column depth (Table 2.11) further demonstrating the presence of fresh material within the lower part of the water column. As discussed in section 2.2.5.1, the $C_{16:1}/C_{16:0}$ ratio is an additional indicator of organic matter quality within the water column, the limitations of which have also been previously discussed (Najdek 1996; Jeffreys et al 2009). Bacterial biomarkers (branched fatty acids) were also observed to increase in relative abundance with depth as did the ratio of C_{27} /C_{29} sterol providing evidence of organic matter reworking by bacteria and invertebrates through the water column (Neto 2002; Table 2.11). The presence of zooplankton biomarkers (unsaturated alcohol $C_{22:1}$) in the POM support the observation of heterotrophic reworking at depth (Sargent and Henderson 1986; Kattner et al 2003). The increase in abundance of labile material and its heterotrophic reworking with depth may be influenced by the turbulent water conditions, which are

known to favour the transport of labile material through the water column (Neto 2002; Huisman et al 2004; Simpson and Sharples 2012).

Seasonally stratified water column structure (CS1-SS and CS2-SS). In a similar way to IS-M, absolute abundances for all functional groups increased between the SCM and bottom mixed layer samples. When relative percentage compositions were assessed for CS1-SS, SCM and bottom waters had similar compositions of phytoplankton biomarkers (C_{14}, C_{16}, C_{18}, $C_{16:1}$, $C_{18:1}$ saturated and mono-unsaturated fatty acids), bacterial biomarkers (branched fatty acids), and PUFAs. Labile POM ($C_{16:1}/C_{16:0}$ ratio and PUFA abundance) increased in relative abundance with depth (Table 2.11) while zooplankton biomarkers (unsaturated alcohols $C_{20:1}$, $C_{21:1}$ and $C_{22:1}$) were present in both SCM and bottom waters increasing with depth (Sargent and Henderson 1986; Najdek 1996; Kattner et al 2003; Jeffreys et al 2009).

At CS2-SS, the most lipids were relatively more abundant at the SCM compared to the surface and bottom mixed layers. At CS2-SS, phytoplankton biomarkers (C_{14}, C_{16}, C_{18}, $C_{16:1}$, $C_{18:1}$ saturated and mono-unsaturated fatty acids) increased in relative abundance with water depth. PUFAs were most abundant at the SCM and indicators of labile organic material ($C_{16:1}/C_{16:0}$; Table 2.11) were observed at depth. Bacterial biomarkers (branched fatty acids, Gillan and Johns 1986; Kaneda 1991) were also most abundant at the SCM. C_{27}/C_{29} sterol ratios showed evidence of invertebrate reworking of organic matter at depth while zooplankton biomarkers were in greatest abundance at the SCM (Neto 2002; Table 2.11).

Stratified shelf break (SB-S). At the shelf break, the majority of the lipid groups had highest absolute concentrations at the SCM compared to surface and bottom mixed layers. There was evidence of heterotrophic reworking of organic matter at the SCM, through the presence of zooplankton biomarkers (unsaturated alcohols $C_{20:1}$ and $C_{22:1}$) and elevated C_{27}/C_{29} sterol ratios (Neto 2002; Table 2.11). Labile material (PUFAs and increasing $C_{16:1}/C_{16:0}$ ratios with depth) and bacterial biomarkers also increased in relative abundance with depth. The increase of labile material at depth could be linked to lateral and down-slope advection processes drawing material towards the benthos. This has previously been observed in polar regions and in canyons where advective processes lead to elevated fluxes of labile material

towards the benthos, which are is rapidly utilised by near-bottom communities (Ritzrau and Thomsen 1997; Huvenne et al 2012).

2.3.4 Further clues to heterotrophic reworking

Biomarkers for heterotrophic activity (herbivorous mesozooplankton) have been used to assess reprocessing of POM in the water column in many regions of the deep sea and at continental margins (e.g. Sargent and Henderson 1986; Hedges et al 2001; Kiriakoulakis et al 2001; Kattner et al 2003; Kiriakoulakis et al 2004; Jeffreys et al 2009). In this study, organic matter heterotrophic re-working was evident at each of the study sites (Figure 2.9). The diagnostic compounds were $C_{20:1}$ and $C_{22:1}$ fatty acids and alcohols, which are known to be common energy reserves for zooplankton (Sargent and Henderson 1986; Kattner et al 2003; Kiriakoulakis et al 2001). The ratio of the C_{27} and C_{29} sterols (C_{27}/C_{29}) also provide evidence of invertebrate reworking of organic matter (Neto 2002). Additional evidence of reworking of organic matter within the water column comes from bacterial biomarkers (branched *iso*- and *anteiso*- fatty acids, particularly C_{15} and C_{17}; these are thought to derive from anaerobic bacteria and their presence in oxygenated waters implies that they derive from microbes associated with anoxic microenvironments (e.g. guts and particle interiors) (Gillan and Johns 1986). The presence of bacterial biomarkers indicates that they are likely to be involved in the remineralisation of POM as they are known to exist as particle-attached organisms (Parkes and Taylor 1983; Cho and Azam 1988; Kiriakoulakis et al 2001). The greatest amount of bacterial biomarkers was observed at the same depth horizons as the high heterotrophic zooplankton biomarker abundance. Previously it has been shown that bacteria play an important role in the particle decomposition of organic matter and this may be a response to the repackaging of POM by zooplankton excretory processes (Cho and Azam 1988).

2.3.5 Total carbon and nitrogen

Most POM samples from all sites and depth horizons in the Irish and Celtic seas had molar C/N ratios similar to the Redfield ratio of 6.6 (Redfield 1934; Redfield et al 1963). However, recently it has been shown that elemental ratios can vary from those originally proposed (Sambrotto and Langdon 1994; Körtzinger et al 2001). Surface waters of the ocean have POM with molar C/N values of 5-8, generally increasing with depth to 8-15, suggesting a more rapid utilisation of proteins than carbohydrates (Redfield 1958; Copin-Montegut and

Copin-Montegut 1985). Here, the molar ratios observed though the water column at each study site varied considerably. POC concentrations varied from 0.7 to 9.6 µM with a mean of 4.0 ± 2.5 µM and PN concentration varied from 0.1 to 1.6 µM with a mean of 0.7 ± 0.4 µM (Table 2.12). The POM molar C/N ratios ranged from 3.5 to 6.8 with a mean of 5.7 ± 0.9. CS1-SS and CS2-SS (stratified water column structures) had the highest C/N ratios, which decreased with depth. At IS-M, the surface water C/N ratio was the lowest of all the sites (3.5) perhaps indicating non-nitrate limiting conditions, and an abundance of labile material with a low C/N ratio. This is consistent with primary production at this site, which was the highest measured at 661 mg C m^{-2} d^{-1}, as compared to the stratified sites (mid-shelf and shelf break) 441 mg C m^{-2} d^{-1} and 446 mg C m^{-2} d^{-1}, respectively (Panton, 2012). Hence, a greater relative abundance of labile material deep in the water column at IS-M might reflect both enhanced primary production and the physical water column dynamics. In stratified regions (both seasonal and shelf break permanent) SCM and surface C/N ratios of POM were highest (Table 2.12) this implies nitrate limitation in surface waters and transfer of labile material to deeper waters.

Table 2.12 Particulate organic carbon (POC), particulate nitrogen (PN) concentrations and C/N ratios for each sampling location and depth horizon.

	IS-M			**CS1-SS**			**CS2-SS**			**SB-S**		
	NµM	CµM	C/N Ratio	NµM	CµM	C/N Ratio	NµM	CµM	C/N Ratio	NµM	CµM	C/N Ratio
Surface	0.2	0.8	3.5	-	-	-	0.9	5.9	6.8	0.1	0.7	5.0
SCM	-	-	-	0.5	3.3	6.7	1.6	9.6	6	0.6	3.6	5.9
Bottom	0.8	4.4	5.3	0.8	5.0	6.1	0.6	3.5	5.1	0.7	3.4	5.1

2.3.6 Natural abundance stable isotopes

The enrichment of nitrogen between trophic levels can help to elucidate food web structures; here evidence of POM being reworked with depth in the water column at each of the study sites was reflected in its isotopic composition (Hedges et al 2001; Canuel et al 1995; Post 2002; Vanderklift and Ponsard 2003). Samples from bottom waters were found to be much more enriched in ^{15}N than those form surface waters (Figure 2.10), suggesting regenerative loss processes through zooplankton grazing and microbial degradation of POM in deeper waters (mean suspended POM $\delta^{15}N$ of bottom waters, 10.48 ± 0.50‰; mean

suspended POM $\delta^{15}N$ of surface waters, 2.32 ± 0.41‰) (Ostrom et al 1997). CS2-SS bottom samples had the highest $\delta^{15}N$ value of 11.73 ± 0.50‰ and CS1-SS had the lowest of 8.99 ± 0.23‰. IS-M and SB-S surface water POM had the lowest values of 1.81 ± 0.44‰ and 1.72 ± 0.73‰, respectively. It is unclear as to why there was such a difference between surface and bottom values at IS-M, since this site was fully mixed but at SB-S the low nitrogen value may reflect nitrate limitation, recycling and utilisation of ammonium sources. However, this could in part be due to patchiness within the pelagic community at these dynamic sites particularly as the carbon values were similar. At other sites, $\delta^{15}N$ values of suspended POM collected in the SCM (mean 4.54‰ ± 0.45) were not very different to those of surface waters. This was particularly noticeable at CS2-SS, suggesting that reworking processes are more uniform in the top 40-50 m of the water column. The results of a one-way ANOVA showed that there was no statistical difference between surface, SCM and bottom $\delta^{15}N$ values at each of the sampling sites ($P = 0.98$).

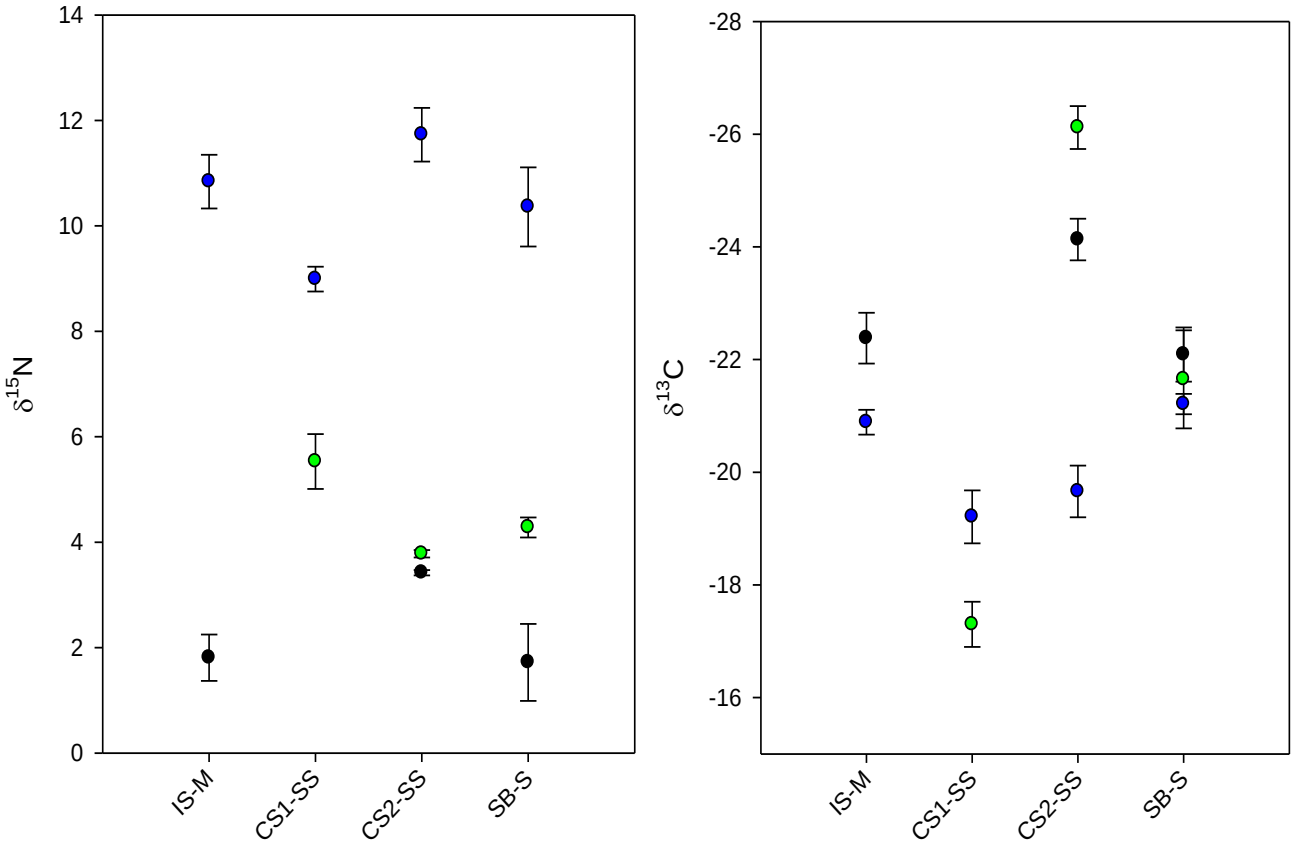


Figure 2.10 Natural stable nitrogen and carbon isotopes from SAPs filters deployed to different horizons of the water column. Blue = bottom, Green = SCM and black = Surface.

$\delta^{13}C$ isotope values can be used to elucidate dominant sources of organic matter in the marine environment (Hedges et al 2001; Canuel et al 1995; Post 2002; Vanderklift and Ponsard 2003). IS-M and SB-S had similar distributions of $\delta^{13}C$ through the water column, showing little enrichment with depth. At IS-M turbulence and mixing within the water column may lead to similar $\delta^{13}C$ through the water column. However, at SB-S the physical structure of the water column is quite different but due to the porosity of the data currently this remains elusive. At seasonally stratified sites, $\delta^{13}C$ values through the water column varied greatly. At CS1-SS the SCM $\delta^{13}C$ value was more enriched than the bottom water sample (-17.3 ± 0.4‰ and -19.21 ± 0.21‰ respectively). At CS2-SS bottom water $\delta^{13}C$ was the most enriched and SCM $\delta^{13}C$ sample was the most depleted (-19.66 ± 0.46‰ and -26.12 ± 0.12‰ respectively). The results of a one-way ANOVA, however, showed that surface, SCM and bottom $\delta^{13}C$ values were not statistically different (P = 0.16).

2.3.7 Multivariate analyses

PERMANOVA tests were conducted for each data set (lipids, pigments, $\delta^{15}N$ and $\delta^{13}C$ and all combined parameters) and significant relationships were apparent in three of them (Table 2.13). Lipid composition was found to vary significantly between site, but not with depth or water column structure (P = 0.006). $\delta^{15}N$ and $\delta^{13}C$ isotope data were found to vary significantly with “water column depth”, but not with “water column structure” or “site” (P = 0.0008). When all parameters were combined and analysed together (lipids/pigments/ $\delta^{15}N$/ $\delta^{13}C$) significant variation between sites, but not water column structure or depth were found (P = 0.0052).

Table 2.13 Main test PERMANOVA output for lipid, pigment and isotope data for each factor that was tested. Statistical significance is shown as a P value for each test (significant P values are highlighted in grey).

Main test	Factor and Statistical significance (P value)
Lipids	Water column structure ; 0.07
	Site; 0.006
	Depth ; 0.7
Pigments	Water column structure; 0.18
	Site; 0.1
	Depth; 0.2
$\delta^{15}N$ and $\delta^{13}C$	Water column structure; 0.9
	Site; 0.8
	Depth; 0.0008
Lipids/pigments/ $\delta^{15}N$/ $\delta^{13}C$	Water column structure; 0.07
	Site; 0.0052
	Depth; 0.7

Significant main test data and factors were further tested by pair-wise analysis. Lipid data for the factor "site" were found not to be significant when tested pair-wise. However, significant differences were observed between the seasonally stratified and permanently stratified sites (Table 2.14).

Table 2.14 Pair-wise PERMANOVA output from lipid data showing the relationships of compounds grouped by water column structure (M = mixed, SS = seasonally stratified and S = stratified), statistical significance is shown as a P value for each comparison (significant P values are highlighted in grey).

Pair wise comparison	Factor: Water column structure Statistical significance (P value)
M, SS	0.19
M, S	0.69
SS, S	0.05

Using MDS to visualise this significant result, some clear separation water column types was apparent (Figure 2.11).

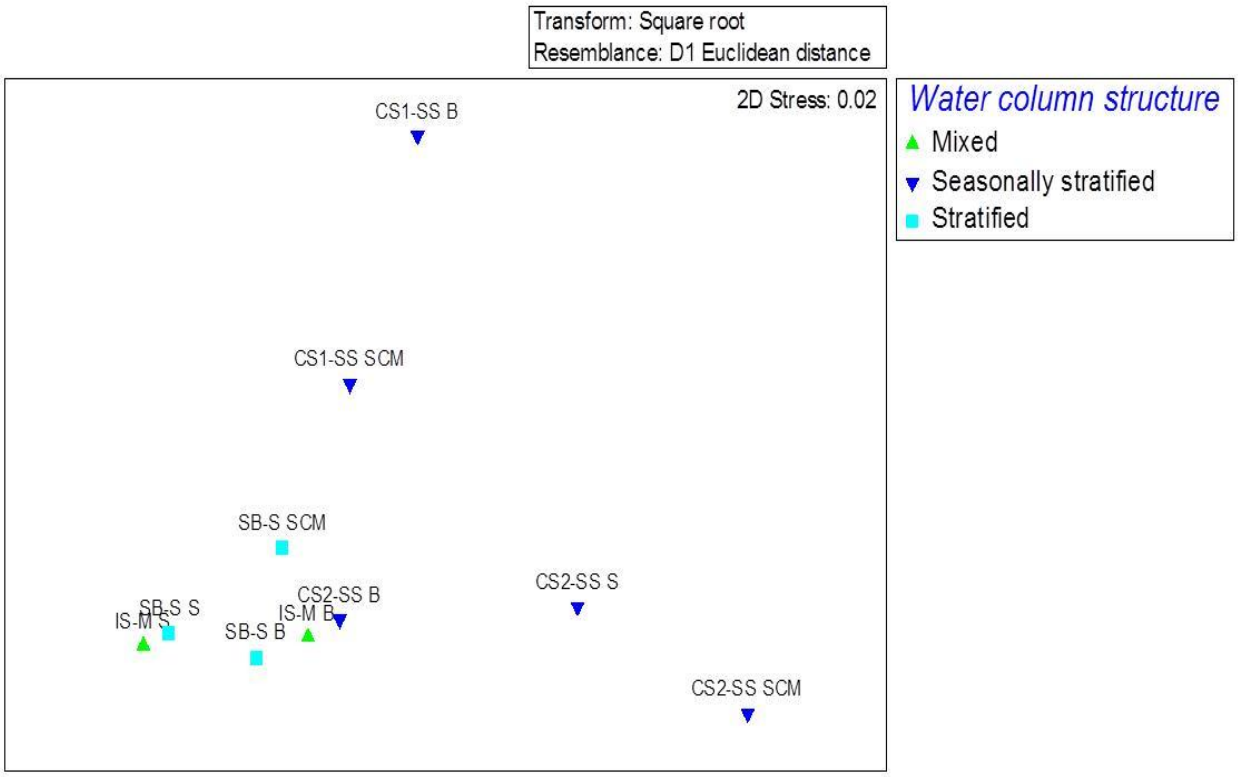


Figure 2.11 MDS plot visualises lipid data, when analysed by water column structure. Mixed water column incorporates IS-M, seasonally stratified incorporates CS1-SS and CS2-SS and stratified incorporates SB-S.

Pair wise tests of nitrogen and carbon isotope data for surface and bottom water samples (P = 0.02) and bottom and SCM water samples (P = 0.02) were significantly different according to the factor “depth” (Table 2.15).

Table 2.15 Pair-wise PERMANOVA output from lipid, pigment and isotope data combined showing the relationships of compounds grouped by depth at which samples was taken (S = surface, SCM = subsurface chlorophyll max, B = bottom) statistical significance is shown as a P value for each comparison (significant P values are highlighted in grey).

Pair wise comparison	**Factor: Depth Statistical significance (P value)**
S, B	0.02
S, SCM	0.09
B, SCM	0.02

Using MDS to visualise this significant result, clear separation of surface and SCM samples away from bottom samples was apparent (Figure 2.12).

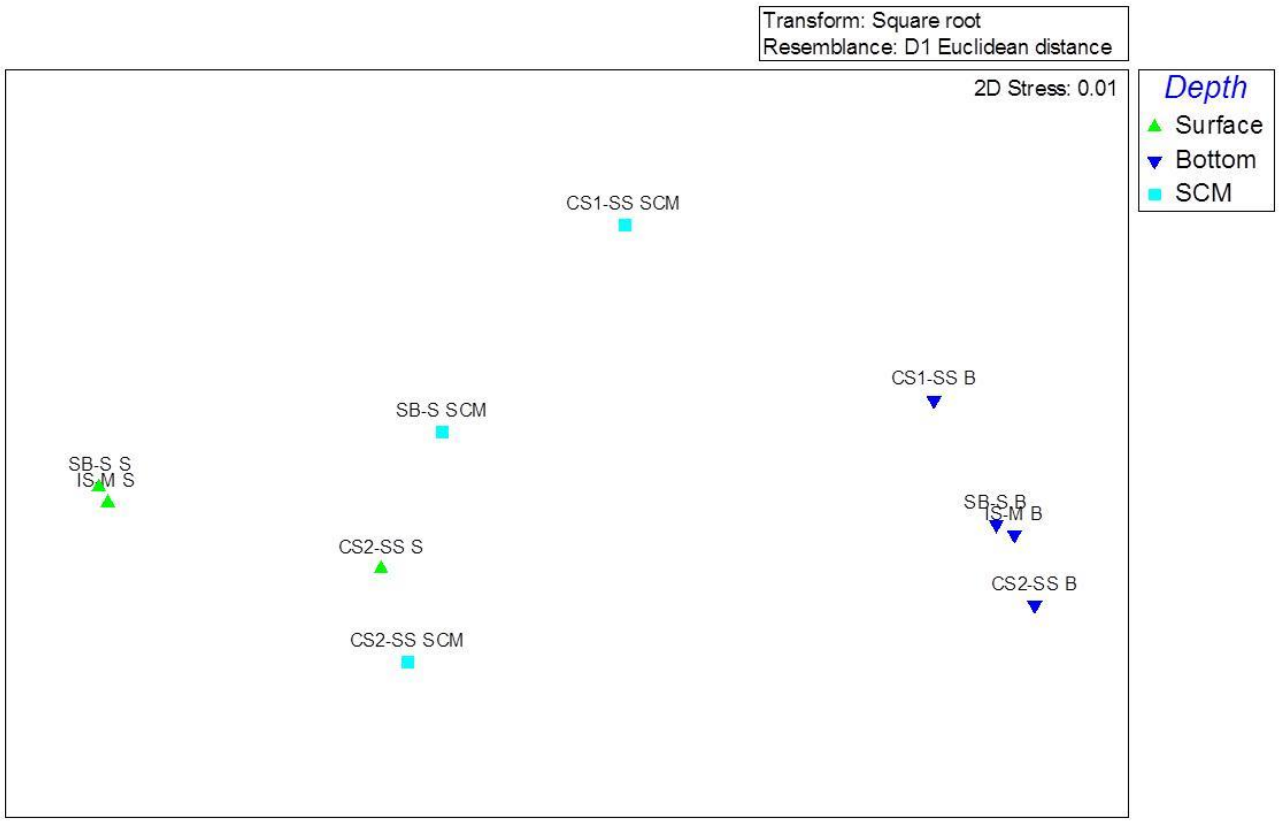


Figure 2.12 MDS plot visualises the nitrogen and carbon isotope data when analysed by depth of sampling. Samples were taken from surface waters, SCM and bottom waters.

All parameters (lipids, pigments and isotopes) for the “site” factor showed significance under the main test conditions (P = 0.0052), however, the pair-wise test for this factor showed no significant differences.

2.4 Synthesis

In this chapter, the distinct structures of the water column across the NW European Continental Shelf have been shown to influence the composition of suspended POM. Different physical conditions appear to be critical in the processing of POM through the water column. Examination of biogeochemical processes using biomarkers and stable isotopes has been used to link the biology with physical processes across the continental shelf.

The distribution of organic matter across the continental shelf is controlled by the physical dynamics and biogeochemical processes (Figure 2.13). In conjunction with the schematic representation of processes (Figure 2.13) occurring across the North West European Continental Shelf the original hypotheses can be re-addressed:

1) **Variation between physically distinct regions of the shelf sea lead to differences in the composition and lability of suspended POM. These differences are reflected in lipid, pigment, isotope and phytoplankton distributions within the water column.**

This hypothesis is confirmed as the composition of POM across the shelf does differ according to the distinct physical water column characteristics. The composition of POM not only varies across the shelf, but also vertically through the water column. These variations are apparent in biomarkers (lipid and pigment) and isotopic and phytoplankton distributions.

Schematic representation of the data (Figure 2.13) shows that in the mixed region where tidal forcing and wind stress caused homogenisation was reflected in the biomarkers. Pigment biomarkers had a uniform distribution throughout the water column, both in terms of composition and a slight increase of abundance was observed with water column depth. Lipid biomarkers showed there to be an increase of labile material with depth, potentially due to turbulent water column conditions leading to aggregation and favouring the sinking of POM down though the water column. In this region of turbulence and tidal influence, resuspension of recently deposited POM from sediments may add to the labile component at depth.

In seasonally stratified regions (Figure 2.13) bacterial and labile POM biomarkers were most abundant at the SCM. It appears that the optimal physical conditions that result in seasonal stratification maintain the concentration of labile material at the SCM. This was supported but pigment abundance data which showed greatest amounts at the SCM (Table 2.7).

At the edge of the continental shelf (the shelf break) the pulses of nutrients and internal mixing create a different dynamic within the water column (Figure 2.13). Rates of primary production are higher than in the mid shelf region, however biomarkers indicate that labile POM increases in abundance with depth, again perhaps due to enhanced aggregation occurring through increased turbulence and advection leading to the sinking of labile material.

2) **Compositional variation in suspended POM is influenced by the distribution of zooplankton within the water column, which is reflected in the biomarker composition.**

Lipid distributions provide qualitative evidence of heterotrophic communities reworking POM at each of the sampling locations. In the turbulent mixed water at IS-M, heterotrophic reworking was observed at depth. This suggested close pelagic-benthic coupling and enhanced sinking of labile material supporting reworking activity.

At the seasonally stratified sites, POM reworking at the phytoplankton-rich SCM was apparent. Pigment biomarkers provided evidence of coccolith-containing POM in the deeper samples. The presence of these biomarkers at depth, suggest that they could derive from organic matter reworking by heterotrophic zooplankton and the sinking of their faecal pellets though the stable stratified water column.

In the upper water column at the shelf break, lipid biomarkers showed strong indication of OM reworking. At depth, pigment biomarkers again identified the presence of coccolith-containing POM. It is possible that advective onshore processes influence the sinking of larger organic matter aggregates hence these biomarkers are observed at depth.

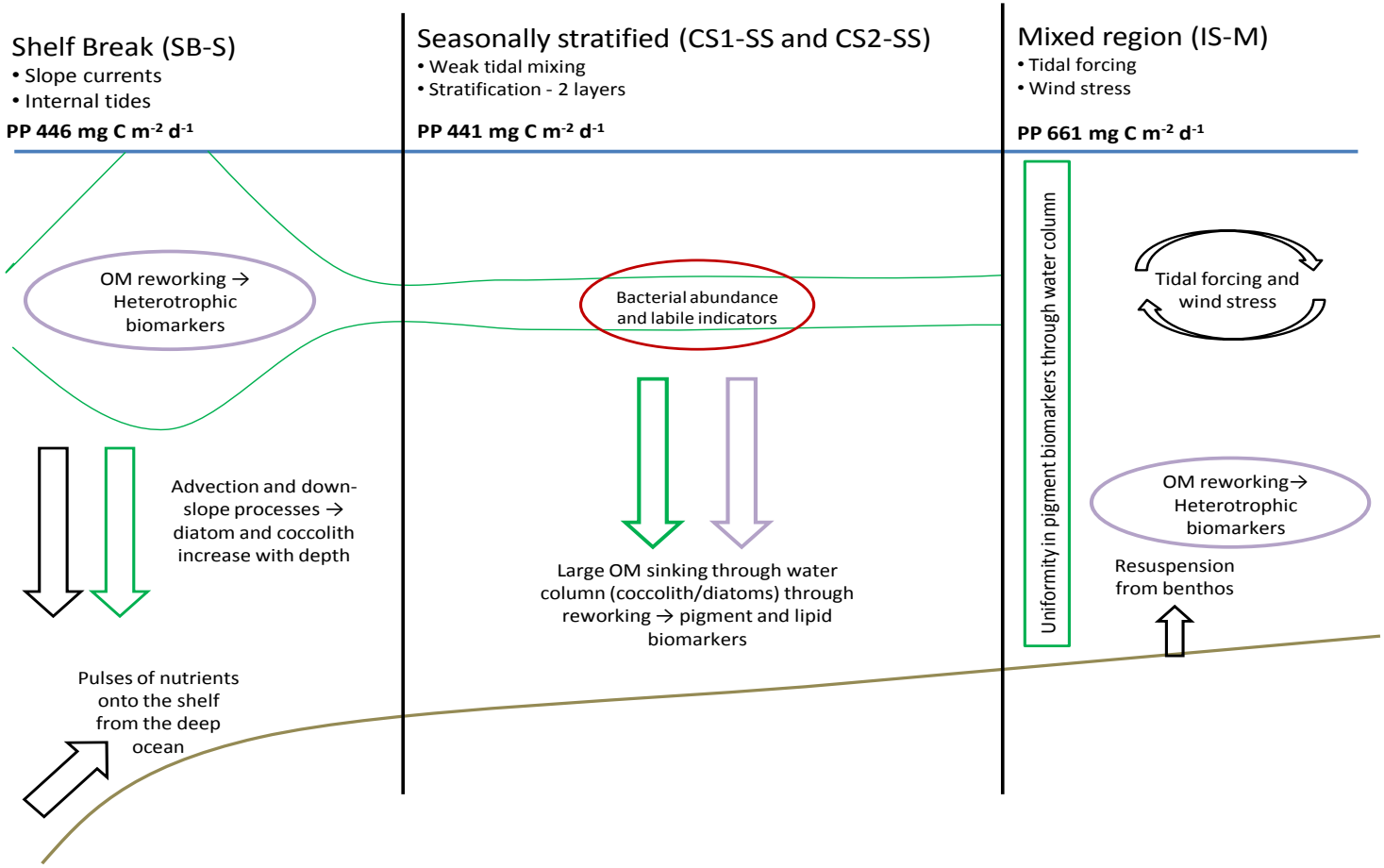


Figure 2.13 Schematic diagram of key physical, biogeochemical and biological processes across the shelf (mixed, seasonally stratified and shelf break regions). Black arrows depict physical dynamics within the water column structure. Purple and green arrows represent the movement of organic matter through the water column elucidated through biomarkers. Purple and red circles represent pools of organic matter and the processes occurring within them from biomarkers.

Biogeochemical techniques enhance the understanding of processes influencing the composition of suspended POM across a shelf sea. The physical structure of the water column is controlled by mixing, tides, temperature and light conditions and ultimately controls nutrient uptake, growth and reproduction. This study has gone some way to exploring adaptations within communities in order to utilise resources most efficiently. These processes are critical in fuelling food webs within marine ecosystems and hence impact the export of carbon and the support of shelf-sea fisheries (Pauly et al 2002; Iken et al 2010). Examination of POM across a temperate continental shelf system has rarely been attempted and best efforts have been made to examine suspended POM across regions with different physical dynamics.

Sampling was conducted at fairly coarse resolution, both in terms of sampling stations and water column horizons. In order to resolve the shelf dynamics more clearly a greater level of replication and finer scale sampling would be required over a seasonal time frame. Sampling at more depth horizons would help resolve POM dynamics. However, there are practical limitations in using the *in situ* sampling methods employed in this study, due to the shallow water depth.

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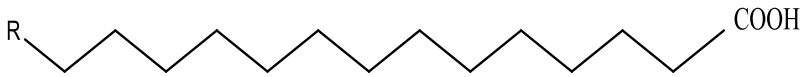
2.6 Appendix

Nomenclature of identified lipids

Fatty acids

$C_{14:0}$ acid: Tetradecanoic acid (R = H)



$C_{15:0}$ acid: Pentadecanoic acid (R = CH_3)

$C_{16:0}$ acid: Hexadecanoic acid (R = C_2H_5)

$C_{17:0}$ acid: Heptadecanoic acid (R = C_3H_7)

$C_{18:0}$ acid: Octadecanoic acid (R = C_4H_9)

$C_{20:0}$ acid: Eicosanoic acid (R = C_6H_{13})

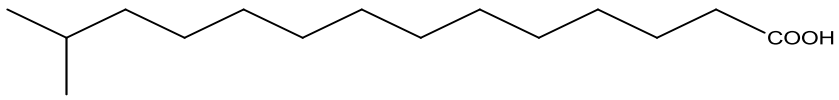
$C_{21:0}$ acid: Heneicosanoic acid (R = C_7H_{15})

$C_{22:0}$ acid: Docosanoic acid (R = C_8H_{17})

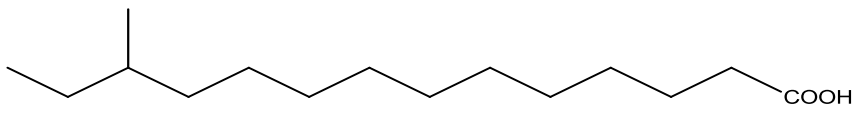
$C_{24:0}$ acid: Tetracosanoic acid (R = $C_{10}H_{21}$)

Branched fatty acids

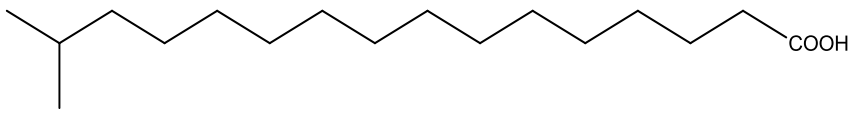
i-$C_{15:0}$ acid: 13-Methyltetradecanoic acid



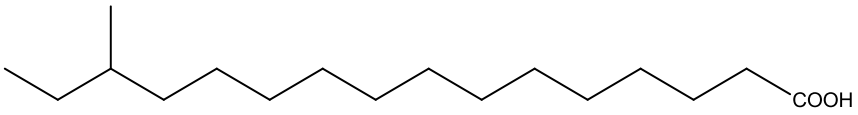
a-$C_{15:0}$ acid: 12-Methyltetradecanoic acid



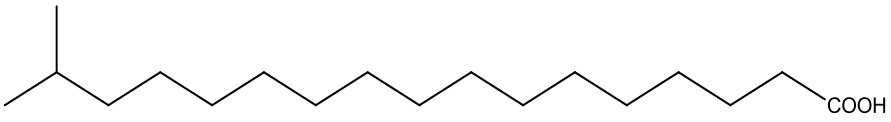
i-$C_{17:0}$ acid: 15-Methylhexadecanoic acid



a-$C_{17:0}$ acid: 14-Methylhexadecanoic acid

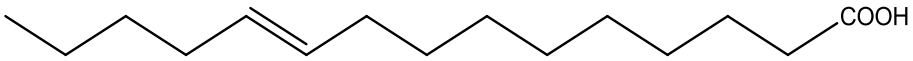


i-$C_{18:0}$ acid: 16-Methylheptadecanoic acid

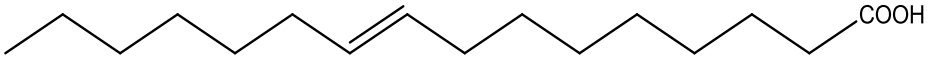


Mono-unsaturated fatty acids

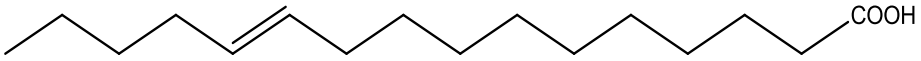
Δ^{10}-$C_{15:1}$ acid: 10-Pentadecenoic acid



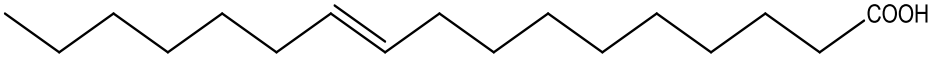
Δ^{9}-$C_{16:1}$ acid: 9-Hexadecenoic acid



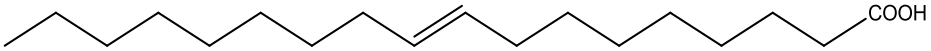
Δ^{11}-$C_{16:1}$ acid: 11-Hexadecenoic acid



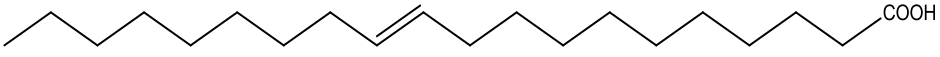
Δ^{10}-$C_{17:1}$ acid: 10-Heptadecenoic acid



Δ^{9}-$C_{18:1}$ acid: 9-Octadecenoic acid



Δ^{11}-$C_{20:1}$ acid: 11-Eicosenoic acid

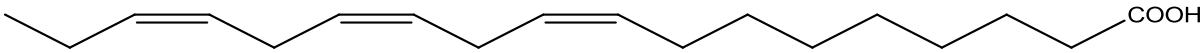


$C_{22:1}$ acid: Docosenoic acid (double bond position unknown)

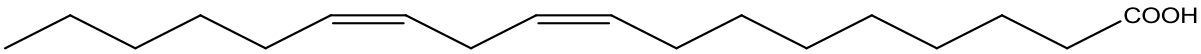
$C_{24:1}$ acid: Tetracosenoic acid (double bond position unknown)

Poly unsaturated fatty acids

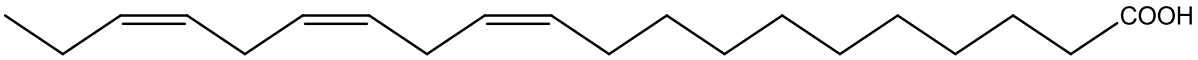
$C_{18:3}$ acid (*n-3*): 9,12,15-Octadecatrienoic acid



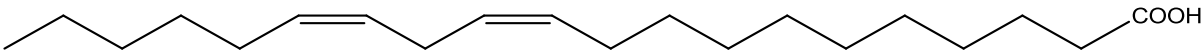
$C_{18:2}$ acid (*n-6*): 9, 12-Octadienoic acid



$C_{20:3}$ acid: 11, 14, 17-Eicosadienoate



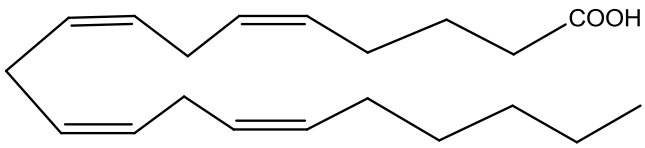
$C_{20:2}$ acid: 11, 14-Eicosadienoate



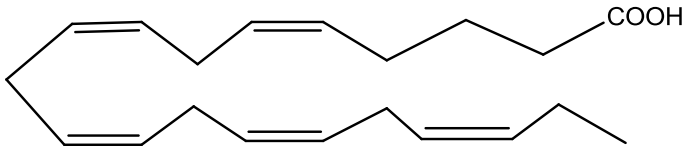
$C_{22:2}$ acid: Docosanoic acid (double bond position unknown)

Highly unsaturated fatty acids

$C_{20:4}$ (*n-6*) acid: 5, 8, 11, 14-Eicosatetraenoic acid

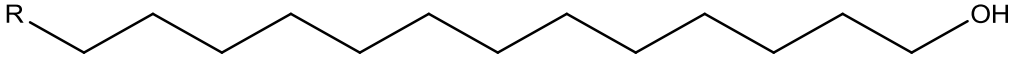


$C_{20:5}$ (*n-3*) acid: 5, 8, 11, 14-Eicosapentaenoic acid



Alcohols

$C_{14:0}$ alcohol: 1-Tetradecanol (R = H)



$C_{15:0}$ alcohol: 1-Pentadecanol (R = CH_3)

$C_{16:0}$ alcohol: 1-Hexadecanol (R = C_2H_5)

$C_{17:0}$ alcohol: 1-Heptadecanol (R = C_3H_7)

$C_{18:0}$ alcohol: 1-Octadecanol (R = C_4H_9)

$C_{20:0}$ alcohol: 1-Eicosanol (R = C_6H_{13})

$C_{21:0}$ alcohol: 1-Heneicosanol (R = C_7H_{15})

$C_{23:0}$ alcohol: 1-Tricosanol (R = C_9H_{19})

Unsaturated alcohols

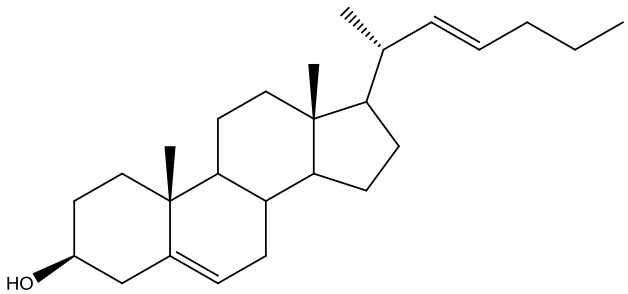
$C_{18:1}$ (double bond position unknown)

$C_{20:1}$(double bond position unknown)

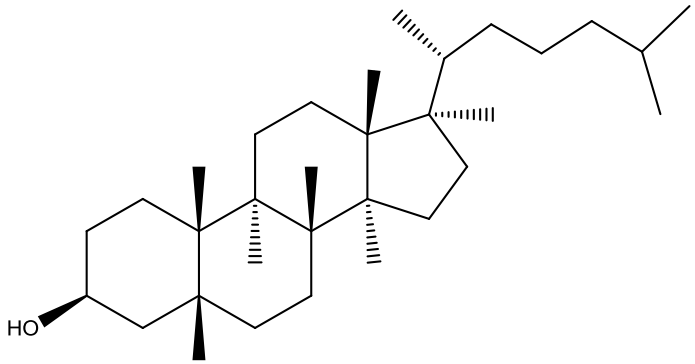
$C_{22:1}$(double bond position unknown)

Sterols

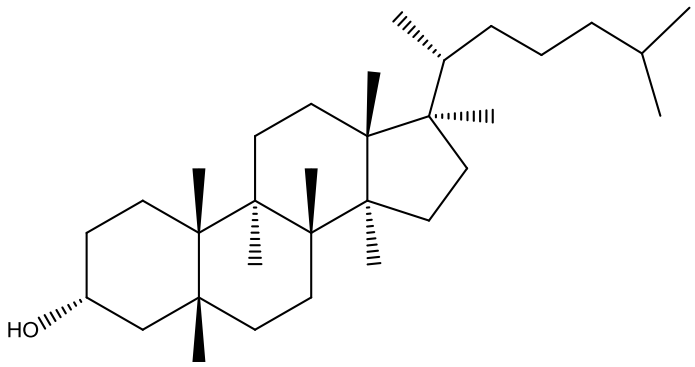
$C_{26}\Delta^{5,22}$ sterol: 26-norcholesta-5,22-dien-3β-ol



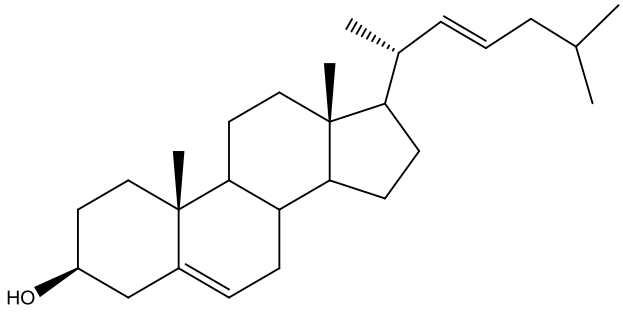
Coprostanol: 5β-cholestan-3β-ol



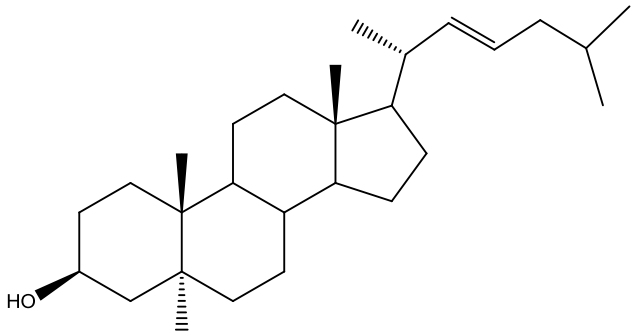
Epicoprostanol: 5β-cholestan-3α-ol



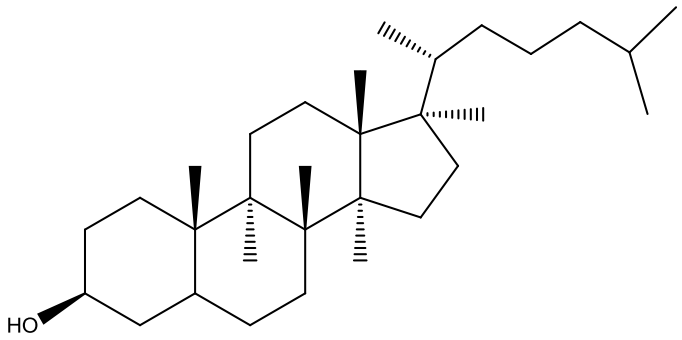
$C_{27}\Delta^{5,22}$ sterol: cholesta-5,22-dien-3β-ol



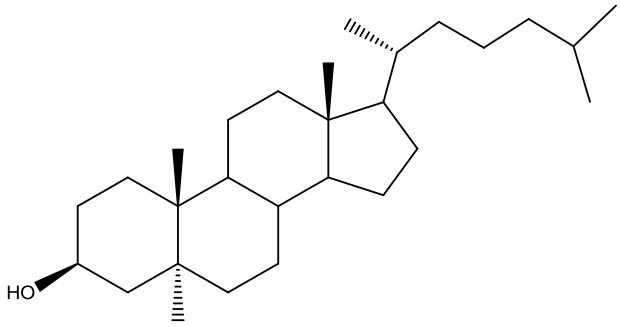
$C_{27}\Delta^{22}$ sterol: cholest-22-en-3β-ol



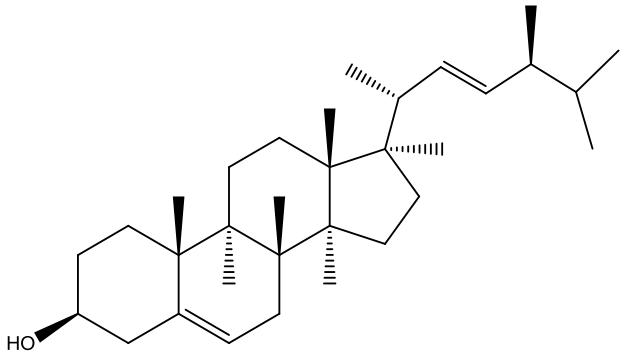
Cholesterol ($C_{27}\Delta^{5}$ sterol): cholest-5-en-3β-ol



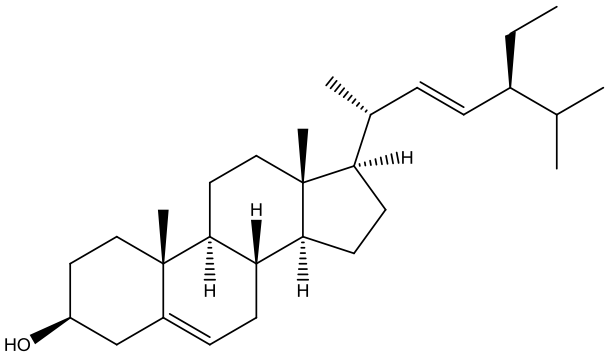
Cholestanol ($C_{27}\Delta^{0}$ sterol): (5αH)-cholestan-3β-ol



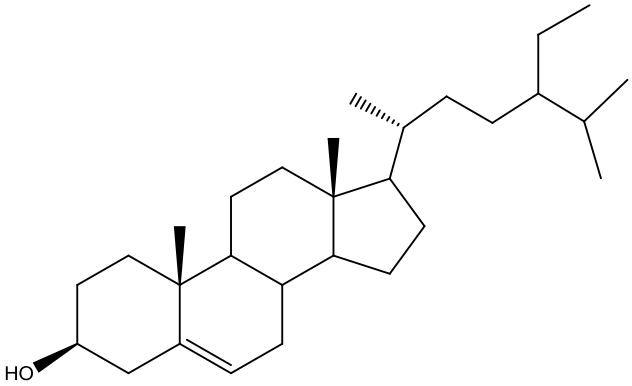
Brassicasterol ($C_{28}\Delta^{5,22}$ sterol): 24-methylcholesta-5,22-dien-3β-ol



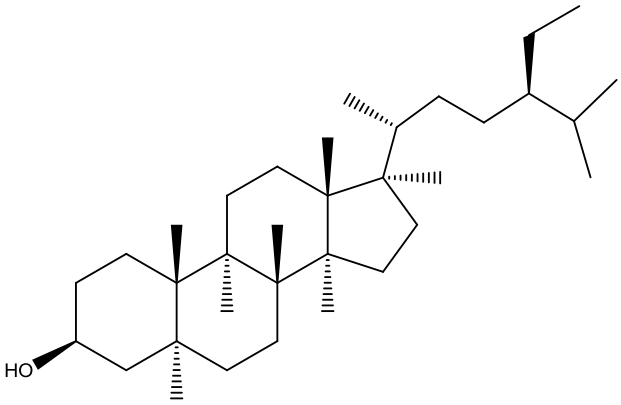
Stigmasterol ($C_{29}\Delta^{5,22}$ sterol): 24-ethylcholesta-5,22-dien-3β-ol



$C_{29}\Delta^5$ sterol: 24-ethylcholesta-5-en-3β-ol



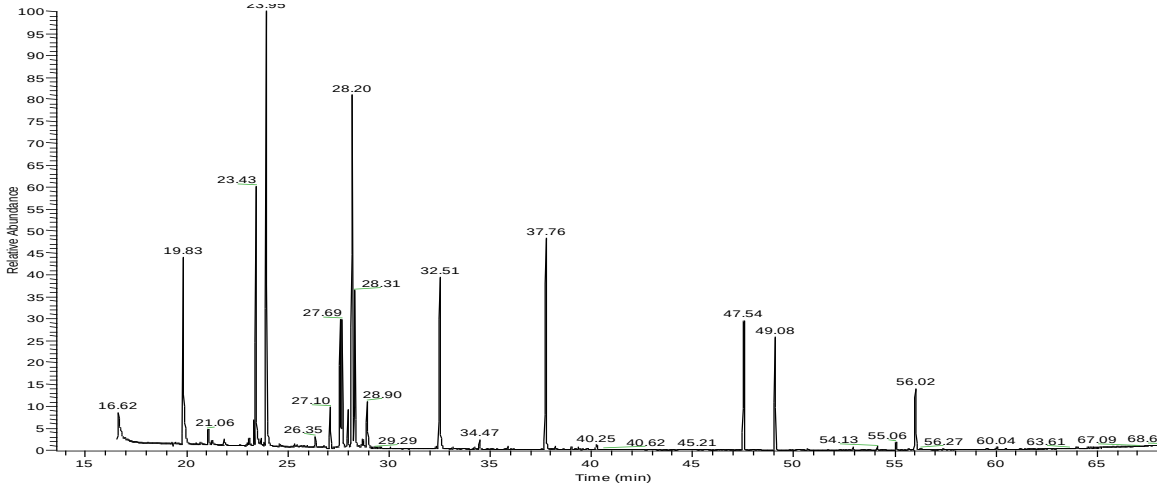
Stigmastanol: 5α(H)-24-ethylcholestan-3β-ol



2.7 Appendix

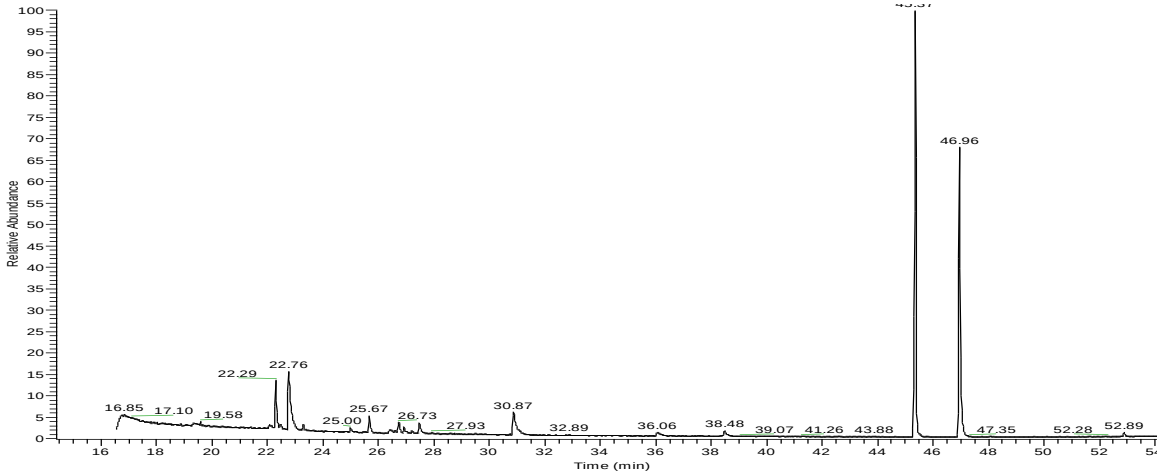
LB-M

Bottom sample

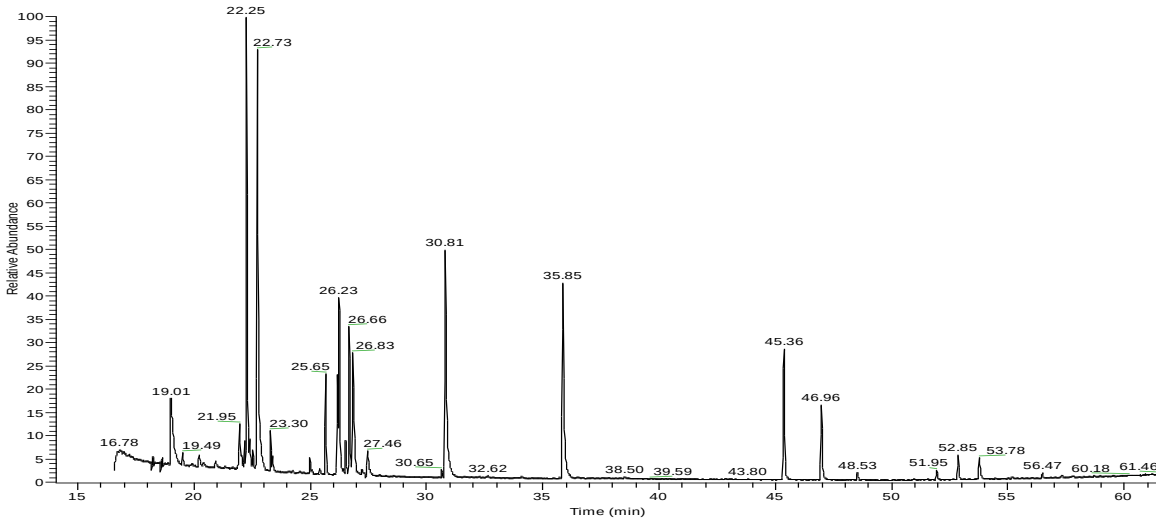


IS-M

Surface sample

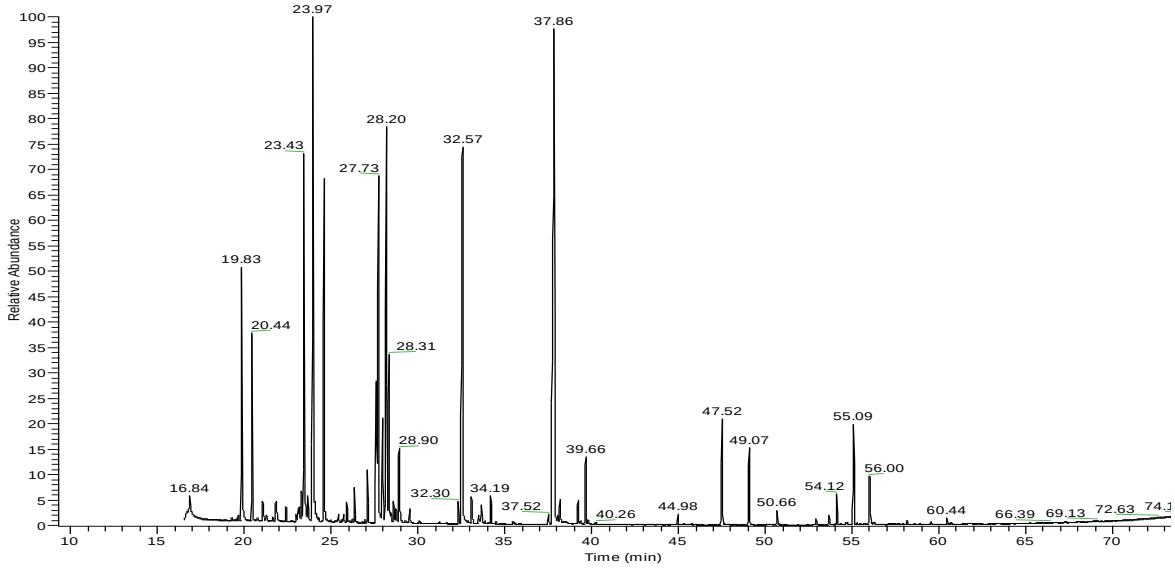


Bottom sample

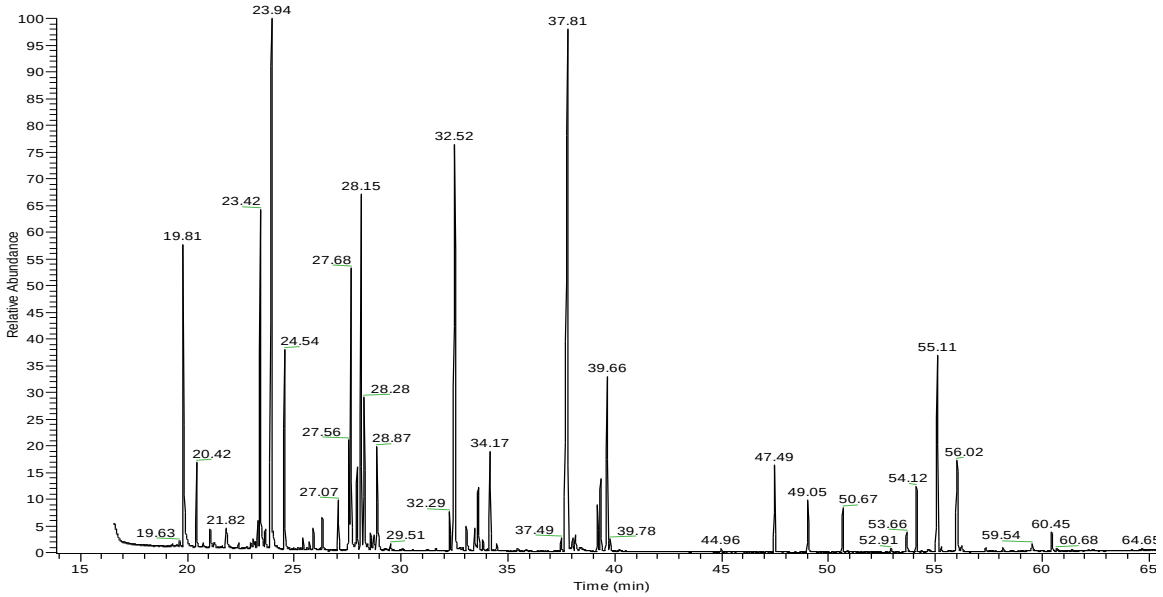


<u>CS1-SS</u>

Surface sample (full annotated chromatograms earlier in this chapter)

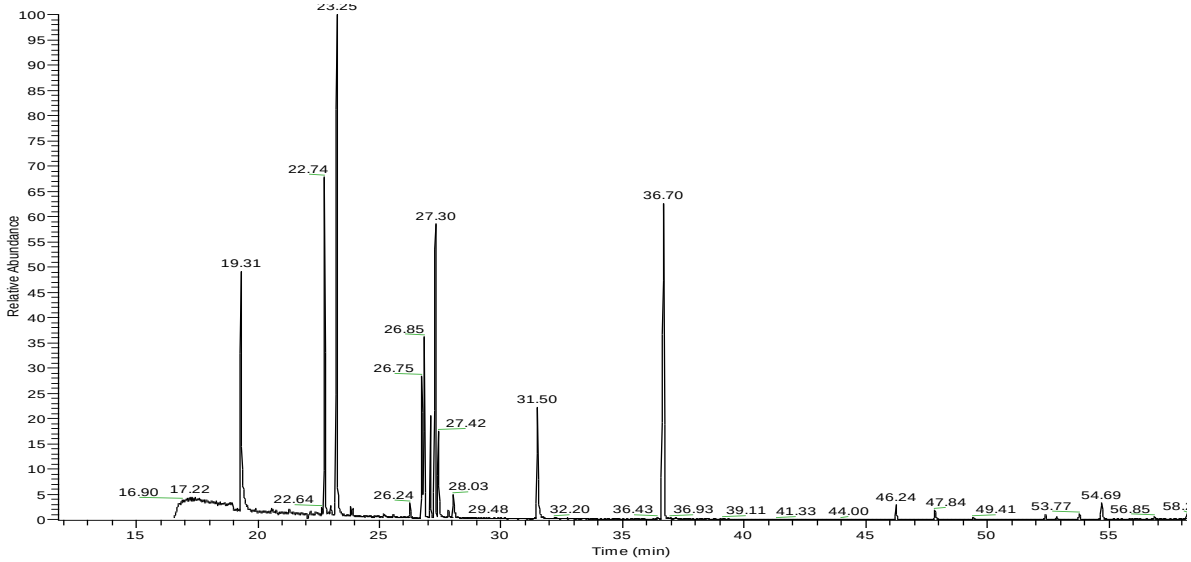


Bottom sample

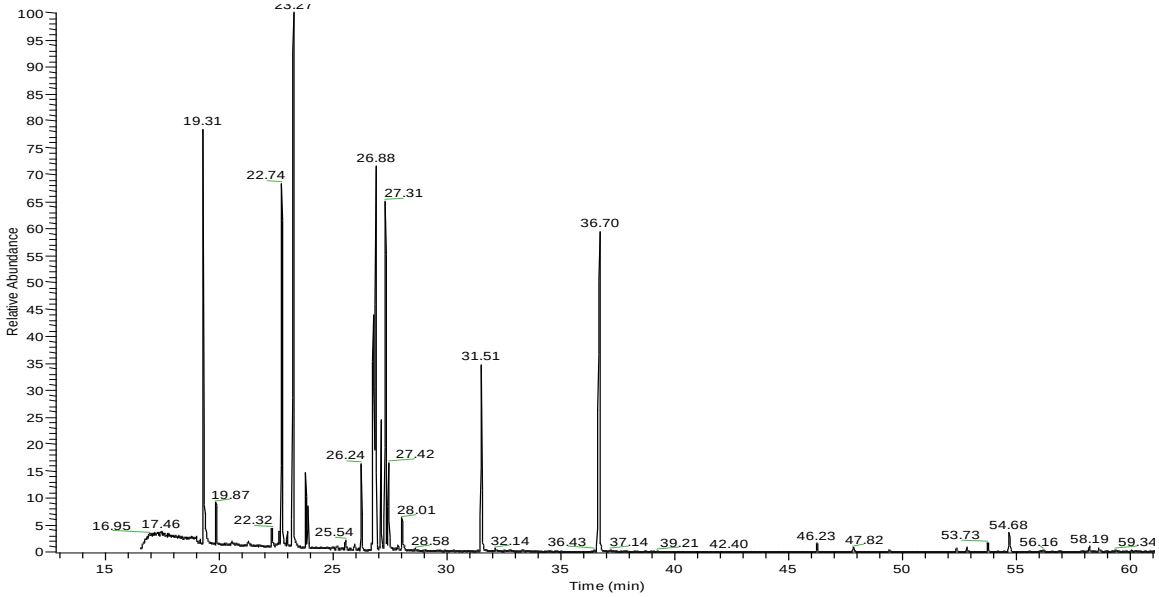


CS2-SS

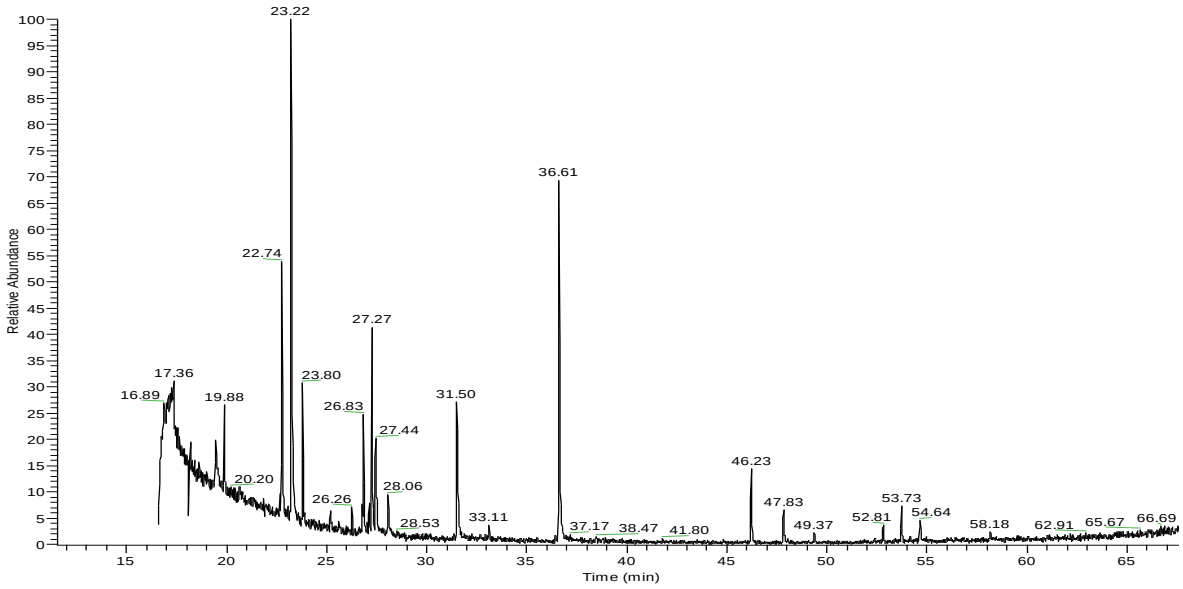
Surface sample



SCM sample

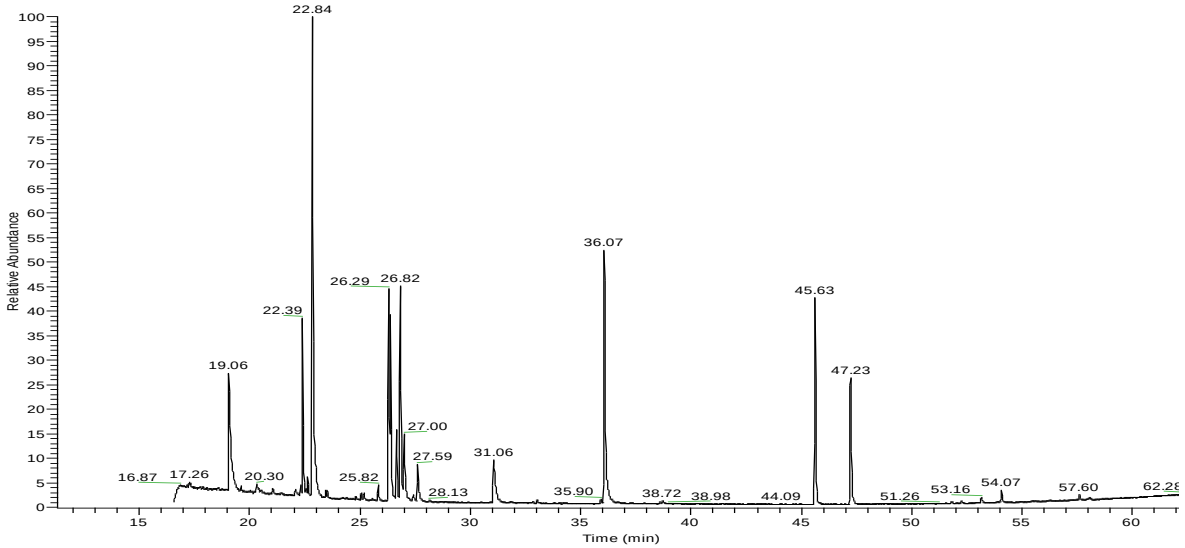


Bottom sample

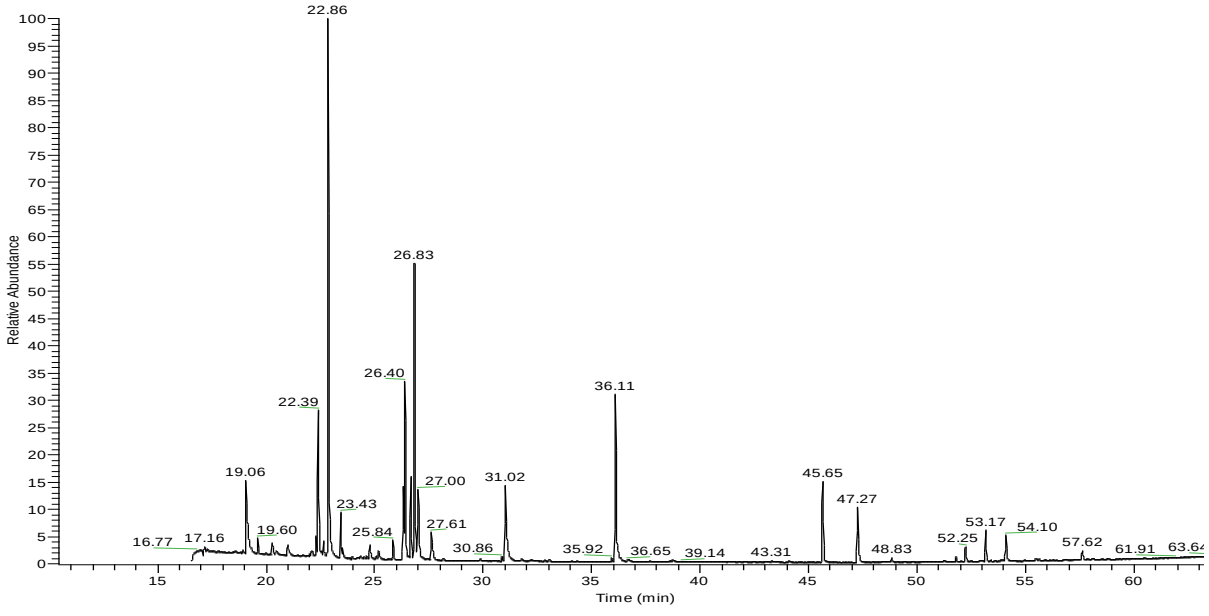


SB-S

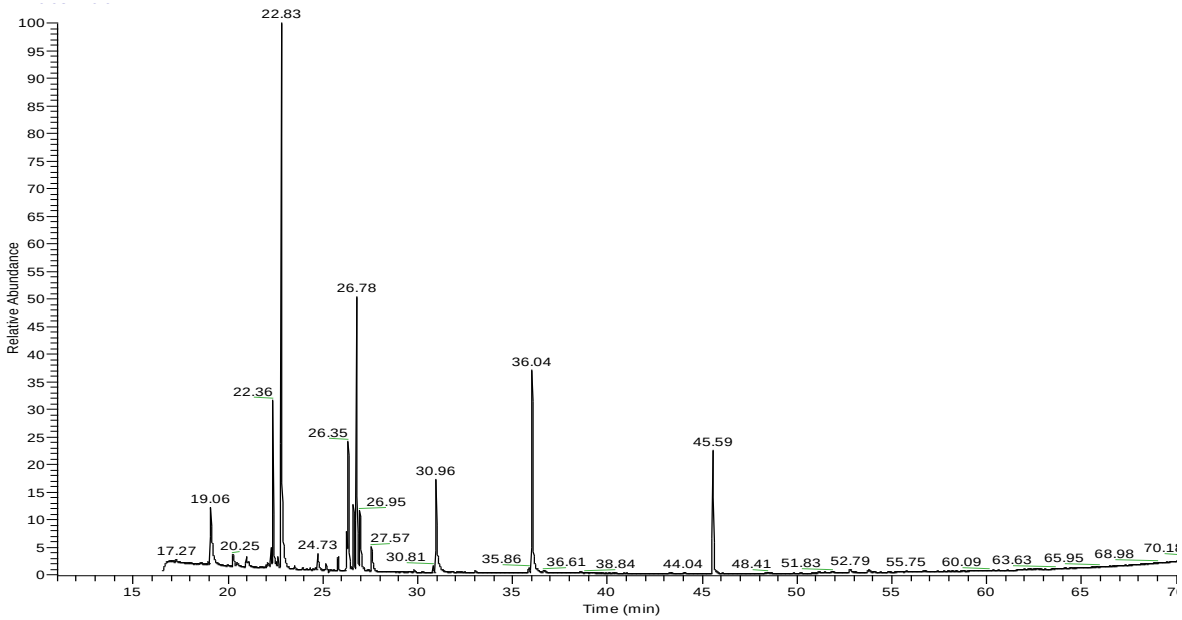
Surface sample



SCM sample



Bottom sample



Chapter 3

Pelagic and benthic community structure analysis across the NW European Continental Shelf – using isotopic techniques to investigate trophic relationships

3.1 Introduction

3.1.1 Background

Continental shelf seas are small in size, accounting for less than 10% surface area of the oceans. However, they are responsible for up to 30% of air-to-sea carbon flux through primary production. Additionally, it has been estimated that up to 40% of particulate carbon sequestration also occurs in these regions (Muller-Kager et al 2005; Chen et al 2009). The enhanced biological production in these regions supports near coast communities by providing up to 90% of global fisheries production (Pauly et al 2002). Carbon fixation, through primary production, supports shelf seas as a key interface in the global carbon cycle as well as acting as an export pathway for terrestrially derived material. In the study region examined here, the North West European Continental Shelf, different physical water column features are observed due to the interaction of tides, mixing events, warming of the water column in the summer months leading to seasonal stratification, and the topographic features of the shelf break.

3.1.2 Shelf sea physics

As previously described in Chapter 2 shelf seas are dynamic regions of the worlds' oceans and due to their shallow depth (0-200m) are strongly influenced by frictional boundary layers at the surface and bottom of the water column (Simpson and Sharples 2012). Tidal forces, wind stress, solar heating and freshwater inputs can cause density gradients within the water column, creating regions with varying water column structure (Simpson and Hunter 1974; Sharples et al 2007; Simpson and Sharples 2012).

The specific dynamics of shelf sea physics are described in Chapter 2 (section 2.1.2) alongside diagrams showing temperature and chlorophyll profiles across the shelf.

3.1.3 Shelf sea biology

Shelf seas are known to be ecologically rich environments and it has been estimated that primary production in the world's shelf seas is about 11 Gt C a^{-1} (Jahnke 2010) compared to a global estimate of 45-60 Gt C a^{-1} (Longhurst et al 1995; Behrenfeld et al 2005). The consequences of the enhanced production and community structure change at the shelf

break can be seen in fish abundance and fishing activity in these shelf break areas. Enhanced phytoplankton growth supports higher trophic levels and this is greatly important to fisheries (Fernandez et al 1993; Wishner et al 1998; Young et al 2001).

Shelf seas also play a key role in the global carbon cycle (Thomas et al 2004). Sequestration of atmospheric carbon through the food web is known as the biological pump; interactions between predators and prey enable organic matter to be processed and ultimately a small amount (less than 1% of annual primary production, ~0.13 mol C m^{-2} $year^{-1}$ in the North Sea) is buried in sediments (De Haas 2002; Rippeth 2005). Particulate organic matter (POM) is often considered to be the base of the food web and sinking POM that falls to the sea floor reflects surface new production (Suess 1980; Mouret et al 2010). The majority (90%) of POM is degraded in the water column and only a small fraction reaches the sea floor. This can be as little as 10% in some areas of the continental shelf although it is important to note that this is likely to be depth dependant (Wollast 1998). The physical dynamics of the water column influence new production, for example at the shelf break where nutrient pulses enhance phytoplankton growth; this in turn can stimulate production at higher trophic levels through increased availability of resources. The quality of organic matter within a marine system is also important and is dependent on numerous factors including the community composition of phytoplankton, particle sinking rates, zooplankton grazing, presence or absence of stratification, boundary layer forces and terrestrial sources (Grebmeier et al 1988).

3.1.4 Stable isotopes and benthic-pelagic coupling

In shelf seas, interactions between pelagic and benthic environments are often dynamic due to physical forces exerted on the two systems and migration of living organisms between the different horizons (Raffaelli et al 2003). The control that the water column has on the vertical distribution of plankton and macrobenthos is key in linking life cycles of these benthic-pelagic communities (Raffaelli et al 2003). As a result, the pelagic and benthic zones are considered to be closely coupled and processes that occur in the pelagic regions are usually expected to affect the benthos and the communities that inhabit it (Raffaelli et al 2003).

In examining benthic and pelagic communities, food web structure and interaction of organisms inhabiting different trophic levels can be examined. Stable isotope techniques prove to be a useful tool in tracing carbon and nitrogen compounds through food webs (Hobson and Welch 1992). Stable nitrogen isotopes are used to determine the trophic relationships between organisms, as from prey to consumer there is an estimated enrichment in $\delta^{15}N$ of 3-4‰ (DeNiro and Epstein 1981; Vander Zanden and Rasmussen 2001; Post 2002). Catabolic and excretory processes play an important role in the enrichment of nitrogen isotopes between trophic levels. Many marine organisms (especially fish and zooplankton) excrete ammonium (NH_4^+) and isotopic discrimination during biochemical processing within the digestive system produces NH_4^+ which is depleted in ^{15}N relative to the source material (Macko et al 1986). This leads to an enrichment of the residual pool of nitrogen within the organism's body (Montoya et al 2002). Stable carbon isotopes are used to examine carbon source and quality due to smaller enrichment of $\delta^{13}C$ between prey and consumer, of 0-1‰ in oceanic systems (Post 2002; Fry 2006; Michener and Lajtha 2007). Due to the enrichment between animals and their consumers, trophic positions can be calculated and food web structures elucidated (Post 2002; Vanderklift and Ponsard 2003). Following on from earlier work in isotope enrichment between trophic levels, estimations have been calculated. In this study the estimation has been derived from laboratory and field investigations on whole benthic organisms (McCutchan et al 2003). These suggest that appropriate trophic enrichments of 2.2‰ per trophic level are reasonable in this particular study (McCutchan et al 2003; Equation 3.2).

3.1.5 Hypotheses and aims

Until now, there have been few studies concerning the role played in biogeochemical processes by pelagic and benthic communities. This is important in further understanding the global importance of shelf seas and their role in carbon sequestration, carbon fixation and food production. The main aim of this study was to investigate pelagic and benthic community structure in regions of contrasting water column structure at the North West European Continental Shelf across five study sites.

Three hypotheses were to be tested.

1) Pelagic and benthic communities are influenced by water column structure and organisms are able to change feeding strategies to cope with different physical conditions in these environments. In regions with increased turbidity and mixing evidence of coupling is stronger and evidence of resuspension of benthic larval species is observed.

2) Changing feeding strategies in different physical conditions leads to individual species displaying different trophic behaviour due to varying carbon sources and specialisation.

3) Dynamics at the edge of the continental shelf supports different biological communities. Evidence of production at higher trophic levels may be apparent through larval and egg populations.

3.1.6 Study sites

The five study sites examined here have contrasting water column structures and dynamics, these were (
Figure **3.1**);

a) Vertically mixed – permanently and periodically (LB-M, IS-M)
b) Mid-shelf seasonally stratified (summer) (CS1-SS, CS2-SS)
c) Shelf break (SB-S)

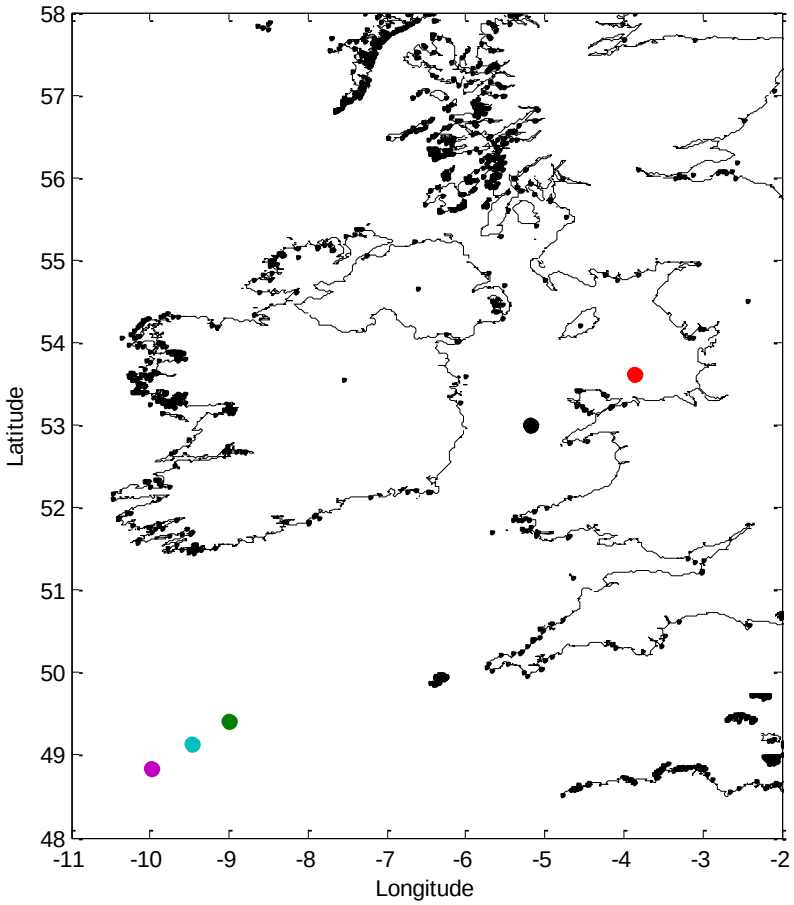


Figure 3.1 Map showing all five survey sites; = LB-M, = IS-M, = CS1-SS, = CS2-SS and = SB-S.

The closest study site to land is in Liverpool Bay (LB-M), a ROFI where inputs from several rivers can reach up to 200 m^3 s^{-1}. The region is also subjected to a strong tidal regime where the range can exceed 10m at spring tides (Howarth and Palmer 2011; Greenwood et al 2011). The Irish Sea study site (IS-M) is not a ROFI but a site where tidal mixing dominates and its close proximity to anthropogenic activity leads to concerns about nutrient enrichment, pollution and potential eutrophication (Gowen and Stewart 2005). At present, despite elevated nutrient levels and some areas of oxygen depletion, the Irish Sea is not considered eutrophic but the impact of anthropogenic nutrients and inputs into the coastal system requires continuous monitoring (Gowen et al 2008). Study sites at the mid-shelf region of the Celtic Sea (CS1-SS and CS2-SS) display characteristic seasonal thermal

stratification, the onset of which drives the spring phytoplankton bloom (Sharples and Holligan 2006). The study site at the edge of the continental margin (SB-S), is away from anthropogenic and freshwater inputs, enhanced mixing, by an internal tide, driving nutrient pulses from the deeper ocean lead to elevated phytoplankton production (Sharples et al 2007).

At each of the five study sites nominal depth (m), latitude and longitude were recorded (Cruise report, Sharples 2011) (Table 3.1) and pelagic and benthic organisms were collected for community composition and isotopic analysis (Table 3.2).

Table 3.1 Date of sampling, nominal depth (m), latitude and longitude at each sampling site.

	Date	Nominal depth (m)	Latitude	Longitude
LB-M	3 June 2010	48	53 36.32N	003 50.96W
IS-M	24 June 2010	104	52 59.96N	005 10.96W
CS1-SS	5 June 2010	137	49 25.11N	008 59.47W
CS2-SS	13 June 2010	159	49 06.94N	009 27.42W
SB-S	20 June 2010	191	48 46.82N	009 58.58W

.

3.2 Methods

3.2.1 Analysis of water column structure

A Seabird CTD instrument profiled temperature (^{o}C), salinity (pss), transmission (%) and calibrated chlorophyll (mg m^{-2}). The flourometer was calibrated in two ways from bulk chlorophyll measurements; one for the shelf edge and one for the Celtic Sea. Samples were taken from all depths of the CTD profile and casts were conducted pre-dawn. Calibration was further verified through calibration of chlorophyll *a* on return to the laboratory (Hopkins and Hickman, NOC). All CTD data were processed using SeaBird Software Processing, Version 7.18c (SBE Data Processing-Win32) (Hopkins, NOC). From the CTD profiles, features of the water column were identified (mixed surface layer (MSL), subsurface chlorophyll max (SCM) and bottom mixed layer (BML)) and the positioning of other instruments (e.g. *in situ* stand alone pumps, SAPs) was informed from these data (Table 3.2).

Table 3.2 Stations with nominal water column depth, horizons at which suspended POM was sampled, vertical zooplankton haul samples and benthic fauna collections.

	Nominal water column depth (m)	sPOM – depth horizon sampled (m)			Meso-Zooplankton		Benthos
		Surface	SCM	Bottom	Full	Partial	
LB-M	48	3.5	-	45	X	X	X
IS-M	104	3	-	90	X	X	X
CS1-SS	137	-	40	100	X	X	X
CS2-SS	159	5	38	100	X	X	X
SB-S	191	3.5	25	120	X	X	

3.2.2 Pelagic and benthic community sampling

Meso-zooplankton were collected using a WP2 net (0.5m diameter, 200µm mesh size). Two hauls at each site were conducted in triplicate, the first was of the full water column (range 48m to 191m) and the second was from the base of the SCM to the surface (typically 40m). Water column samples were transferred into 500mL HDPE bottles and preserved with a buffered formaldehyde solution (4%); an unpreserved subsample was also frozen (-20^{o}C). In

the data analysis estimations of community composition were calculated for upper and lower horizons of the water column (above and below the SCM). Lower water column composition was calculated by subtracting partial water column data from the full water column data. It is noted that this only provides an estimate of species composition in the lower water column. However, bulk percentage abundances from this method may prove useful in disseminating community structure distribution in the water column. Benthic fauna were collected using a $0.1m^3$ Day grab; this was sorted to retain macrofauna with a 0.5mm sieve (internal measurement) and whole samples were preserved in buffered formaldehyde solution (4%). Five replicate Day grabs were made at each site. Water column and benthic fauna were sorted, identified to the highest taxonomic level possible and numerated (Hobson and Welch 1992; McCutchan et al 2003). Fauna were categorised by phyla and by feeding guild, based on the literature (Table 3.3).

Table 3.3 Feeding guild classifications and the associated literature used in this study.

Feeding guild	Literature source
Water column suspension feeders (WSF)	Bellan G (2001)
Water column herbivorous suspension feeders (WH/SF)	Revis N & EN Okemwa (1988)
	Costello MJ et al (2001)
Water column predators (WP)	Bellan G (2001)
Water column omnivores (WO)	Costello MJ et al (2001)
Water column grazers (WG)	Hansson HG (2001)
Benthic predator (BP)	Bellan G (2001)
	Gofas S, Le Renard J, Bouchet P (2001)
	Türkay M (2001)
Deposit feeders (DF)	Bellan-Santini D, Costello MJ (2001)
	Gofas S, Le Renard J, Bouchet P (2001)
Versatile feeders (DF/O/BP/S)	Hayward PJ, Ryland JS (Ed.) (1990)
	Bellan-Santini D, Costello MJ (2001)
	Gofas S, Le Renard J, Bouchet P (2001)
	Hansson HG (2001)
	Türkay M (2001)
Deposit/Suspension feeders (DF/SF)	Backeljau T (1986)
	Hayward PJ, Ryland JS (Ed.) (1990)
	Bellan G (2001)
	Bird GJ (2001)
	Gofas S, Le Renard J, Bouchet P (2001)
	Watling L (2001)
Benthic grazers (G)	Gofas S, Le Renard J, Bouchet P (2001)
Suspension feeders (SF)	Howson CM, Picton BE (Ed.) (1997)
	Bellan G (2001)
	Bellan-Santini D, Costello MJ (2001)
	Gofas S, Le Renard J, Bouchet P (2001)
	Hayward PJ (2001)
	Huber M (2010)

3.2.3 Collection of suspended particulate organic matter

Suspended particulate organic matter (suspended POM) was collected using the method described in Chapter 2 (section 2.2.3).

3.2.4 Benthic and pelagic community structure analysis

Having identified pelagic and benthic fauna, species abundances were calculated from replicate samples (pelagic n = 3, benthic n = 5) and displayed as percentage composition and standardised per vertical water column haul and per benthic grab. The Shannon-Wiener diversity index (H') was calculated for each study site using multivariate statistical software PRIMER 6 (Clarke and Gorley 2006) which allows calculation of these biodiversity indices from quantitative species lists. This index of diversity is a commonly used measure of species diversity as it accounts for the number of species in a community (richness) and the relative abundance of species (evenness). The minimum value for the Shannon-Wiener index is 0, this would indicate a community with a single species. A high diversity index shows that species richness and evenness within a community is also high (Molles 1995).

3.2.5 Stable Isotope analysis

Suspended POM samples, collected on GF/F filters, was sub sampled from the large filters using a 1cm diameter punch. Punches were taken from the edge and centre of the filter to ensure representative samples were collected. Suspended POM samples were pre-treated with acid to remove inorganic carbon fractions prior to isotope analysis. De-carbonation pre-treatment was conducted using concentrated hydrochloric acid (11.4N; 250mL) in the base of a desiccator the fumes de-carbonating the samples overnight. De-carbonation of samples is known to impact isotope values (Goering et al 1990; Bunn et al 1995). Therefore, samples were prepared and run in two batches, one for carbon analysis (de-carbonated) and the other for nitrogen analysis (non de-carbonated). Quantitative analysis in a previous study has shown that carbon isotope values can decrease by up to 1‰ when comparing non de-carbonated to de-carbonated (Larrain et al 2003). Samples for isotope ratio mass spectrometry were wrapped in tin foil and folded into pellets prior to analysis. Pelagic and benthic animal tissue samples were thoroughly rinsed in MilliQ water (18.2MΩ cm^{-1}) and freeze dried (organisms were freeze dried whole to reduce the bias in gut content and body tissue for light and heavy isotopes respectively) prior to isotope analysis. Samples were

homogenised and stored in glass tubes in a desiccator ensuring dry atmospheric conditions until analysis. Samples were not extracted for lipids, the result of which can bias carbon measurements, but the often small quantities of tissue precluded lipid extraction. De-carbonation of samples was not carried out due to small quantities of tissue and to avoid losses of nitrogen. As samples were not de-carbonated there was a risk of carbonate affecting overall carbon isotope values (particularly for organisms with high carbonate intake or morphology). In order to mitigate this, organisms with values of $\delta^{13}C$ -10‰ or more are presumed to have potentially high carbonate content so have been excluded from the data set.

Formaldehyde was used as preservative for both pelagic and benthic species, previous studies have shown that this preservation method can alter $\delta^{15}N$ values by up to 2‰ and $\delta^{13}C$ values by up to 2.9‰ (Edwards et al 2002; Carabel et al 2009). In this study, to assess the impact of formaldehyde preservation, a number of preserved samples were compared with fresh frozen material (sub-sample from pelagic and benthic collections). The maximum difference observed between preserved and frozen samples for $\delta^{15}N$ was 1.46‰ and for $\delta^{13}C$ was 1.88‰. Statistical analysis of these data showed that $\delta^{15}N$ and $\delta^{13}C$ isotopes did not vary significantly between preserved and frozen samples ($\delta^{15}N$ t-test $P = 0.91$, $\delta^{13}C$ t-test $P = 0.24$). As a result no correction has been made to nitrogen or carbon isotope values where preserved samples were analysed.

Samples for isotope ratio mass spectrometry were transferred to tin capsules and loaded into an auto-sampler prior to combustion using Costech ECS 4010 CHNSO elemental analyser. Here the samples are combusted with oxygen (generating temperatures of 1700-1800^{o}C) breaking the sample into its elemental constituents. Excess oxygen is absorbed through copper wire prior to passing through A GC separation (NC 3m stainless steel column) and producing electrical signals proportional to the amount of element in the sample. Separated gases ($C0_2$, N_20) are then transferred via a Thermo Scientific Conflo IV to a Thermo Scientific Delta V Advantage continuous flow isotope ratio mass spectrometer where a report for each element, on a weight basis, is generated. Sample isotopic ratios are reported according to Equation 3.1:

Equation 3.1 $$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000\ ‰$$

Where X is ^{13}C or ^{15}N of the sample and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

In order to determine trophic levels of organisms from nitrogen isotopes, a number of equations have been developed based on assumptions of enrichment between trophic levels. Recent estimates of enrichment, which are based on trophic levels in benthic food webs and account for whole organisms being analysed indicate an enrichment level of 2.2‰ (McCutchan et al 2003; Equation 3.2);

Equation 3.2 $$TL = 1 + \frac{D_{org} - D_{POM}}{2.2}$$

Where TL = trophic level of the consumer, $D_{org} = \delta^{15}N$ of the consumer's tissue, $D_{POM} = \delta^{15}N$ of the particulate organic matter.

Mean suspended POM values (from all depth horizons sampled at each site) were used as a baseline for organic matter to estimate the number of trophic levels. Equation 3.2 accounts for whole organism sampling and has been used in this instance to help provide an estimate of the number of trophic levels at each study site. The determination of trophic levels has proven to be useful when comparing interactions between organisms at each study site and consequently carbon cycling and utilisation through each physically distinct region of the shelf sea. At a site with a greater number or continuum of trophic levels one can hypothesise that processing of organic matter may go through a more complex pathways than those sites with fewer trophic levels.

3.2.6 Statistical and multivariate analyses

Statistical analyses were carried out using SigmaPlot (version 11) software. Community composition data for pelagic and benthic environments were tested for normality and variance using Shapiro-Wilk normality test and equal variance tests. Statistical significance was tested between sites and water column structure using one-way ANOVA and t-tests (or rank tests if the normality test failed). In analysing the community composition at each site it was hypothesised that there would be a statistical difference in faunal composition between the sites, specifically between the mixed and stratified water columns. Data from isotope analyses were also tested in a similar way (Shapiro-Wilk normality test followed by

one-way ANOVA or t-test, or rank tests if the normality test failed) following the same hypothesis that there would be a statistical difference of isotope values between sites.

Multivariate analyses were used to assess the relationships between community composition, stable isotope data and different water column dynamics. The multivariate statistical software package PRIMER 6 + PERMANOVA was used (Clarke and Gorely 2006). Due to overall small sample size, conventional analysis of similarity (ANOSIM) could not be carried out to assess the relationships between sample sites and water column structures, so PERMANOVA analyses were carried out instead. This analysis was better suited to the data set as the number of replicates and sample sites were limited, the PERMANOVA analysis does not assume normal distributions and Euclidian distance unlike ANOVA and ANOSIM tests. Four analyses were carried out on the data sets (Table 3.4). Data were analysed as a whole (main test) and then pair-wise interactions were calculated to establish where the significant interactions occur for each of the two factors “site” and “water column structure”. These two factors were used as they group the data differently in each case. The factor “site” tests the five sampling locations separately assessing any similarities between them. The factor “water column structure” groups the sampling locations with the same physical water column dynamics (LB-M and IS-M was grouped as mixed water columns, CS1-SS and CS2-SS were grouped as seasonally stratified and SB-S was categorised as shelf break) assessing similarities between physical structure. Following PERMANOVA analysis, multidimensional scaling (MDS) was conducted in order to highlight similarities in the data through spatial representation. Data that are similar are represented by points that are close together and those that are dissimilar are further apart.

Table 3.4 List of variables that PERMANOVA analysis was conducted on and the resulting statistical significance of each test for site and water column structure.

Data tested	Factor: Site Statistical significance	Factor: Water column structure Statistical significance
$\delta^{15}N$, $\delta^{13}C$ and community structure	No	No
Community structure	No	No
Carbon isotopes	No	No
Nitrogen isotopes	Yes	Yes

3.3 Results

3.3.1 Physical water column structure

CTD profiles for each of the study sites across the continental shelf (Figure 3.2) show chlorophyll (mg m^{-3}), salinity (pss), temperature (^{o}C) and transmission (%). LB-M and IS-M show mixed water column structures with homogenous physical parameters. CS1-SS and CS2-SS show seasonal stratification with clear SCM and thermocline dynamics. SB-S also shows a slight SCM and shallow thermocline.

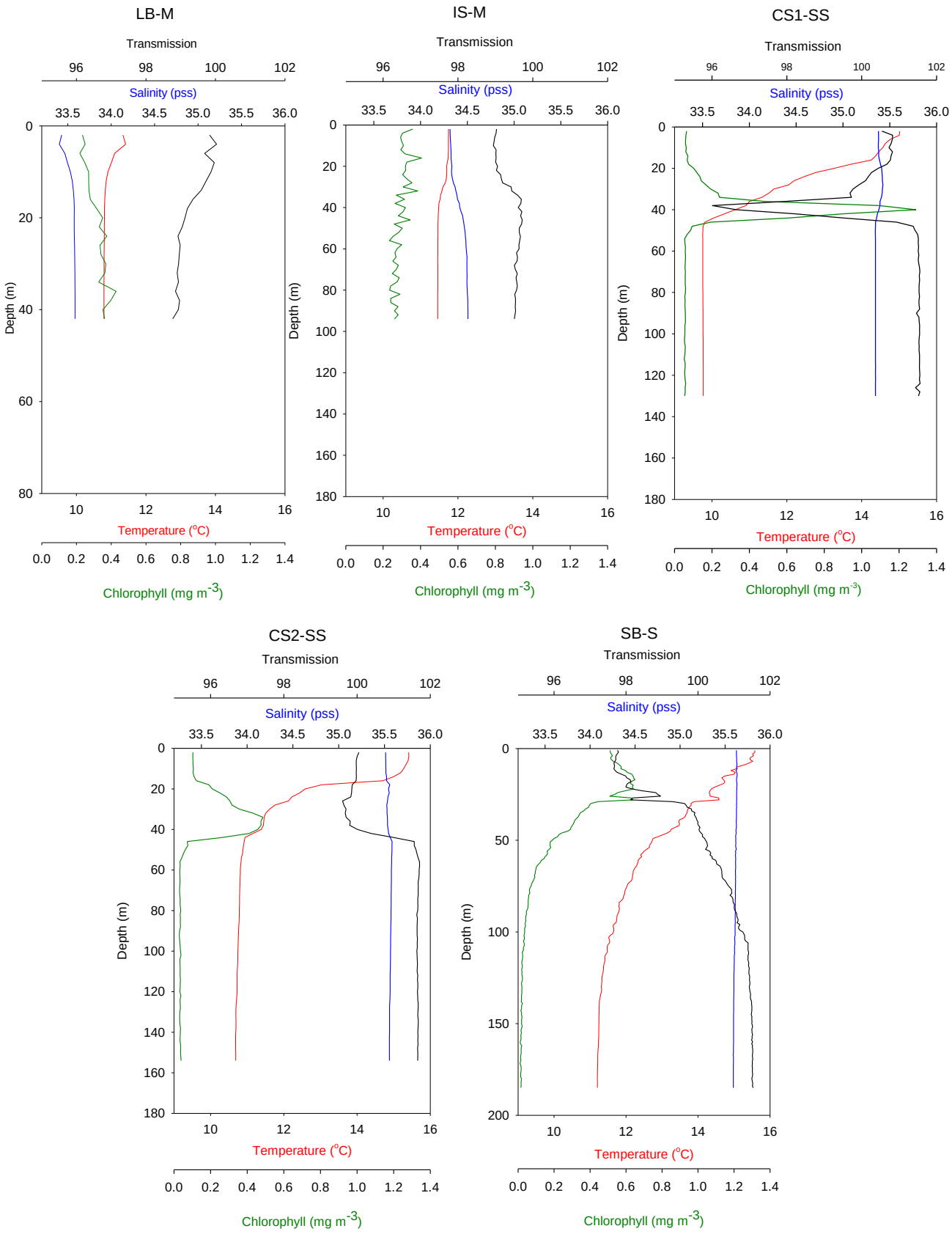


Figure 3.2 Chlorophyll, salinity and transmission measurements from CTD casts. LB-M and IS-M sites defined as mixed water columns and CS1-SS and CS2-SS defined as stratified water columns due to the presence of a thermocline and subsurface chlorophyll max (SCM). SB-S shows typical stratification at the continental shelf edge.

Table 3.5 Summary of pelagic and benthic data per vertical net haul grouped by phyla and feeding guild expressed as percentage composition (balck numbers) and mean counts with standard deviation (red numbers). Pelagic data from upper and lower water column horizons, in stratified regions, are also shown alongside full water column net hauls. Note there is no data for benthic samples at site SB-S. Note upper and lower water column may not equal full water column values as separate hauls were taken, leading to slightly different compositions.

	LB-M		IS-M		CS1-SS			CS2-SS				SB-S		
	Pleagic	**Benthos**	**Pelagic**	**Benthos**	**Pelagic**			**Benthos**	**Pelagic**			**Benthos**	**Pelagic**	
					Full	DCM	Low		Full	DCM	Low		Full	DCM	Low
Phyla															
Annelida		5.67 9 (± 0)	4.13 94 (± 1.7)	20.51 43 (± 0.6)				45.10 64 (± 5)	0.84 2 (± 0.5)	0.01 1 (± 0.1)	0.04	13.59 13 (± 0.6)	0.84 25 (± 9.5)	0.17 5 (± 1.2)	0.67
Arthropoda	36.0 122 (± 3.1)	5.32 9 (± 0.7)	88.16 2030 (± 6.32)	17.31 30 (± 0.9)	96.1 3209 (± 8.9)	85.01 2839 (± 11)	16.1	8.74 5 (± 0)	93.37 2643 (± 10.7)	81.71 2313 (± 7.6)	11.66	44.8 71 (± 7.2)	97.1 2887 (± 11.4)	85.48 2542 (± 13.9)	11.84
Bryozoa		5.32 15 (± 1.1)		13.17 45 (± 4.4)								3.6 5 (± 0.58)			
Chaetognatha	1.82		0.07 2 (± 0.5)		1.25 42 (± 2.1)	0.90 30 (± 5.7)	0.35		2.74 78 (± 9.5)	2.55 72 (± 9.5)	0.19		1.04 31 (± 6.3)	0.48 14 (± 3.3)	0.56
Chordata						0.02 1 (± 0.1)			0.75 21 (± 2.7)	1.26 35 (± 2.1)			0.35 10 (±1.7)	0.16 5 (± 1.3)	0.19
Cnidaria								1.75 2 (± 0)							
Ctenophora	6.20 1 (± 0.2)		0.36 8 (± 1.4)		0.01 0.3 (± 0.1)				0.2 6 (± 2.2)	0.13 4 (± 0.5)	0.07		0.20 6 (±3)	0.15 4 (± 0.7	0.06
Dinoflagellata	46.7 188 (± 11.4)														
Echinodermata	3.14 1 (± 0)	12.55 53 (± 12.5)	0.07 2 (± 0.2)	9.00 21 (± 3.4)	2.32 77 (± 1.7)	3.28 109 (± 2)		14.69 25 (± 3.5)	1.77 50 (± 5)	1.08 31 (± 2.8)	0.67	24.41 41 (± 3.4)	0.33 10 (± 3.2)	0.12 4 (± 2.1)	0.21

Mollusca	6.20 2 (± 0)	71.13 238 (± 8.1)	7.21 166 (± 3.1)	37.01 85 (± 2.8)	0.34 11 (±1.5)	0.14 5 (± 0.3)	0.05	61.71 25 (± 0.38)	0.10 31 (± 9.6)	0.26 7 (± 3.3)	0.84	13.59 14 (± 1)	0.17 5 (± 2.2)	0.07 2 (± 1.2)	0.10
Porifera				3.00 7 (± 0.6)											
Total abundance	322 (± 16.5)	324 (± 22)	2303 (± 45)		3340 (± 52)			121 (± 9)	2831 (± 117)			144 (±12)	2974 (± 110)		
% error of total abundance	5.1	6.9	7.1	5.5	6.9			7.3	6.9			8.8	6.8		
Feeding guild															
Benthic predator		1.42 2		8.00 19 (± 0.7)				8.74 5				3.09 2			
Deposit feeder		8.16 13 (± 0.7)		22.81 55 (± 2.1)				5.24 3				15.45 9			
Versatile		16.81 60 (± 6.25)		16.31 34 (± 2.3)				58.04 89 (± 3.4)				34.91 52 (± 2.01)			
Deposit/suspension feeders		11.35 22 (± 5.7)		16.21 30 (± 1.1)				15.73 10 (± 0)				33.99 64 (± 7.16)			
Suspension feeders		55.89 217 (± 7.2)		30.18 79 (± 2.7)				10.49 13 (± 0.58)				12.56 16 (± 0.77)			
Benthic grazers		6.38 10 (± 0)		6.50 14 (± 3)				1.75 1							
W/C grazers	49.83 189 (± 5.7)		0.51 12 (±0.4)		2.65 89 (± 1.6)	3.42 114 (± 1.4)	2.31		2.85 81 (± 6.1)	1.34 38 (± 3)	0.07		0.50 15 (± 2.8)	0.19 6 (± 1.7)	1.51
W/C suspension feeders	6.20 2 (± 0)		9.96 229 (± 4.4)				6.12								
W/C herbivorous suspension feeders	21.65 75 (± 5.4)		85.97 1980 (± 12)		95.6 3196 (± 20.7)	84.88 2835 (± 20.8)	3.06		92.69 2624 (± 17.3)	81.34 2303 (± 12.8)	49.17		96.7 2876 (± 19.1)	85.13 2532 (± 16.7)	11.35
W/C omnivores	14.30 47 (± 1.5)		2.03 46 (± 2.8)		0.01 1 (± 0.1)		3.14		0.28 7 (± 1.1)	0.29 8 (± 0.6)			0.15 4 (± 0.9)	0.07 2 (± 1.2)	0.07

W/C predator	8.02 8 (± 1)	1.53 36 (± 1.2)	1.66 55 (± 1.4)	1.03 34 (± 3.1)	4.38	4.18 118 (± 3.5)	4.04 114 (± 2.7)	0.80	2.66 79 (± 4)	1.23 37 (± 1.8)	0.58

3.3.2 Community structure analysis

A total of 137 invertebrate and vertebrate taxa (pelagic and benthic) were identified across 11 phyla at the five study sites. The taxa were grouped into 11 feeding guilds. Sampling for both pelagic and benthic communities was conducted in replicate (n = 3 for pelagic samples and n = 5 for benthic samples) and their compositions have been presented in two ways as mean percentages and mean total abundance (with standard deviations) for zooplankton haul and Day grab data (Table 3.5). Data for pelagic and benthic communities have assessed by phyla and feeding guild groups. It is important to examine both classification types particularly when assessing plasticity ad diversity of communities across the continental shelf. Full tabulated data for individual species can be found in Appendix 3.6; Table 3.9;Table 3.10.

Shannon-Wiener Index is an inclusive index taking species richness and evenness into account. Here, it has been observed that benthic communities are more diverse than the pelagic communities (Figure 3.3). Pelagic and benthic communities at LB-M showed to be more similar in diversity than those of other study sites. IS-M benthos had the highest diversity index but the pelagic fauna were the least diverse. SB-S was observed to have the lowest pelagic diversity of all the study sites.

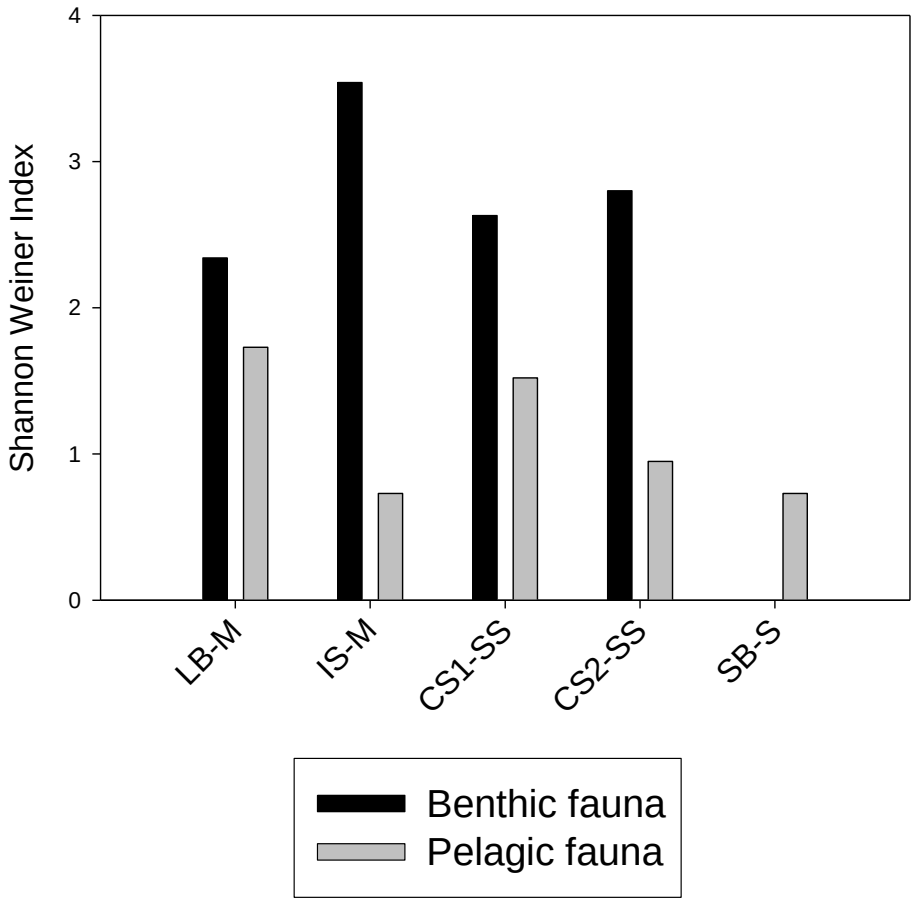


Figure 3.3 Shannon-Weiner Index for each site, pelagic species represented by grey bars and benthic fauna represented by black bars.

In order to establish the variability in the number of species in the benthic and pelagic environments at each site total abundances have been presented below (Figure 3.4). Abundance of pelagic communites were much greater than benthic communites in all sites, other than LB-M. Benthic community abundance appears to decrease across the shelf from the coastal region to the mid shelf.

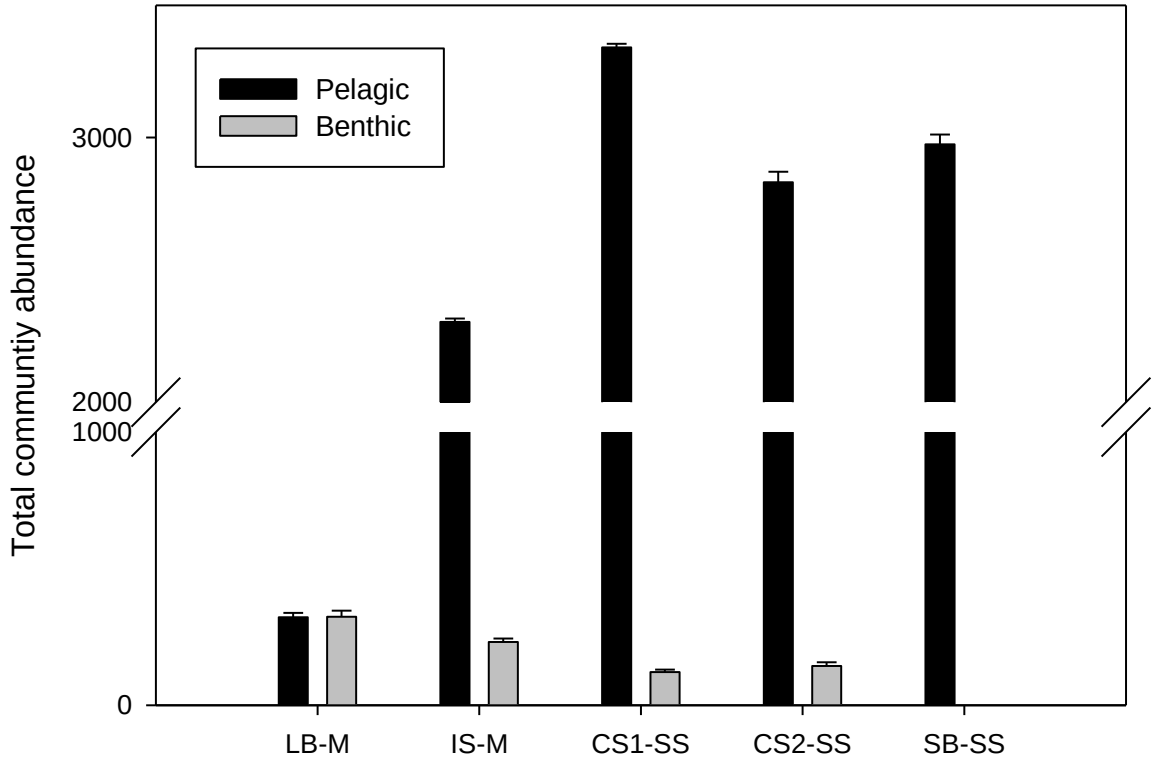


Figure 3.4 Total community abundance for each sampling site, data are presented as mean values with standard deviation presented as error bars. Black bars represent the pelagic data and gray bars represent the benthic data.

Community composition in the water column from vertical net hauls (Figure 3.5; Table 3.5; individual species abundances by % composition are shown in Appendix 3.6; Table 3.9) has been further assessed though total abundances and % composition and are summarised below;

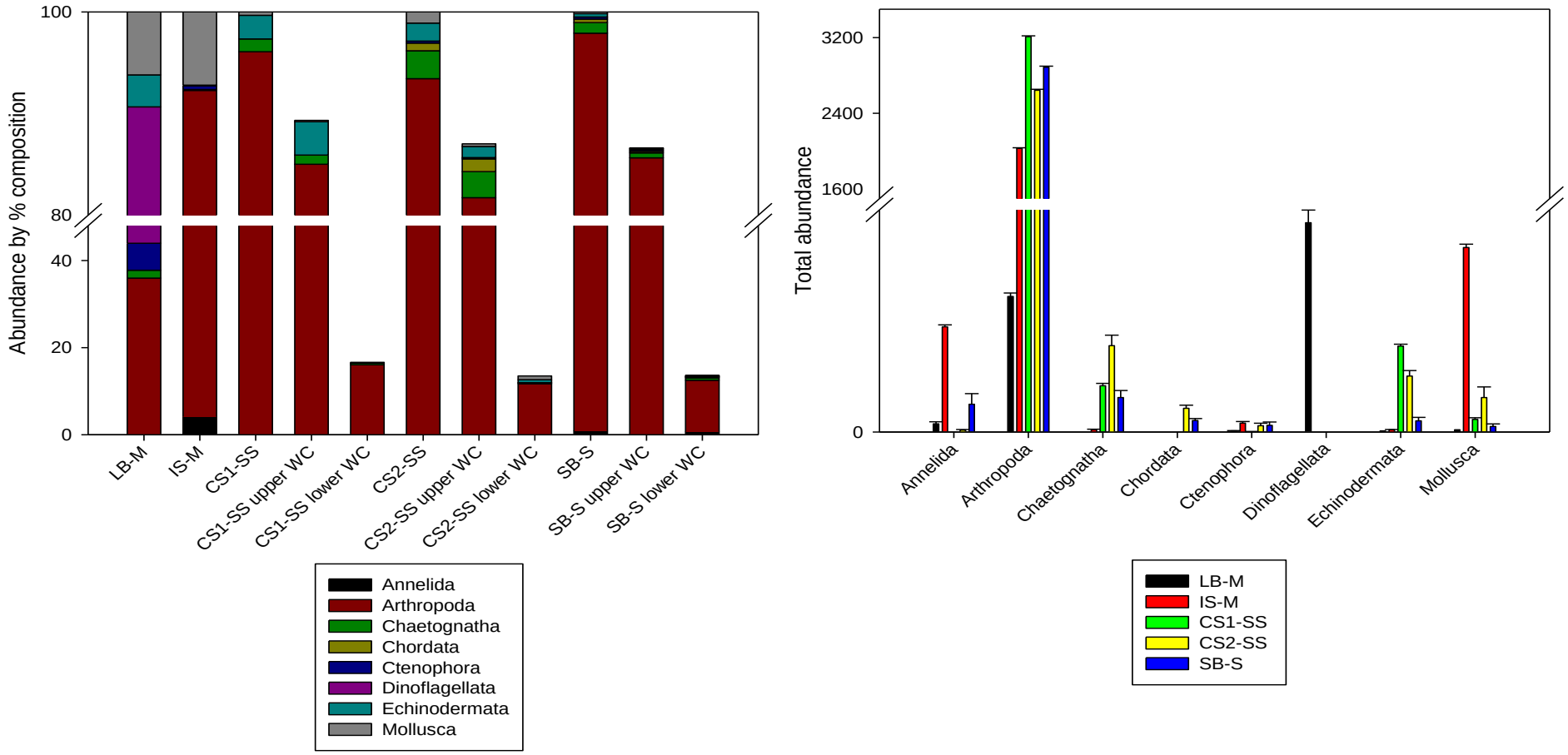


Figure 3.5 Pelagic community data grouped by phyla and displayed as relative abundance by % composition and total abundance. In the % composition graph, sites with mixed water column structures (LB-M and IS-M), data are shown for the full water column. In stratified regions (CS1-SS, CS2-SS and SB-S) full water column, upper water column (from the base of the SCM to the surface) and lower water column (base of the SCM to the benthos) data are shown.

Mixed water column (LB-M and IS-M). These sites showed the lowest contribution from the phyla Arthropoda when compared to the other sites. The dominant species found within this phylum were copepods. They were observed to contribute to the total composition of this phylum by 18% in LB-M and 83% at IS-M. A dominance of the phylum Mollusca was observed in mixed regions, particularly molluscs at the larval stage of the life cycle. Phylum Mollusca contributed up to 7% in the mixed regions compared to a maximum of 1% in the stratified regions. Each phyla showed greater total abundances at IS-M when compared to LB-M, with the exception of Dinoflagellata. Total abundances at mixed sites were generally lower in each phyla group when compared to stratified sites, with the exception of Mollusca and Annelida (Dinoflagellata only observed at LB-M).

Stratified water column (CS1-SS, CS2-SS and SB-S). At these sites the phyla Arthropoda dominated the overall community composition, this was clear from the total abundance data which showed greatest abundance of all phyla groups. The dominating species at these sites within the Arthropoda phylum was copepods, and they were observed to contribute to community composition by 90% or greater. In addition to dominance of adult copepod species copepod naupli were also observed to increase in relative abundance from less than 2% on the shelf to more than 4% at the shelf edge. In phyla Mollusca and Chordata a presence of larval life stages from species that inhabit higher trophic levels were observed. At all three stratified sites up to 80% of planktonic species inhabited the upper part of the water column and showed similar species dominance to the full water column samples.

Community composition of the water column was also examined by feeding guild (Figure 3.6; Table 3.5; individual species abundances by % composition Appendix 3.6; Table 3.9) and were further assessed by % composition and total abundance. Feeding guilds were assigned from literature evidence (Table 3.3).

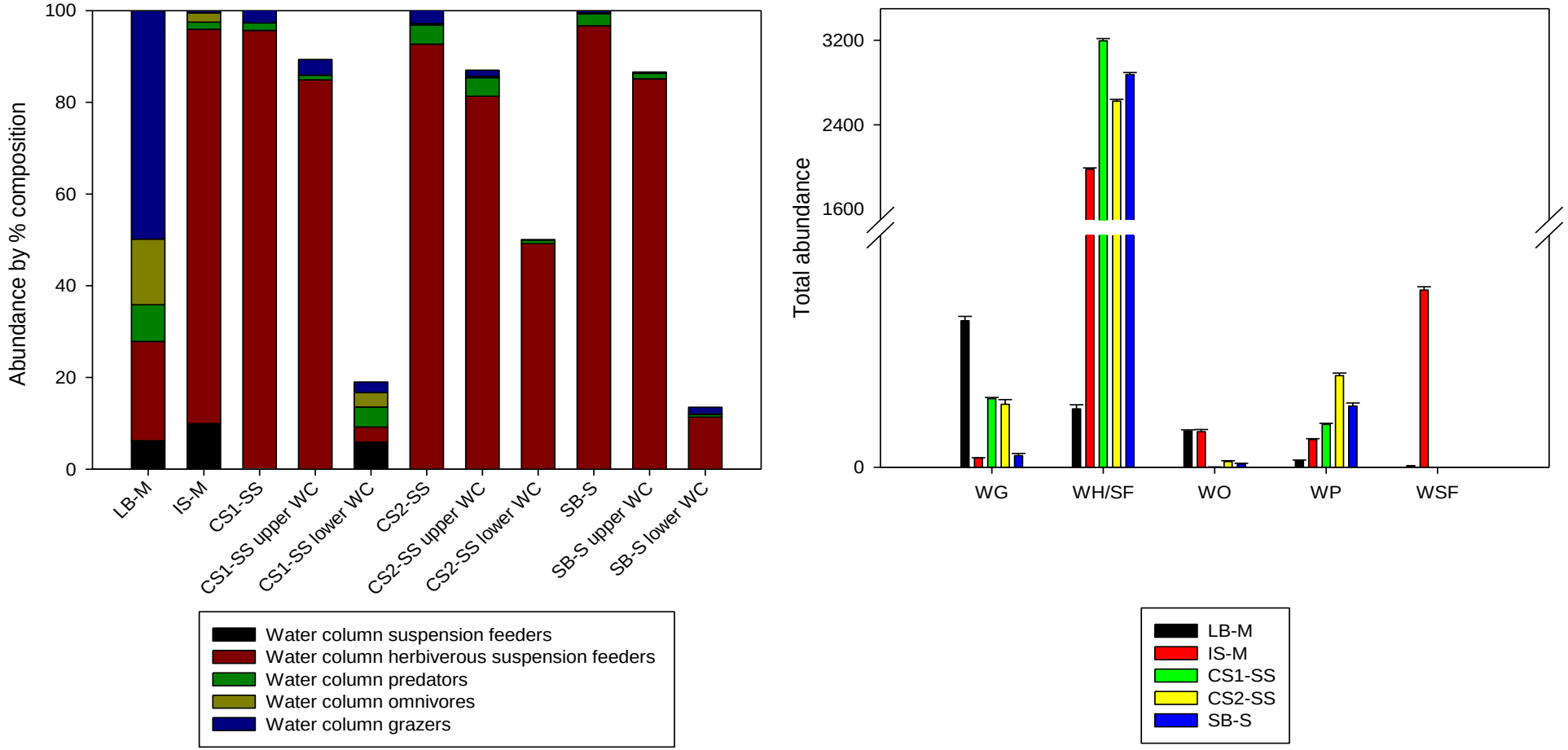


Figure 3.6 Pelagic community data grouped by feeding guild and displayed as relative abundance by % composition and total abundance. In the % composition graph, sites with mixed water column structures (LB-M and IS-M) data is shown for the full water column, in stratified regions (CS1-SS, CS2-SS and SB-S) full water column, upper water column (from the base of the SCM to the surface) and lower water column (base of the SCM to the benthos) data are shown.

Mixed water column (LB-M and IS-M). Water column suspension feeders were observed in the full water column net hauls at these sites accounting for up to 10% of the community structure, species responsible for this were juvenile bivalves and annelids (*Sabellaria* spp*)*. LB-M had the largest contribution from water column grazers than other sites, this is clearly observed from the total abundances. Mixed water column sites showed larger total abundances of water column omnivores than stratified sites.

Stratified water column (CS1-SS, CS2-SS and SB-S). In stratified waters, the herbivorous suspension feeders dominated community composition, (≥ 80% by composition); the large abundance of copepod species in these regions are responsible for this distribution. Upper water column data showed stratified regions to be dominated by herbivorous suspension feeders, this was also found to be true from the total abundance data. In sites with stratified water column water column predators were found to be more abundant than in mixed water columns. The lower water column samples across the stratified regions had smaller abundance of pelagic species. The lower water column of site CS1-SS showed an even contribution from each of the five feeding guilds, this was not observed at the other sites.

Community structure of the benthos when grouped by phyla (Figure 3.7; Table 3.5; individual species abundances by % composition Appendix 3.6;Table 3.10) is also presented according to the overlying water column structure.

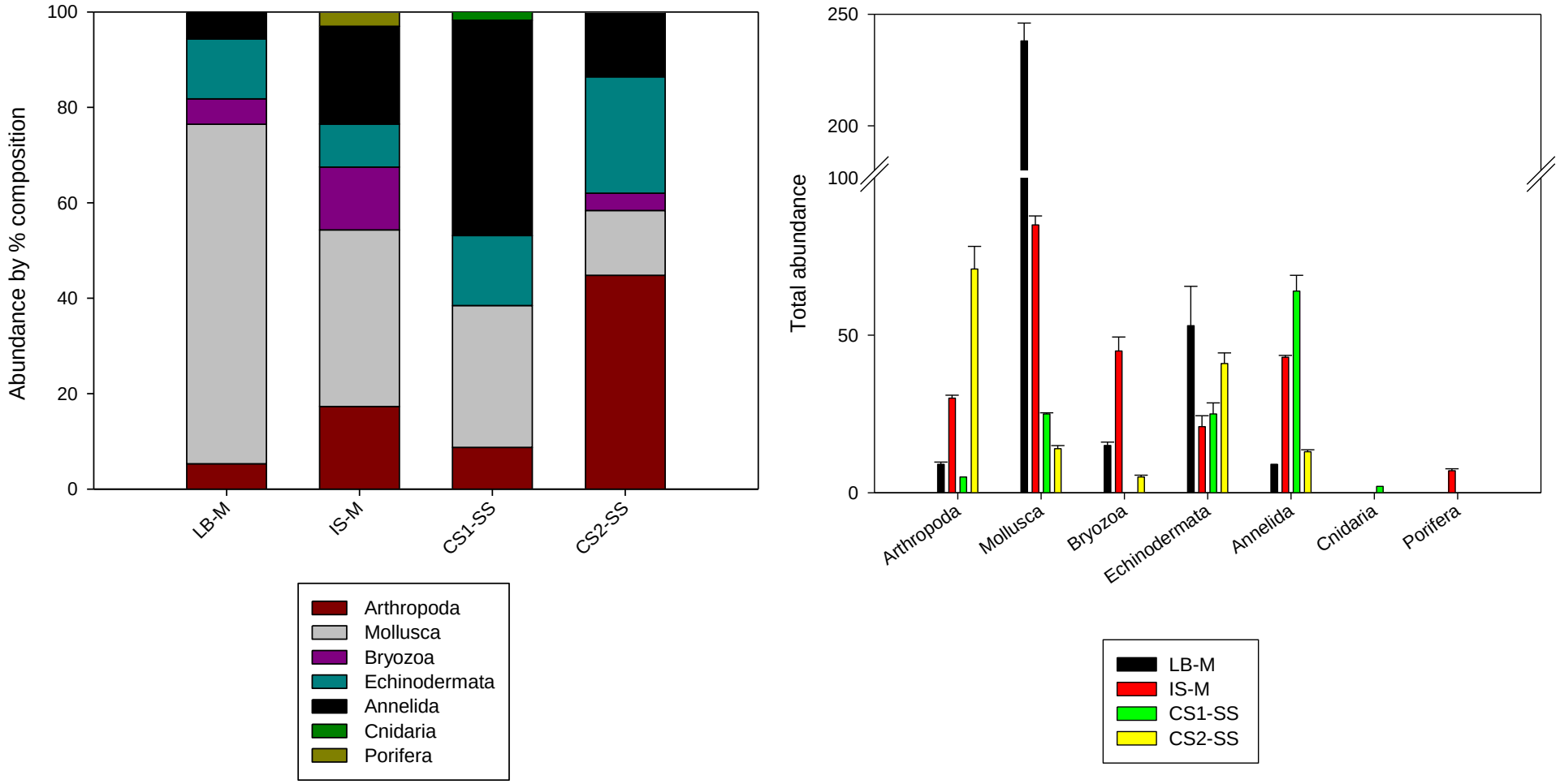


Figure 3.7 Benthic community data grouped by phyla and relative abundance by % composition and total abundance.

Mixed water column (LB-M and IS-M). These sites were dominated by the phylum Mollusca, *Parvicardium scabrum* dominated site LB-M contributing approximately 44% of community abundance. From total abundance data it is clear that mixed water column sites have the greatest Mollusca abundance decreasing with distance across the continental shelf. Mixed water columns also showed to have the greatest abundance of Bryozoa.

Stratified water column (CS1-SS and CS2-SS). Annelida dominate site CS1-SS with 45% abundance by composition. Additionally, the abundance of Annelida at CS1-SS was the greatest of all other sites. CS2-SS was observed to have a much more even community structure with a slight dominance of the Arthropoda.

Community structure of the benthos grouped by feeding guild (Figure 3.8; Table 3.5; individual species abundances by % composition Appendix 3.6;Table 3.10) and were further assessed by % composition and total abundance. Feeding guilds were assigned from literature evidence (Table 3.3).

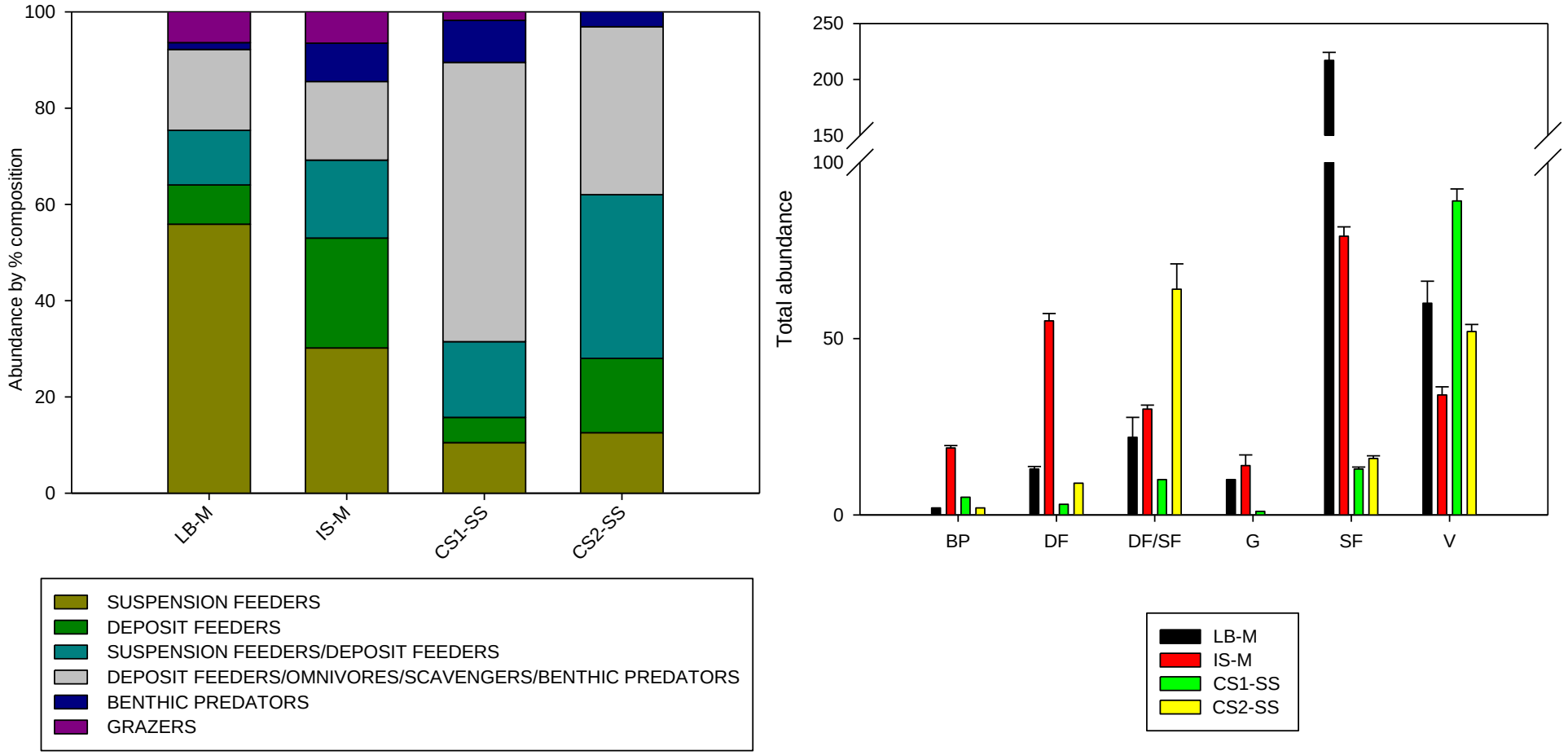


Figure 3.8 Benthic community structure grouped by feeding guild and displayed as % composition and total abundance for each sampling site.

Mixed water column (LB-M and IS-M). Suspension and deposit feeding organisms contribute over 50% of the overall community composition at each of the sites with mixed water column structure. This feeding guild is much more abundant in mixed water columns than stratified regions.

Stratified water column (CS1-SS and CS2-SS). Organisms that are able to utilise several feeding mechanisms, versatile feeders, contributed ~60% at CS1-SS and ~35% at CS2-SS. Scaphopods are molluscs that are capable of feeding via several mechanisms; at CS1-SS they contributed up to 30% of the total community composition. Versatile omnivorous species dominated at CS2-SS, particularly the pea urchin (*Echinocyamus pusillus*) and brittle stars (*Ophiura affinis)* which contributed up to 25% community composition.

The pelagic and benthic community structures of each of the five sites are subtly different. Coupling between pelagic and benthic communities is evident from mollusc communities in regions of mixed water column structure as adult communities are dominant in the benthos and pelagic juvenile stages were present in the net hauls (Table 3.5, Figure 3.5 and Figure 3.7). The total abundances and the percentage contributions of each phyla and feeding guild to the community show that there is much variation across the continental shelf and very few clear patterns. Statistical analyses (ANOVA and Kruskall-Wallis) support this and reveal no significant difference between the communities at the five study sites showing that community composition is more homogenous across the shelf than first anticipated in the original hypotheses. Table 3.5 shows a summary of the community composition from pelagic and benthic communities across the shelf and data are presented as percentage compositions and total abundance (with standard deviations). This table also provides an indication of the variability at each of the sampling sites across sampling repetitions. Variability within the benthic community appears to be greater than pelagic communities, with the greatest observed at CS2-SS of 8.8%. Lowest variability was observed at LB-M with a value of 5.1%.

3.3.3 Stable isotope analysis

The stable isotopic composition of nitrogen and carbon were determined in 23 individual taxa assigned to 8 feeding guilds from LB-M. Nitrogen isotopic compositions ranged over 18.44‰ with a mean value (± standard deviation) of 12.2 ± 0.2‰ while the carbon isotopic range was 9.86‰ with a mean value (± standard deviation) of -19.7 ± 0.5‰. At IS-M, 37 individual taxa were assigned to 19 feeding guilds, nitrogen isotopes range over 11.94‰ with a mean value (± standard deviation) of 9.3 ± 0.4‰ and carbon isotopes range over 11.95‰ with a mean value (± standard deviation) of -19.7 ± 0.7 ‰. At CS1-SS, 26 taxa were assigned to 9 feeding guilds, nitrogen isotopes range over 9.24‰ with a mean value (± standard deviation) of 7.1 ± 0.3‰ and carbon isotopes range over 15.04‰ with a mean value (± standard deviation) of -19.9 ± 0.5‰. At CS2-SS, 33 taxa were assigned to 10 feeding guilds, nitrogen isotopes range over 11.24‰ with a mean value (± standard deviation) of 6.22 ± 0.3‰ and carbon isotopes range over 9.32‰ with a mean value (± standard deviation) of -20.6 ± 0.4‰. At SB-S, 13 taxa were assigned to 5 feeding guilds, nitrogen isotopes range over 10.24‰ with a mean value (± standard deviation) of 7.8 ± 0.5‰ and carbon isotopes range over 4.26‰ with a mean value (± standard deviation) of -19.7 ± 0.3‰. (Note that only pelagic fauna were analysed at this site). Summary of all data is shown in Table 3.6 and fully tabulated data for individual species are shown in Appendix 2.6; Table 3.11. It is important to note that samples with $\delta^{13}C$ of more than -10‰ were removed, methods section 3.2.5).

$\delta^{15}N$ and $\delta^{13}C$ values of suspended POM, collected using SAPs, showed a range of values at each of the study sites (note that at LB-M the pump failed). As samples were collected at two or three depths through the water column, mean $\delta^{15}N$ and $\delta^{13}C$ values have been calculated for each site (Table 3.6, full tabulated values are presented in Appendix 2.6; Table 3.11). Mean $\delta^{15}N$ values (± standard deviation) for POM at IS-M were 6.3 ± 0.5‰, CS1-SS were 7.2 ± 0.4‰, CS2-SS were 6.3 ± 0.2‰ and SB-S were 5.5 ± -.5‰. Mean $\delta^{13}C$ values (± standard deviation) for POM at IS-M were -21.6 ± 0.3‰, CS1-SS were -18.3 ± 0.4‰, CS2-SS were -23.3 ± 0.4‰ and SB-S were -21.6 ± 0.5‰. POM values have been plotted on following plots for reference purposes but more detailed analysis of the POM data has been discussed in Chapter 2.

Table 3.6 Summary nitrogen and carbon isotope data for each study site, expressed with standard deviation for each phylum and feeding guild, note n.d signifies no data.

	LB-M		IS-M		CS1-SS		CS2-SS		SB-S	
Phyla	**δ15N**	**δ13C**	**δ15N**	**δ13C**	**δ15N**	**δ13C**	**δ15N**	**δ13C**	**δ15N**	**δ13C**
POM	n.d	n.d	6.3	-21.6 (± 0.4)	7.3 (± 0.4)	-18.3(± 0.4)	6.3 (± 0.2)	-23.3 (± 0.4)	5.5 (± 0.6)	-21.7 (± 0.5)
Annelid	14.6(± 0.1)	-18.5 (± 0.5)	9.1 (± 0.4)	-7.6 (± 0.4)	10.1 (± 0.4)	-9.1 (± 0.5)	5.8	-19.1	6.2	-20.1 (± 0.4)
Arthropoda	11.9 (± 0.8)	-20.9 (± 0.5)	9.0 (± 0.2)	-7.6 (± 1)	7.9 (± 0.1)	-5.3 (± 1.0)	6.1 (± 0.4)	-3.5 (± 0.4)	7.5 (± 0.3)	-3.4 (± 0.3)
Cetnophora	14.5 (± 0.2)	-18.2 (± 0.8)	n.d	n.d	n.d	n.d	n.d	n.d	12.0 (± 0.3)	-17.8 (± 0.4)
Chaetognatha	n.d	n.d	13.1	-18.6	10.5 (± 0.5)	-23.3 (± 0.7)	9.8 (± 0.4)	-21.9 (± 0.4)	8.1 (± 1.0)	-18.8 (± 0.5)
Chordata	16.0	-15.6	10.4	-19.0	n.d	n.d	6.2 (± 0.2)	-6.2 (± 0.4)	7.6	-0.1 (± 0.4)
Echinodermata	n.d	n.d	8.8 (± 0.3)	-12.7 (± 0.0)	2.4 (± 0.2)	-8.9 (± 0.5)	5.2	-23.3	6.6	-20.0
Mollusca	3.6 (± 0.4)	-20.7	9.2 (± 0.4)	-0.8 (± 0.8)	6.0 (± 0.3)	-0.02 (± 0.6)	6.1 (± 0.4)	-3.3 (± 0.4)	n.d	n.d
Porifera	n.d	n.d	9.2 (± 0.8)	-1.1 (± 3.0)	n.d	n.d	n.d	n.d	n.d	n.d
Feeding Guild										
POM Mean	n.d	n.d	6.3 (0.5)	-21.6(± 0.3)	7.3 (± 0.4)	-18.3 (± 0.4)	6.3 (± 0.2)	-23.3 (± 0.4)	5.5 (± 0.6)	-21.7 (± 0.5)
Benthic predator (BP)	15.2 (± 0.1)	-17.3 (± 0.8)	11.1 (± 0.6)	-9.7 (± 1.3)	9.7 (± 0.2)	-6.1 (± 1.0)	7.1	-18.8	n.d	n.d
Deposit feeder (DF)	10.5 (± 0.8)	-19.9 (± 0.4)	8.2 (± 0.3)	-4.0 (± 0.5)	8.9 (± 0.2)	-17.1 (± 0.6)	7.3 (± 0.3)	-9.3 (± 0.7)	n.d	n.d
Versatile (DF/O/BP/S)	17.1	-20.00	9.1 (± 0.3)	-12.5 (± 0.0)	5.8 (± 0.4)	-12.4 (± 0.4)	6.9 (± 0.4)	-6.5 (± 0.6)	n.d	n.d
Deposit/suspension feeders (DF/SF)	4.7 (± 0.3)	-20.0 (± 0.0)	8.8 (± 0.7)	-1.5 (± 0.5)	7.6 (± 1.0)	-6.0 (± 1.5)	5.8 (± 0.3)	-10.1 (± 0.6)	n.d	n.d
Suspension (SF)	11.4 (± 0.2)	-21.3 (± 0.6)	8.7 (± 0.3)	-1.2 (± 0.6)	7.0 (± 0.2)	-11.3 (± 0.2)	5.9 (± 0.5)	-1.9	n.d	n.d
Water column grazers (WG)	n.d	n.d	n.d	n.d	3.8 (± 0.0)	-0.7 (± 0.5)	5.7	-22.2	6.6	-20.00
Water column herbivorous suspension feeders (WH/SF)	12.2 (± 0.2)	-20.5 (± 0.3)	10.4 (± 0.2)	-19.7 (± 0.9)	8.1 (±0.1)	-27.1 (± 0.7)	6.6 (± 0.4)	-8.3 (± 0.5)	6.6 (± 0.3)	-7.2 (± 0.3)
Water column omnivores (WO)	12.9	-20.3	10.4	-19.0	n.d	n.d	4.2	-20.9	8.3	-0.2 (0.4)
Water column predator (WP)	14.5 (± 0.2)	-18.5 (± 0.5)	11.4	-18.7	9.1 (± 0.3)	-8.2 (± 0.9)	5.8 (± 0.3)	-0.6 (± 0.3)	8.6 (± 0.6)	-3.5 (± 0.4)

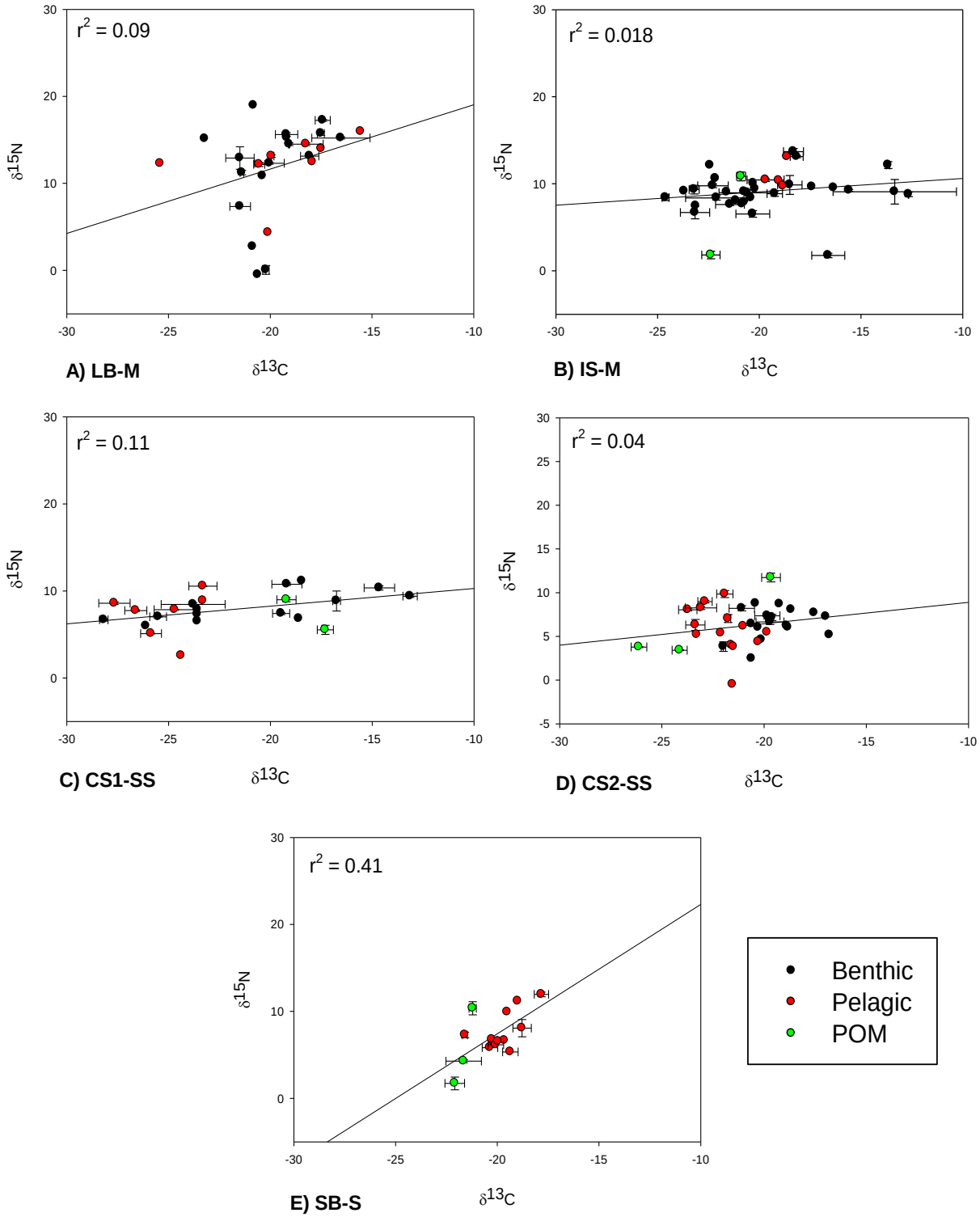


Figure 3.9 $\delta^{15}N$ and $\delta^{13}C$ bi-plots for each survey site, each data point represents an individual organism with error bars from triplicate analysis. ● = benthic organisms, ● = pelagic organisms and ● = suspended POM.

Nitrogen and carbon isotopes were plotted against each other in order to evaluate the linearity of the trophic assemblages and links between prey and consumers. Linear

regression was used and returned weak positive correlations at all sampling sites, but they were not all statistically significant (Figure 3.9). Weakest associations between carbon and nitrogen isotopes were observed at the sites with mixed water columns, LB-M (r^2 = 0.09, P = 0.13) and IS-M (r^2 = 0.03, P = 0.4). CS1-SS had the strongest correlation but it was not statistically significant (r^2 = 0.11, P = 0.09). CS2-SS had the weakest correlation of the stratified sampling sites and was also not significant (r^2 = 0.04, P = 0.79). SB-S was the only site to have a significant correlation (r^2 = 0.41, P = 0.01). At seasonally stratified sites CS1-SS and CS2-SS despite the weak positive correlation, pelagic species appear depleted in $\delta^{13}C$ compared to benthic species (Figure 3.9). This was not the case at LB-M or IS-M, where pelagic and benthic organisms were grouped together when nitrogen and carbon isotopes were plotted together.

Species were further grouped according to phyla and feeding guild in order to assess trophic dynamics. Data were first assessed using mean nitrogen and carbon values for each phylum and feeding guild (for both pelagic and benthic systems), then individual values were assessed and feeding guilds of individuals within specific phyla were highlighted

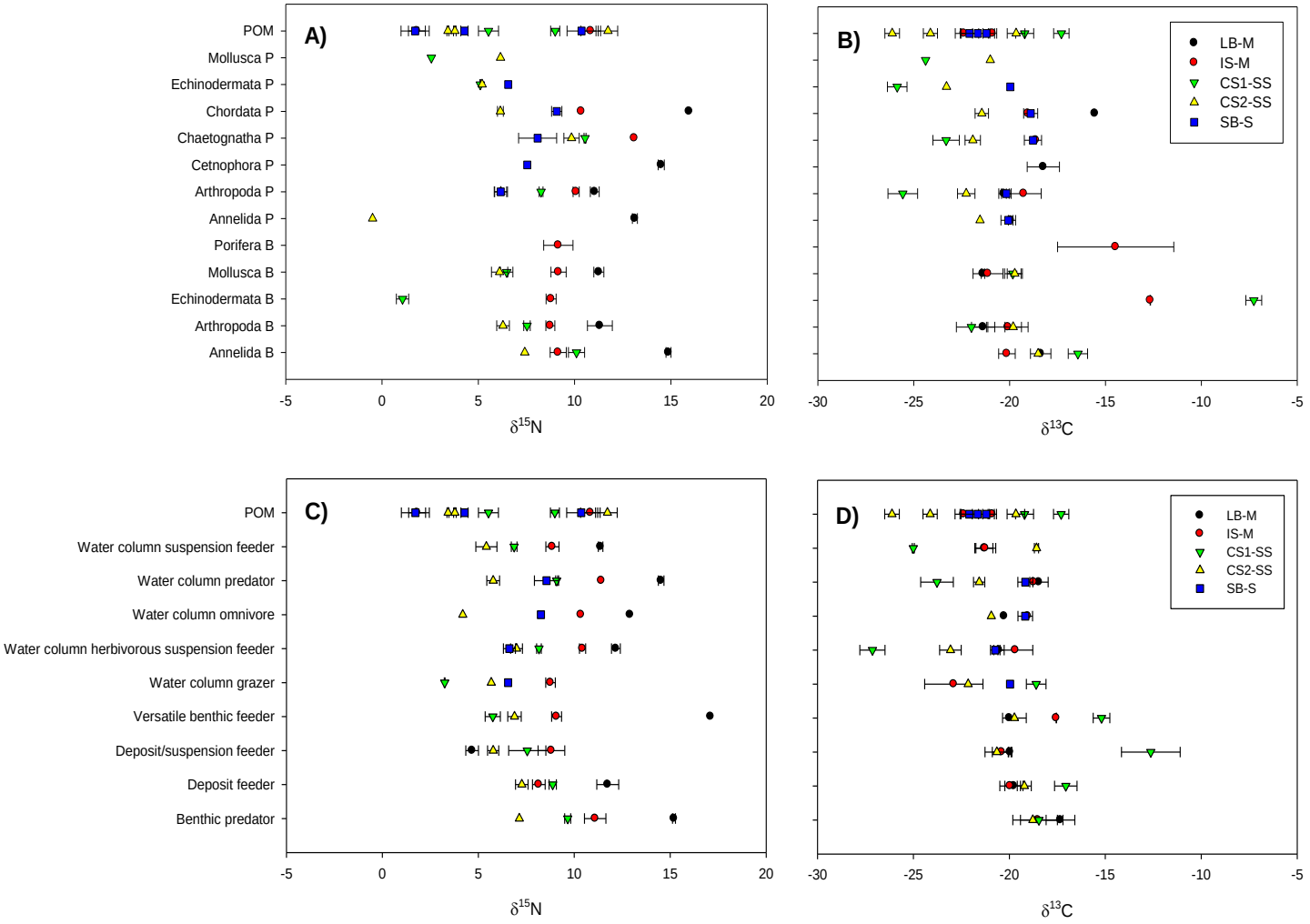


Figure 3.10 Mean $\delta^{15}N$ and $\delta^{13}C$ values plotted for each phylum and feeding guild, error bars shown from each phylum. Plots A and B show data for mean phylum values and plots C and D show data for mean feeding guild values. Suspended POM values are plotted for each water depth sampled.

Mean $\delta^{15}N$ values of phyla in regions with mixed and stratified water column structures were 10.77 ± 0.34‰ and 6.39 ± 0.33‰, respectively (Figure 3.10). This difference was found to be statistically significant (t-test = 4.771, p = < 0.001), implying potentially different trophic positioning of species in the physically distinct water column structures. This observation was examined further by specifically determining $\delta^{15}N$ differences in pelagic Arthropoda species, which were chosen due to the abundance of individual species across all study sites. The mean $\delta^{15}N$ value of this phylum in mixed regions was 10.56 ± 0.2‰ while that in the stratified regions was 6.86 ±0.25‰. These differences were found to be statistically significant (t-test 3.816, p = 0.032). The mean $\delta^{13}C$ value of phyla in regions of mixed verses stratified water column structure were -19.05 ± 0.67‰ and -20.78 ± 0.47‰, respectively. The data were not normally distributed so following a Mann-Whitney Rank Sum test the difference was found to be statistically significant (p = 0.038) implying utilisation of isotopically different carbon sources in the physically distinct water column structures. At CS2-SS and SB-S sites (Figure 3.10) there was a close association between the $\delta^{13}C$ values of the fauna and the water column suspended POM, suggesting strong coupling of carbon between pelagic POM, pelagic and benthic organisms.

The mean $\delta^{15}N$ values of feeding guilds in regions with mixed and stratified water column structures were 10.49 ± 0.33‰ and 6.69 ± 0.34‰ respectively (Figure 3.10). This difference was found to be statistically significant (t-test = 4.554, P = < 0.001), much like when the data were grouped according to phyla suggesting different trophic positioning of feeding guilds in the physically distinct water column structures. The mean $\delta^{13}C$ value of feeding guilds in regions of mixed versus stratified water column structure were -19.94 ± 0.57‰ and -20.45 ± 0.51‰, respectively. The data were not found to be statistically different implying that the carbon sources between the physically distinct regions, when the community is analysed though feeding guild, were not isotopically different.

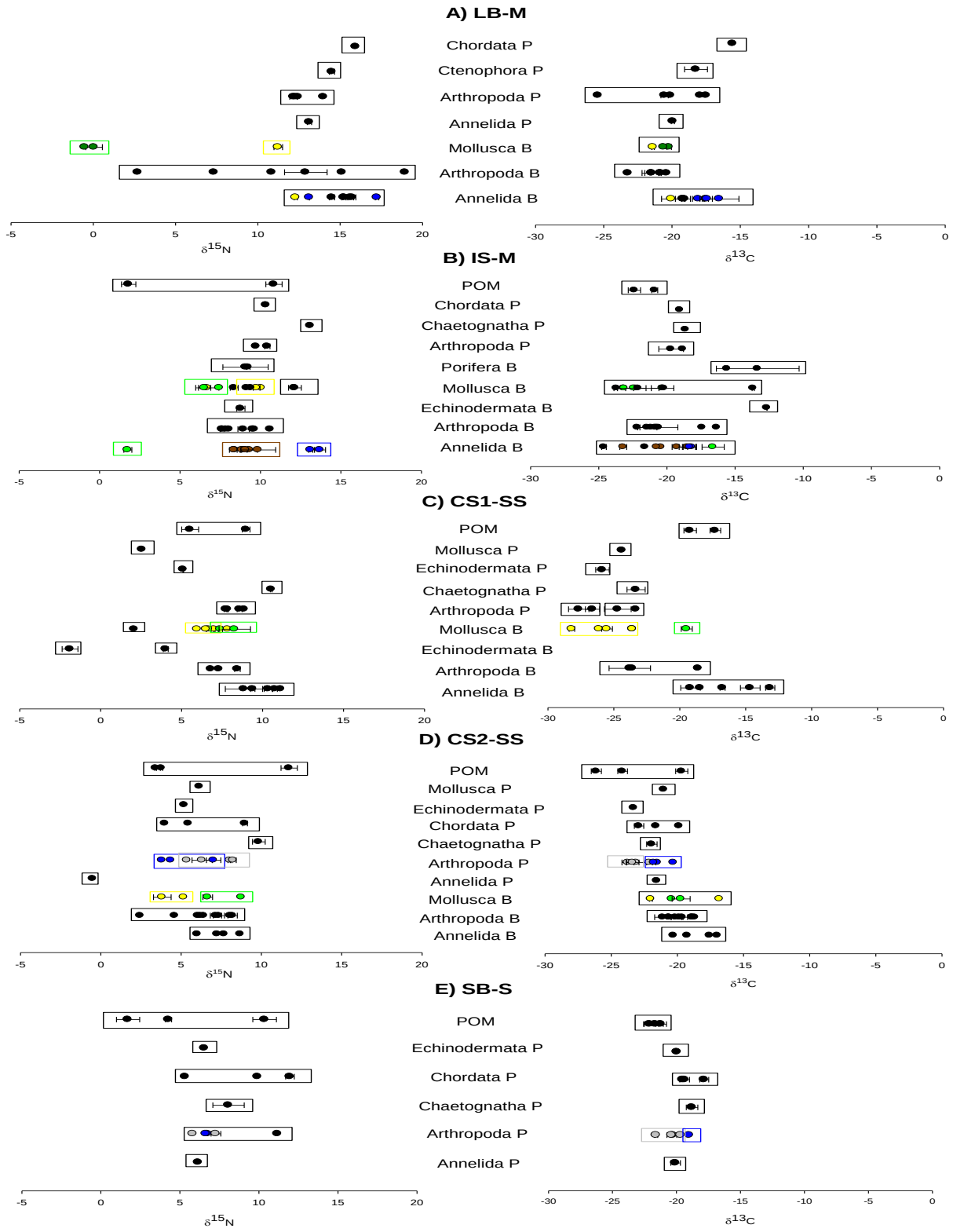


Figure 3.11 Individual $\delta^{15}N$ and $\delta^{13}C$ data showing pelagic and benthic species, grouped by phyla, with error bars from triplicate analysis of each organism. sPOM values have also been plotted for each water depth sampled. Specific individuals have been highlighted in colour to represent feeding guild. ● = benthic suspension feeders, ● = benthic deposit feeders, ● = benthic suspension/deposit feeders, ● = predators and ● = water column herbivorous suspension feeders.

Interactions between suspension and deposit feeders in benthic Molluscs differ depending on the structure of the overlying water column. In mixed waters deposit feeders had lower $\delta^{15}N$ than suspension feeders. However, in stratified regions the opposite trend was observed (Figure 3.11).

$\delta^{15}N$ values of benthic annelids show clear separation of feeding guilds in IS-M while source material from $\delta^{13}C$ values did not show patterns or significant separation (Figure 3.11). Within this phylum, predators were the most enriched feeding guild, deposit feeders were the least enriched. Organisms that can feed via deposit and suspension feeding were intermediate compared to the mean $\delta^{15}N$ values of the deposit feeders of this phylum 1.76 ± 0.25‰, compared to those of the multi feeding suspension/deposit feeding guild 9.096 ± 0.63‰ and predator feeding guild 13.41 ± 0.24‰. Differences between guilds within the phylum were tested for significance using a one-way ANOVA and were found to be significantly different ($p = < 0.001$) demonstrating trophic separation of organisms depending on feeding guild.

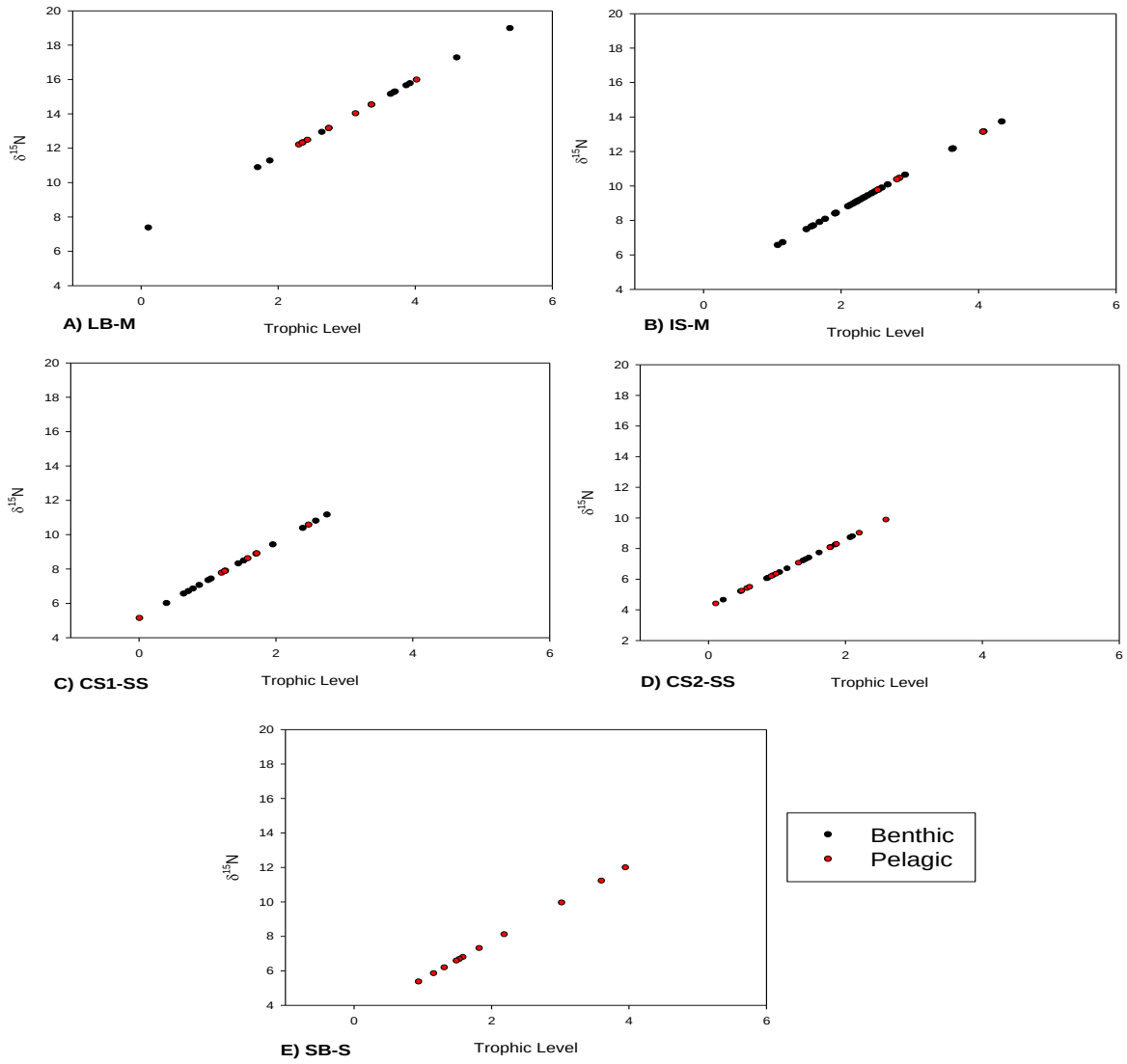


Figure 3.12 Trophic level verses $\delta^{15}N$ values, derived from McCutchan et al (2003). Mean sPOM values were used to calculate trophic levels. ● = Benthic organism, ● = pelagic organisms.

Trophic level versus $\delta^{15}N$ (Figure 3.12), derived using the enrichment calculation published by McCutchan et al (2003) (Equation 3.2), showed the spread of trophic levels to vary between water column structures. Mean pelagic suspended POM values were used in calculating trophic levels for pelagic and benthic organisms; as a result the relative range of trophic levels observed at the study sites have some value, while specific values are more uncertain. Study sites with mixed water column structures (LB-M and IS-M Figure 3.12), had a spread of estimated trophic levels of 5.25, whereas those with stratified water columns (CS1-SS, CS2-SS and SB-S; Figure 3.12) had a smaller range of 3.15.

3.3.4 Multivariate analysis

In each of the main tests that were conducted, only nitrogen isotope data showed any significant relationships between the two factors “site” and “water column structure” (Table 3.4). The significance for the main test using factor “site” was found to be P = 0.002. As a result of this significance pair wise analysis was then conducted (Table 3.7).

Table 3.7 Pair-wise PERMANOVA output from nitrogen isotope data showing the relationships of the data grouped by site, statistical significance is shown as a P value for each comparison (significant P values are highlighted in grey).

Pair wise comparison	**Factor: Site Statistical significance (P value)**
IS-M, CS1-SS	0.0107
IS-M, CS2-SS	0.0001
IS-M, LB-M	0.221
IS-M, SB-S	0.0904
CS1-SS, CS2-SS	0.0791
CS1-SS, LB-M	0.0174
CS1-SS, SB-S	0.6748
CS2-SS, LB-M	0.0008
CS2-SS, SB-S	0.053
LB-M, SB-S	0.1433

Significant differences were observed between IS-M and both Celtic Sea sites (CS1-SS and CS2-SS) (P = 0.0107 and P = 0.0001) and between LB-M and both Celtic Sea sites (CS1-SS and CS2-SS) (P = 0.0174 and P = 0.0008). The highest level of significance was observed when IS-M and LB-M were compared with CS2-SS (P = 0.0001 and P = 0.0008, respectively).

Visualisation of this data through the use of MDS shows separation of LB-M and IS-M sampling sites to the left of the plot away from the CS1-SS and CS2-SS data points which are scattered more towards the right hand side of the plot (Figure 3.13).

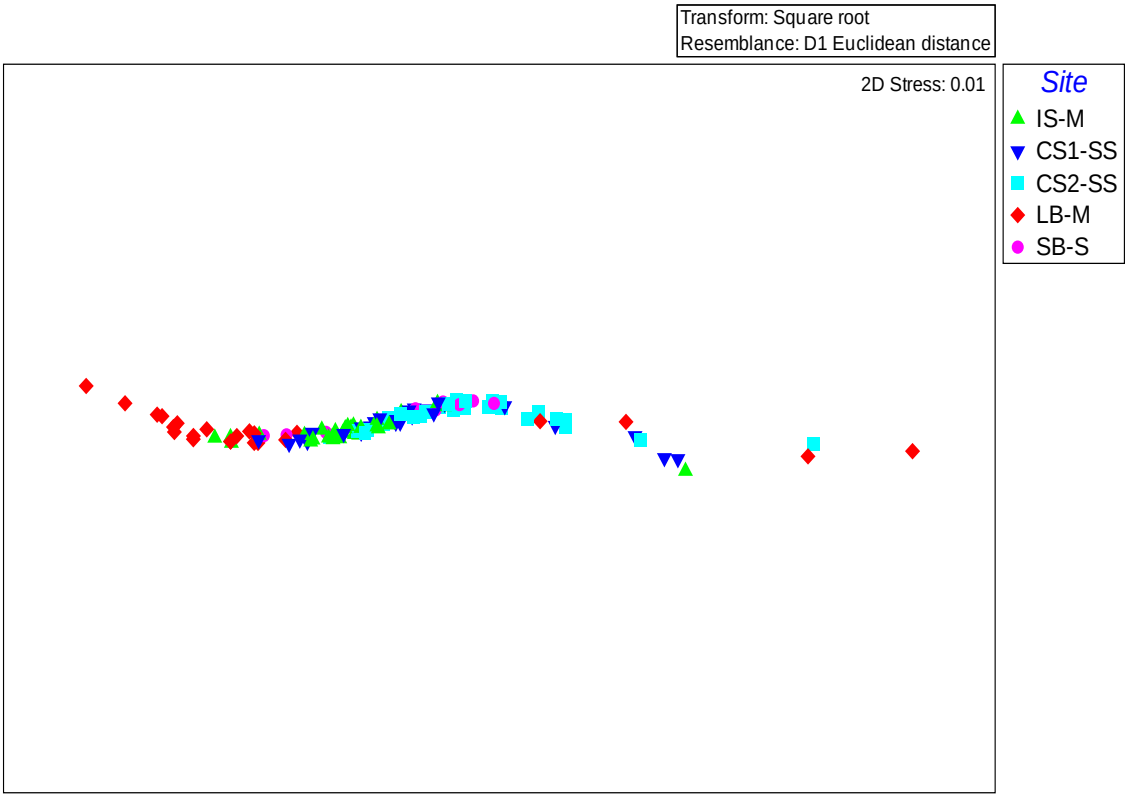


Figure 3.13 MDS plot visualising the data when grouped and analysed for each individual sampling site.

As for the "site" factor, the main test was conducted for the factor "water column structure" which was also found to be significant (P = 0.002). Following this significant result pair wise analysis was conducted. The results of the pair-wise analysis are shown in Table 3.8. Significance was only observed between mixed (LB-M; IS-M) and seasonally stratified waters (CS1-SS; CS2-SS) (P = 0.0001).

Table 3.8 Pair-wise PERMANOVA output from nitrogen isotope data showing the relationships of data grouped by water column structure (M = mixed, SS = seasonally stratified and S = stratified), statistical significance is shown as a P value for each comparison (significant P values are highlighted in grey).

Pair wise comparison	Factor: Water column structure Statistical significance (P value)
M, SS	0.0001
M, S	0.0856
SS, S	0.2345

Visualisation (Figure 3.14) shows some clear separation and clustering of the mixed water column data (LB-M and IS-M) (with the exception of a few outliers to the right of the MDS plot area.

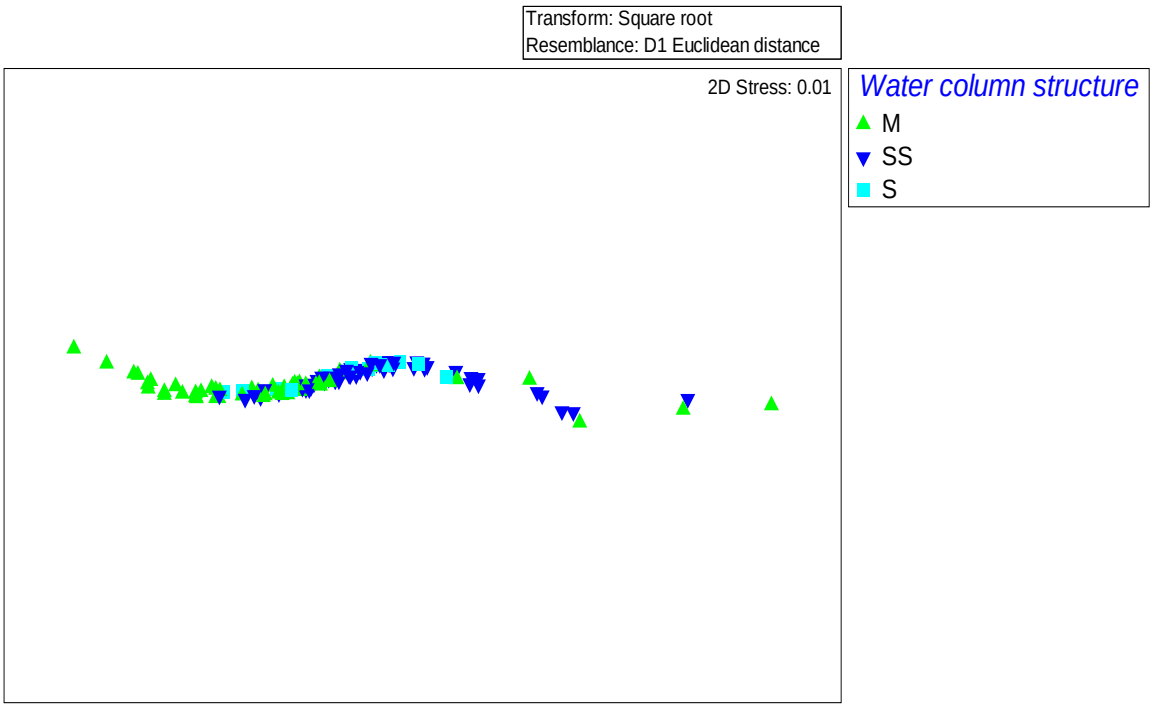


Figure 3.14 MDS plot visualising the data when grouped and analysed by water column structure. Mixed water column incorporates LB-M and IS-M, seasonally stratified incorporates CS1-SS and CS2-SS and stratified incorporates SB-S.

3.4 Discussion

This study has described a multidisciplinary approach using ecological and biogeochemical techniques to characterise communities within the ecosystem across contrasting physical regimes across the NW European Continental Shelf. It has been observed that water column dynamics affect the pelagic and benthic community structure. However, the NW European Continental Shelf community is very patchy and adaptations to changing dynamics are observed. Here the influence of physical dynamics on the community is discussed.

3.4.1 The influence of physical dynamics on the community structure – examining variation across the shelf

Shelf seas account for a small proportion of the world's oceans (~10%) (Muller-Kager et al 2005). The changing physical dynamic across the NW European Continental Shelf has been well documented (Simpson and Hunter 1974; Sharples et al 2007; Simpson and Sharples 2012). Here, the variations in pelagic and benthic communities have been described and the specific regions which individual species inhabit investigated; observable changes towards the edge of the shelf and the wider implications of these changes are discussed as well as the critical link between the pelagic and benthic communities and co-habitation in these two environments. From individual species data, variation across the shelf in both the pelagic and benthic communities (calculated across all sampling replications) was found to be fairly consistent across the continental shelf. In the benthos, less variability was observed in the mixed water columns. This was also true for LB-M pelagic species. In these mixed regions it seems that there is less variation and that the communities are more homogenous in both pelagic and benthic ecosystems. In regions of stratified water column structure, variation in the benthic community was greater between sampling replications. This may be due to patchiness in the community, which is further discussed below. All of the variations between sampling replicates may be due to localised fluctuations in the community (for example horizontal advection in the pelagic community) and the difficulty in exactly replicating sampling conditions (for example, a Day grab is not truly quantitative).

3.4.1.1 Patchiness in the community

The community structures of both pelagic and benthic environments across the five study sites were not found to be statistically different in composition. Compositional analysis and total abundances of phyla and feeding groups highlight the variability within the shelf sea environment here the apparent patchiness of the communities was clear. Patchiness across a large region, such as a continental shelf, has been recognised in previous studies and results from adaptation of individual organisms in terms of feeding, foraging and reproductive behaviour (Gili and Coma 1998; Raffaelli et al 2002). The present study suggests that the benthic environments of stratified regions were dominated by organisms with versatile feeding strategies (Figure 3.7). This versatility has been observed previously for fish species, where generalists dominate the Celtic Sea enabling them to switch between prey types depending on resource availability balancing the cost of prey capture and handling with the benefit of prey quality and energy intake (Pinnegar et al 2003; Trenkel et al 2005). In the mid-region of the Celtic Sea, where seasonal stratification occurs periodically, nutrient availability and predator-prey relationships may vary throughout the year. In previous studies, it has been noted that physical variation in the water column structure can affect the amount of energy being passed between trophic levels and the distribution of predators (Hunt et al 2002; Hunt and Stabeno 2002). Consequently, it could be suggested that the dominance of animals with versatile feeding strategies may be a response to the changing quality of POM sinking through the water column in these regions. Water column conditions vary through the year potentially affecting food supply and quality. These factors may have contributed to the patchiness in communities across the shelf sea environment from coastal regions to the shelf break.

3.4.1.2 Change at the shelf break

Survey sites represent a transect across the NW European Continental Shelf (
Figure 3.1) from coastal sites (LB-M and IS-M) to the shelf break (SB-S). Previously, copepod populations across the shelf have been observed to change due to the varying hydrodynamic conditions. In shallow coastal waters, smaller copepod species dominate (e.g. *Pseudocalanus* spp.) due to specific reproductive and feeding adaptations (Lindley 1986; Williams et al 1994). These small copepod species are not as dependent on high food concentrations, so can reproduce when resources are low, and they are capable of

tolerating high levels of water column turbidity (Tester and Turner 1989; Williams et al 1994). In seasonally stratified waters and at the shelf break, larger species respond to the onset of primary production at the SCM creating a more direct pathway to higher trophic level organisms (Cushing 1989; Madden et al 1999). Previously, it has also been observed that the phytoplankton community structure changes at the shelf break from being dominated by small picoplankton to larger eukaryotes (Sharples et al 2007). These changes in community composition are influenced by processes of nutrient upwelling providing more food and resources in these regions to phytoplankton communities (Sharples et al 2007). In this study the zooplankton community shows similar changes across the shelf. *Pseudocalanus* spp. dominated LB-M and IS-M, (which is easily adaptable to the low nutrient and turbid conditions of the mixed water column). With distance across the shelf away from the coastal sites a greater proportion of larger, more typically oceanic copepod species were observed (e.g. *Eucalanus* spp. and *Paracalanus* spp.). These species are more capable of tolerating higher salinity conditions and increased nutrient supply at the SCM for the onset of egg production (Madden et al 1999; Johnson and Allen 2005) compared to those present at coastal study sites.

Across the study region from LB-M to SB-S an increase of larval planktonic species, such as fish eggs, gastropod larvae and copepod naupli, were also observed (Table 3.5). At the shelf edge hydrodynamic properties are different and increased internal tides support enhanced primary production, and possibly more importantly, support the production of larger phytoplankton cells in surface waters (Holligan 1981; Sharples et al 2009). It has been suggested that this in turn supports fish larvae and zooplankton production (Piatt and Springer 2003; Skarðhamar and Svendsen 2005). Enhanced primary production and growth of larger phytoplankton cells creates ideal conditions for fish spawning grounds and this is reflected by high fishing intensity often observed at continental margins (Young et al 2001; Sharples et al 2007; Sharples et al 2009).

3.4.1.3 Benthic-pelagic coupling

Interactions between the pelagic and benthic environments are critical for organisms that inhabit them (Herring 2002). Fluxes of living organisms between the water column and benthos occur daily and seasonally, creating the critical links between them (Raffaelli et al

2003). As previously described, the water column structure controls vertical distribution of phytoplankton and macrobenthos (Raffaelli et al 2003). It has previously been observed that most benthic organisms spend part of their life cycle in a planktonic stage and this process of inhabiting both environments at different stages of development aids the pelagic-benthic coupling (Marcus and Boero 1998; Raffaelli et al 2002). Planktonic life stages of some benthic species are able to adapt to changing physical conditions in the water column. Bivalve larvae are able to migrate vertically in the water column in order to reach optimum feeding horizons (Bayne 1976). It has been observed that planktonic larvae in mixed water columns are likely to be more evenly distributed through the water column than in stratified regions (Raby et al 1994). In the present study, regions with mixed water column structure showed greater contributions from pelagic life stages of benthic organisms (for example mollusc, echinoderm and annelid larvae) than their stratified counterparts. The greater proportion of larval life stages in these regions suggests enhanced coupling between pelagic and benthic horizons. This could be aided by increased tidal forcing and wind stress maintaining these mixed water column structures (Simpson and Sharples 2012).

3.4.2 An examination of physical and trophic dynamics using carbon and nitrogen stable isotopes

Carbon and nitrogen isotopes have previously been used to determine trophic relationships between prey and consumers due to known enrichment between trophic levels (DeNiro and Epstein 1981; Vander Zanden and Rasmussen 2001; Post 2002). Here, these tools have aided assessment of interactions between different phyla, linearity in pelagic and benthic systems, and trophic adaptations between different regions of the continental shelf sea.

3.4.2.1 Interpreting trophic relationships and carbon input from $\delta^{15}N$ and $\delta^{13}C$ – interaction of phyla at different regions of the shelf

In the present study, the mean nitrogen isotope values for individual phyla were significantly enriched (> 4‰) at the mixed sites when compared to the stratified sites. Carbon isotope mean values for individual phyla also showed significant enrichment of 1.7‰ in the mixed verses stratified water columns. Seasonal stratification in the Celtic Sea causes nutrient limitation in the surface waters. This reduces nitrate availability to the system and an alternative inorganic pathway of ammonium assimilation may occur

(McCarthy et al 1977; Sarimento and Gruber 2006). There is evidence for this occurring in the Celtic Sea as δ^{15}N-nitrate is enriched at the mid shelf when compared to the shelf break (Pers. Comm. Mahaffey). This could lead nitrogen isotopes in pelagic and benthic organisms to be depleted when compared to regions with mixed water column structure. Consequences of nitrate limitation have been observed during summer stratification in the Bering Sea, where nutrient availability in the water column is reduced by stratification, therefore restricting resources to higher trophic levels (Sambrotto et al 1986; Whitledge et al 1986; Ladd and Stabeno 2012). Mixed water column structures were observed to have enriched carbon isotope values. Bacterial remineralisation within the water column can also affect the organic matter pool that is utilised by pelagic and benthic organisms. It is known that bacterial remineralisation in pelagic and benthic environments can enrich carbon values by up to 2.3‰ compared to the original carbon source (Coffin et al 1990).

3.4.2.2 Linearity within the pelagic and benthic system

Assessing the linearity of the pelagic and benthic communities through correlating carbon and nitrogen values has provided some clues as to the partitioning of carbon through the food chain. In Polunin et al (2001), strong positive correlations were observed in deep-sea communities where marine snow was found to be the primary source material. In contrast, δ^{15}N/δ^{13}C correlations in the Mediterranean littoral zone have much weaker relationships, due to the diversity of organic source material (Pinnegar and Polunin 2000; Polunin et al 2001). In this case, organic material was originating from primary production within the water column, sea grasses and microalgae contributing to the diverse structure between trophic levels of the food web (Pinnegar and Polunin 2000). In the present study, correlations at all sites were found to be positive but weak. LB-M and IS-M were found to have the weakest correlations which suggest that the food sources to the system are complex with more than one trophic pathway (Polunin et al 2001; Drazen et al 2008). At CS1-SS, CS2-SS and SB-S a stronger association and positive relationship suggests a more linear food chain, with a single photosynthetic food source and less trophic complexity (Polunin et al 2001; Drazen et al 2008).

Analysis of the estimated number of trophic levels within each distinct physical region revealed potential changes within the complexity of food web structure. In other studies,

near shore regions with mixed water column structure have shown increased complexity within the food web due to anthropogenic and freshwater inputs into these systems (Gowen and Stewart 2005; Howarth and Plamer 2011). In the present study, a greater number of estimated trophic levels were identified in mixed when compared to stratified sites (Figure 3.12). One can hypothesise that systems with a greater number of trophic levels have increased capacity for energy transfer between organisms and therefore for increased efficiency in carbon transfer from the atmosphere and ultimately burial in sediments (Dickman et al 2008). It has been long been argued that that there is a positive relationship between the number of trophic levels and productivity (Oksanen et al 1981). Hence regions of mixed water column structure in the Celtic Sea are likely to be more productive than stratified waters. This was observed to be true from the estimation of primary production. Primary production in the mixed regions was 661 mg C m^{-2} d^{-1}, whereas in the stratified regions production ranged from 371-446 mg C m^{-2} d^{-1} (Panton 2012).

3.4.2.3 Utilising resources – adaptation to different physical environments

Benthic data suggest that material is being processed in the water column differently under mixed and stratified waters. Benthic molluscs and annelids are known to utilise resources in different ways by changing feeding strategies depending on water column structure and food available to them (Jaksić 1981). In this study, greater enrichment in ^{15}N of suspension feeders in mixed water columns was observed suggesting that these organisms are consuming larger particles of organic matter, possibly at a higher trophic level than their counterparts in stratified regions (Jennings et al 2002). In mixed waters, suspension feeders could also be actively selecting larger particles as they are recycled back into the boundary layer following resuspension from the benthos (Davenport and Bax 2002). Turbidity within the water column and boundary forces routinely re-suspend material into the water column forcing the suspension feeders to adapt to the dynamic conditions, change feeding strategy and harness nutrient resources differently to ensure optimum resource utilisation is obtained (Gili and Coma 1998; Simpson and Sharples 2012).

3.4.3 Conclusion

Five contrasting site across the North West European Continental Shelf were studied in order to attempt to understand pelagic and benthic community structure. Despite the small

size of shelf seas, they contribute significantly to primary production, carbon export and global fisheries consequently it is important to understand the interactions of organisms that inhabit them under different physical conditions (Pauly et al 2002).

Community composition and isotope techniques were employed to address the three original hypotheses posed earlier in the chapter:

1) Pelagic and benthic communities are influenced by water column structure and organisms are able to change feeding strategies to cope with different physical conditions in these environments. In regions with increased turbidity and mixing evidence of coupling is stronger and evidence of resuspension of benthic larval species is observed.

The variation in physical water column structure across the NW European Continental Shelf requires organisms to locate themselves in regions that they are best adapted for to ensure feeding and reproductive strategies are maintained. As a result the patchiness in pelagic and benthic communities is observed as organisms spatially arrange themselves to maximise these feeding and reproductive potentials.

2) Changing feeding strategies in different physical conditions leads to individual species displaying different trophic behaviour due to varying carbon sources and specialisation.

Variation between the different physical environments was found to be statistically significant for the nitrogen isotopes particularly between the mixed and seasonally stratified regions. These variations were influenced by nutrient availability determined by water column structure. Mixed regions and the shelf break were statistically more similar than first imagined, potentially due to the increased mixing and nutrient fluxes in these regions.

3) Dynamics at the edge of the continental shelf supports different biological communities. Evidence of production at higher trophic levels may be apparent through larval and egg populations.

In sampling across the shelf, subtle changes in community structure were observed in the zooplankton community, complementing earlier observations of phytoplankton community

change at the shelf break. This re-emphasises the importance of the shelf edge for maintaining production at higher trophic levels and sustaining fish stocks.

Sampling was conducted at fairly coarse resolution, both in terms of sampling stations and water column horizons. In order to resolve the shelf dynamics more clearly a greater level of replication and finer scale sampling would be required over a seasonal time frame. Pelagic dynamics could be further resolved by sampling at more depths within the water column and attempting to pin-point zooplankton communities more accurately. This could be done with the use of a more advanced vertical net haul, for example a MultiNet. This system incorporates several zooplankton nets vertically pulled though the water column at the same time with each net opening at different depth horizons. Variability and patchiness of the benthic community could be further assessed by sampling with coring devices as well as grabs. The use of coring devices alongside grabs would help reduce sampling bias often experienced with grab sampling. The depth at which the grab enters the sediment (bite profile) is a common source of error and complimentary core sampling would help reduce this error and achieve consistency of sampling depth. A coring device that is capable of collecting multiple cores with one deployment reduces replication error associated with grab sampling. With this level of sampling effort it may be possible to better assess the patchiness of the community and investigate the plasticity of organisms that inhabit this dynamic environment.

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3.6 Appendix

Table 3.9 Complete species pelagic list expressed as percentage composition per vertical net haul. Data shown as upper and lower water column horizons, in stratified regions, are also shown alongside full water column net hauls.

Phyla/Species	Feeding guild	LB-M	IS-M	CS1-SS			CS2-SS			SB-S		
				Portion of the water column			Portion of the water column			Portion of the water column		
				Full	Upper	Lower	Full	Upper	Lower	Full	Upper	Lower
Annelida												
Sabellaria spp	WSF		3.19									
Polychaete larvae	WP		0.91									
Tomopteris spp	WP		0.03				0.06	0.01	0.05	0.84	0.17	0.67
Arthropoda												
Podon spp	WO	4.38		0.01		0.01						
Evadue spp	WO	6.78	1.32									
Mysid Parathemisto	WP		0.16	0.13	0.06	0.07	0.45	0.29	0.15	0.34	0.35	0.01
Mysid	WP			0.27	0.07	0.20	0.19	0.07	0.12	0.01		0.02
Crab larvae	WO	3.14	0.71					0.01		0.02		
Sand crab larvae	WO						0.05		0.05			10.04
Pseudocalanus spp	WH/SF	18.51	83.77	59.9	64.91		67.63	59.9	7.69	82.36	72.32	
Paracalanus spp	WH/SF			35.7	19.97	15.79	24.79	21.3	3.49	9.34	9.58	0.38
Eucalanus spp	WH/SF						0.14	0.07	0.07	0.86	0.48	1.39
Copepod naupli	WH/SF	3.14	2.20				0.13	0.02	0.11	4.14	2.75	
Chaetognatha												
Sagitta spp	WP	1.82	0.07	1.25	0.89	0.35	2.74	2.56	0.19	1.04	0.48	0.56
Chordata												
Fish eggs	WP						0.54	0.98		0.22	0.09	0.13
Salps	WO						0.21	0.28		0.12	0.07	0.06
Ctenophora												
Ctenophore	WP	6.20	0.36	0.01		0.01	0.20	0.10	0.07	0.20	0.15	0.06
Dinoflagellata												
Noctiluca spp	WG	46.69										
Echinodermata												
Pluteus	WG	3.14	0.07	2.18	2.89		1.55	0.95	0.60	0.25	0.12	0.12
Echinocyamus pusillus	WG			0.14	0.39		0.20	0.13	0.07	0.09		0.09
Adult brittle star	WO						0.02		0.02			
Mollusca												
Juvanile bivalve	WSF	6.20	6.78									
Gastropod larvae	WG		0.43	0.34	0.14	0.20	1.1	0.26	0.84	0.17	0.07	0.10

Total	**100**	**100**	**100**	**89.33**	**16.63**	**100**	**86.99**	**13.51**	**100**	**86.62**	**13.36**

Table 3.10 Complete benthic species list expressed as percentage composition per Day grab. Note there is no benthic data for SB-S.

Phyla/Species	Feeding Guild	LB-M	IS-M	CS1-SS	CS2-SS
Annelida					
Lumbrineris laterilli	BP			3.50	
Lumbrineris spp	DF/O/BP/S				2.32
Golfingia spp	DF/SF	0.71			
Hubrechtella dubia	DF			5.24	
Aphrodita aculeata	BP	0.71			
Anaitides maculata	DF	0.71			
Glycera rouxi	DF/O/BP/S	0.71	1.00		2.01
Glycera tridactyla	DF/O/BP/S			2.62	
Syllidae (family)	DF/O/BP/S	1.42			
Nephtys cirrosa	BP			1.75	
Nephtys hombergi	BP		1.00		
Paranaitis kosteriensis	BP	0.71			
Sphaerosyllis bulbosa	DF		1.00		1.54
Bispira volutacornis	SF	0.71			
Circeis spp	SF		1.00		4.63
Sabella pavonina	SF		1.00		
Chaetopterus variopedatus	DF/SF		1.00		
Fabricola spp	SF				
Nicolea venustula	DF/SF		3.70		
Pectinaria auricoma	DF		2.00		
Trichobranchus glacialis	DF		2.30		
Unknown polycheate spp	DF/SF		6.50		3.09
Arthropoda					
Amphilochidae (family)	DF		3.00		
Apherusa spp	SF	1.42			
Bathyporeia spp	DF	0.71	1.00		6.18
Bathyporeia guilliamsoniana	DF	1.06		3.50	
Iphimedia spp	DF		1.00		3.09
Phoxocephalus spp	DF/SF				1.54
Pontocrates spp	DF/O/BP/S	0.71			1.54
Urothe spp	DF	1.42			
Unknown spp	DF/O/BP/S		2.30		1.54
Calanoid copepod	DF/SF				21.63
Pseudocuma spp	DF/SF				1.54
Galathea strigosa	DF/O/BP/S		1.00		
Carcinus maenas	BP		4.50	1.75	1.54

	Pasiphaea spp	DF/O/BP/S		1.00		
	Pasiphaea sivado	DF/O/BP/S				3.09
	Pagurus bernhardus	BP				
	Eurydice spp	BP		2.50		1.54
	Nymphon spp	DF/O/BP/S		1.00		
	Pseudoparatanais spp	DF/SF			3.50	1.54
Bryozoa						
	Cryptosula pallasiana	SF	1.06	2.67		2.06
	Smittoidea reticulata	SF	4.26	8.00		1.54
	Unknown spp	SF		2.50		
Cnidaria						
	Sea anemone	DF/O/BP/S			1.75	
Echinodermata						
	Echinocyanms pusillus	DF/O/BP/S	12.55	3.00	9.97	14.37
	Ophiura affinis	DF/O/BP/S		6.00	4.72	10.04
Mollusca						
	Emarginula fissura	DF				1.00
	Glycymeris glycymeris	SF				3.00
	Epitonium spp	DF/O/BP/S		1.75		
	Eulima bilineata	G	0.71			
	Melanella alba	G	0.71			
	Turritella communis	SF		3.50		
	Astarte sulcata	SF				2.30
	Retusa obtusa	BP		1.75		
	Lepidochitona cinereus	DF				3.00
	Patella spp	G				1.00
	Hiatella arctica	SF				1.00
	Brachystomia eulimoides	G				1.00
	Odostomia plicata	G	3.55			
	Odostomia spp	G		1.75		
	Odostomia unidentata	G	0.71			
	Limaria hians	DF/SF			1.54	1.00
	Alvania punctura	DF			1.54	
	Aporrhain pespelecani	DF		1.75	1.54	
	Lacuna vincta	DF/SF	0.71			
	Lunatia catena	DF/SF	2.13	6.99	4.63	
	Trivia monacha	DF/SF				1.00
	Boreotrophon truncatus	DF/SF		1.75		1.00
	Colus islandicus	DF/SF		1.75		
	Neptunea antiqua	DF/O/BP/S				1.00
	Oenopota turricula	DF/O/BP/S	1.42			

	Trophonopsis muricatus	G	0.71			1.00
	Nucula nitidosa	DF				5.50
	Palliolum tigerinum	SF		1.75		
	Angulus tenuis	SF	1.42			
	Arctica islandica	SF		1.75		
	Circomphalus casina	SF			1.54	
	Dosinia exoleta	SF	0.71			
	Parvicardium scabrum	SF	44.68	1.75	2.78	5.70
	Spisula elliptica	SF	1.63			
	Tellina donacina	DF/SF	2.84			
	Tellina pygmaea	DF/SF	0.71			
	Lutraria lutraria	SF		1.75		
	Calliostoma zizyphinum	G				2.50
	Gibbula magus	G				1.00
	Gibbula tumida	DF	2.84			2.00
	Jujubinus montagui	DF	1.42			1.00
	Margarites groenlandicus	DF/SF	4.26			2.00
	Margarites helicinus	DF/SF		1.75		
	Scaphopod tubes	DF/O/BP/S		31.99		
Porifera						
	Alcyonium digitatum	BP		1.00		
	Sycon ciliatum	SF		3.00		

Table 3.11 Complete pelagic and benthic species list for carbon and nitrogen stable isotope data, expressed with standard error. Note there is no isotope benthic species data for SB-S.

		LB-M		IS-M		CS1-SS		CS2-SS		SB-S	
Phyla/Species	**Feeding guild**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**
POM											
POM Surface	POM			1.8 (± 0.4)	-22.4 (± 0.5)			3.4 (± 0.1)	-24.1 (± 0.4)	1.7 (± 0.7)	-22.1 (± 0.5)
POM SCM	POM					5.5 (± 0.5)	-17.3 (± 0.4)	3.8 (± 0.1)	-26.1 (± 0.4)	4.3 (± 0.2)	-21.7 (± 0.9)
POM Bottom	POM			10.8 (± 0.5)	-20.9 (± 0.2)	9.0 (± 0.2)	-19.2 (± 0.5)	11.7 (± 0.5)	-19.7 (± 0.5)	10.4 (± 0.8)	-21.2 (± 0.2)
Annelida											
Anaitides maculata	DF	15.7 (± 0.2)	-17.5 (± 0.2)								
Aphtodita aculeata	BP	13.1 (± 0.0)	-18.1 (± 0.5)								
Bispira volutacoruis	SF	12.3 (± 0.1)	-20.0 (± 0.7)								
Chaetopterus variopedatus	DF/SF			9.1	-20.7						
Circeis spp	SF			9.0	-21.6			7.3	-17.0		
Glycera tridactyla	DF/O/BP/S					10.8 (± 0.1)	-19.2 (± 0.7)				
Glyceridae rouxi	BP	17.2 (± 0.1)	-17.4 (± 0.4)	13.7 (± 0.3)	-18.3 (± 0.5)						
Golfingia spp	DF/SF	14.5 (± 0.2)	-19.1 (± 0.0)								
Hubrechetella dubia	DF					10.4 (± 0.3)	-14.7 (± 0.8)				
Lumbrineris latreilli	DF/O/BP/S					8.9 (± 1.2)	-16.8 (± 0.2)				
Lumbrineris spp	DF/O/BP/S							8.9	-19.2		
Nephtys cirrosa	BP					11.1	-18.5				
Nephtys hombergi	BP			13.1 (± 0.1)	-18.2 (± 0.3)						
Nicolea venustula	DF/SF			8.9 (± 0.3)	-19.2 (± 0.4)						
Paranaitis kosterieusis	BP	15.2 (±0.1)	-16.5 (± 1.4)								
Pectinaria auricoma	DF			8.4	-20.4						
Sabella pavonia	SF			8.4 (± 0.3)	-24.6 (± 0.2)						
Sphaerosyllis bulbosa	DF			1.8 (± 0.3)	-16.6 (± 0.8)			7.7	-17.6		
Syllidae (family)	DF/O/BP/S	15.3	-19.2								
Trichobranchus glacialis	DF			9.3 (± 0.5)	-23.2 (± 0.3)						
Unknown bristle worm	BP					9.4 (± 0.1)	-13.1 (± 0.4)				
Unknown plotcheate	DF/SF			9.9 (± 1.1)	-18.5 (± 0.6)			6.0	-20.3		
Polycheate larvae	WP	13.1 (± 0.1)	-19.9 (± 0.1)					-0.5	-21.5	6.2	-20.1 (± 0.4)
Arthropoda											
Amphillochidae (family)	DF			7.6 (± 0.1)	-21.4 (± 0.7)						
Apherusa spp	SF	10.9	-20.4								
Bathyporeiaguilliamsoniana	SF	7.3 (± 0.0)	-21.5 (± 0.5)			7.3	-23.6				
Bathyoreia spp	SF	15.1	-23.2	7.9	-20.7			7.2 (± 0.4)	-19.2 (± 0.1)		
Calanoid copepod	DF/SF							8.2 (± 0.3)	-21.1 (± 0.6)		

Carcinus maenas	BP			8.9 (± 0.4)	-20.6 (± 1.4)	8.5 (± 0.2)	-23.8 (± 1.6)	8.1	-18.7		
Eurydice spp	BP			10.6	-22.2			6.2	-18.9		
Hippomedon denticulatus	DF	2.7	-20.9								
Iphimedia spp	DF			7.7	-20.8			6.1	-18.83		
Nymphon spp	DF/O/BP/S			9.63	-17.4						
Pasiphaea sivado	DF/O/BP/S							7.4 (± 0.4)	-19.8 (± 0.6)		
Pasiphaea spp	DF/O/BP/S			9.5	-16.3						
Pontcrates spp	DF/O/BP/S	18.9	-20.8								
Psedoparatanais spp	DF/SF					6.8	-18.6	6.4	-20.6		
Pseudocuma spp	DF/SF							2.5	-20.6		
Pseudoparatanais	DF/SF							4.6	-20.1		
Unknown amphipod	DF/O/BP/S			8.1	-21.2						
Urothe spp	DF	12.9 (± 1.3)	-21.5 (± 0.7)			8.9	-23.3	3.8	-21.5		
Amphipod	WP									7.3 (± 0.3)	-21.6 (± 0.1)
Copepod	WH/SF	12.2 (± 0.2)	-20.5 (± 0.3)	10.4 (± 0.2)	-19.7 (± 0.9)	8.6 (± 0.2)	-27.7 (± 0.8)	8.0 (± 0.1)	-23.7 (± 0.5)		
Copepod large	WH/SF							6.3 (± 0.6)	-23.4 (± 0.5)		
Copepod pink	WH/SF					7.7 (± 0.1)	-26.6 (± 0.5)			6.8	-20.3
Copepod small	WH/SF							4.4	-20.3	11.2	-18.9
Crab larvae	WO	12.5	-17.9					5.4	-22.1	5.8	-20.4 (± 0.4)
Eucalanus spp	WH/SF										
Evadue spp	WO	12.3	-25.4			7.8 (± 0.1)	-24.7 (± 1.0)	7.0 (± 0.5)	-21.8 (± 0.1)	6.7	-19.7
Mysid	WP		-20.1	9.7	-18.8						
Podon spp	WO	13.9	-17.5								
Cetnophora											
Ctenophore	WP	14.5 (± 0.2)	-18.2(± 0.8)							12.0 (± 0.3)	-17.8 (± 0.4)
Chaetognatha											
Arrow worm	WP			13.1	-18.6	10.5 (± 0.5)	-23.3 (± 0.7)	9.8 (± 0.4)	-21.9 (± 0.4)	8.1 (± 0.9)	-18.8 (± 0.5)
Chordata											
Fish egg	WP	15.9	-15.6					9.0 (± 0.2)	-22.9 (± 0.4)	9.9	-19.5
Fish larvae	WP							5.5	-19.9		
Salps	WO			10.4	-19.0			4	-21.6	5.3	-19.4 (± 0.4)
Echinodermata											
Echinocyanms pusillus	DF/O/BP/S					-1.9	-6.9				
Ophiura affinis	DF//BP/S			8.8 (± 0.3)	-12.7 (± 0.0)	4.0 (± 0.2)	-7.6 (± 0.6)				
Pleutus	WG					5.1 (± 0.0)	-25.9 (± 0.5)	5.2	-23.3	6.6	-19.9
Mollusca											
Aporrhais pespelecani	DF					7.4 (± 0.1)	-19.5 (± 0.4)	6.7 (± 0.3)	-19.7 (± 0.7)		
Arctica islandica	DF/O/BP/S					7.0 (± 0.0)	-25.5 (± 0.4)				

Astarte salcata	SF			9.8 (± 0.2)	-22.3 (± 0.8)				
Brachystomia scalaris	WG					2.1	-5.6		
Calliostoma zizyphinum	WG			9.2	-23.7				
Circomphalus casina	SF							3.8 (± 0.6)	-22.0 (± 0.1)
Dosinia exoleta	SF	11.2 (± 0.3)	-21.4						
Emarginula fissura	DF			12.1	-22.4				
Glycymeris glycymeris	SF			10.1	-20.3				
Lepidochitona cinereus	DF			12.1 (± 0.4)	-13.7 (± 0.1)				
Limaria hians	DF/SF			7.5	-23.1				
Lutraria lutraria	SF					6.0	-26.1		
Netunes antiqua	DF/O/BP/S			9.4	-20.2				
Nucula nitidosa	DF			6.5 (± 0.4)	-20.3 (± 0.8)				
Palliolum tigerinum	SF					7.9	-23.6		
Parvicardicum scabrum	SF			6.7 (± 0.7)	-23.2 (± 0.7)			5.2	-16.8
Patella spp	WG			8.4 (± 0.3)	-22.1 (± 1.5)				
Phoxocephalus spp	DF							8.8	-20.4
Retusa obtusa	DF/SF					8.3 (± 0.9)	-6.7 (± 1.5)		
Tellina donacina	DF/SF	0.1 (± 0.5)	-20.2						
Tellina pygmaea	DF/SF	-0.5	-20.6						
Turritella communis	SF					6.7 (± 0.2)	-28.2 (± 0.2)		
Gastropod larvae	WG					2.6		6.2	-21.0
Porifera									
Alcyonium digitatum	BP			9.1 (± 1.4)	-13.3 (± 3.0)				
Sycon ciliatum	SF			9.2 (± 0.1)	-15.6				

Chapter 4

Wind events and simulation experiments – impact of SCM erosion on biological and biogeochemical parameters

4. Introduction

4.1.1 Background

This chapter focuses on the consequences of a wind-induced mixing event, both through observation of a natural event (*in situ* observations) and through an experimental simulation on-board ship at single site, CS1 (

Figure **3.1**). In Chapter 2, the physical dynamics of shelf seas have been described, illustrating the importance of frictional boundary layers, tidal currents, wind stress and solar heating to the structure of the water column described (Simpson and Hunter 1974; Sharples et al 2007; Simpson and Sharples 2012). It is clear that in shelf seas in the summer months, seasonal stratification acts as a barrier between surface waters and high light-availability and nutrient-rich deeper waters (e.g. Burchard and Rippeth 2009).

4.1.2 The dynamics of wind-induced mixing events

Wind-induced mixing events are known to disrupt seasonal stratification and to inject nutrients from bottom waters into the upper water column (Walsh et al 1978; Kiørboe and Nielsen 1990). Surface wind stress enhances shear at the base of the surface layer which triggers instability and turbulence within the thermocline. This results in vertical mixing and as observed at CS1, stratification is weakened and the surface layer deepened (Burchard and Rippeth 2009). The partial breakdown of the stratified water column structure has key impacts on nutrient fluxes, utilisation and primary production (Sharples et al 2007). Periods of turbulence influence the distribution of organic matter and organisms within the water column (Rothschild and Osborn 1989; Visser et al 2001). During mixing, impacts on zooplankton communities have also been noted with them reacting to an increase of primary production in hours to days (Nieksen and Kiørboe 1991).

4.1.3 Aims and hypotheses

The aim of this study was to investigate the impact of a wind-induced mixing event on the composition of suspended POM and community structure. I*n situ* observations and experimental incubations were undertaken.

Two hypotheses were tested:

1) In the event of mixing within a stratified water column (even if there is only minimal erosion of the DCM and the thermocline remains intact) the distribution of POM becomes dispersed differently through the water column. This is apparent through the composition of POM and biomarker evidence.

2) During a mixing event within the stratified region of the shelf sea, chlorophyll is distributed more evenly thorough the water column. As a result of this chlorophyll homogenisation through the water column pelagic organisms are also observed to orient themselves differently optimising food intake and ultimately growth and reproduction.

Simulation experiments were carried out under realistic conditions in order to verify *in situ* field observations. It was expected that an increased growth of phytoplankton stimulated through nutrient flux would affect populations of heterotrophic organisms (zooplankton + bacteria).

4.1.4 Study site

The seasonally stratified site, CS1, has been described previously (Chapter 2;

Figure **3.1**; Figure 3.2). Details of the date of sampling, nominal depth (m), latitude and longitude for the *in situ* sampling (before and after the wind event, CS1-pre and CS1-post) and incubation experiments are shown in Table 4.1.

Table 4.1 Date of sampling, nominal depth (m), latitude and longitude at each sampling site.

	Date	**Nominal depth (m)**	**Latitude**	**Longitude**
CS1-pre	5 June 2010	137	49 25.11N	008 59.47W
CS1-post	9 June 2010	135	49 25.55N	008 59.73W
Incubation	6 June 2012	146	49 24.47N	008 59.16W

4.2 Methods

4.2.1 *Analysis of the water column structure and water collection for incubation experiments*

CTD profiles were constructed using the method described in Chapter 2 (section 2.2.1). Depths and horizons of POM samples and subsequent analyses are recorded in Table 4.2.

Table 4.2 Sampling stations detailing total depths and horizons of suspended POM sampling and subsequent analyses performed on filters; total functional lipids (TFL), stable isotope analysis (SIA) and high pressure liquid chromatography analysis (HPLC) and benthic and pelagic community structure.

	Nominal water column depth (m)	sPOM – depth horizon sampled (m)			TFL	SIA	HPLC/ Phyto. community	Benthic and pelagic community
		Surface	SCM	Bottom				
CS1-pre	13	-	40	100	X	X	X	X
CS1-post	135	5	50	100	X	X	X	X
Incubation control	146	10L of SCM water (37m)			X		X	
Incubation treatment	146	9L of SCM water (37m) spiked with 1L BML water (70m)			X		X	

Two incubation experiments were carried out on deck at ambient sea surface temperature and at 4% surface incident light intensity (simulating light levels at the subsurface chlorophyll maximum). Polycarbonate carboys (10 L) were used for incubations. These were pre-washed with 10% hydrochloric acid, deionised water and the seawater sample. Non-nutrient addition incubations were used to assess the community composition in an isolated scenario, without injection of deep nutrients; water from 37 m water depth (SCM) was used and incubated for 96 h. Water for treatment incubations was also collected via the CTD from the SCM and spiked with 1 L of water from 70 m (BML). Phytoplankton species identification, total functional lipids and pigment analyses were conducted for each incubation experiment (Table 4.2). Sampling was conducted at time points 0 h, 24 h, 48 h and 96 h. Each incubation, both with and without nutrient addition, was conducted in replicate, so each carboy could be sacrificed after each sampling effort.

4.2.2 Phytoplankton community analyses (phytoplankton community structure sample collection and analysis conducted by A. Panton, UOL).

Phytoplankton community structure through pigment analysis was determined using the same methodology described in Chapter 2 (section 2.2.4;Table 2.5). Microscopy was conducted using the method described by Utermohl (1931) and identification carried out according to Tomas (1997) (Chapter 2; section 2.2.2).

4.2.3 Collection of suspended particulate organic matter (sPOM)

Suspended particulate organic matter (sPOM) was collected using the *in-situ* stand alone pumps (SAPs; Challenger Oceanic), as described in Chapter 2 (section 2.2.3;Table 2.3).

4.2.4 Pigment extraction and high pressure liquid chromatography (HPLC) analysis (HPLC analysis partly conducted by N. Carr, UOL)

Pigment extraction for HPLC analysis was carried out according to Chapter 2 (section 2.2.4). To ascertain phytoplankton community structure and taxa in both the natural *in situ* samples and in the simulation experiments, the biomarker approach was used based on the literature (Table 4.3). This approach was used in conjunction with the phytoplankton microscopy data.

Table 4.3 Pigments extracted during the HPLC process, the biomarkers that they represent and the literature sources.

Pigment	Biomarker	Literature
19 – butanoyloxyfucoxanthin	Some prymnesiophytes (e.g. coccoliths and *Phaeocystis*), marine chrysophytes (e.g. *Pelagococcus*)	Vesk and Jeffrey 1987 Jeffrey and Wright 1994
19 – hexanoyloxyfucoxanthin	Prymnesiophytes (e.g. coccoliths), some dinoflagellates	Arpin et al 1976 Jeffrey and Wright 1994
Alloxanthin	Cryptomonads (mostly photosynthetic but have an ability to be mixatrophic)	Chapman 1966 Pennington et al 1985
Chlorophyll a	All photosynthetic algae and higher plants	Scheer 1991
Chlorophyll b	Higher plants, green algae, symbiotic prochlorophytes	Scheer 1991
Chlorophyll C3	Some prymnesiophytes (e.g. coccoliths), diatoms and chrysophytes	Vesk and Jeffrey 1987 Jeffrey and Wright 1994
Fucoxanthin	Diatoms (major pigment), prymnesiophytes (e.g. coccoliths), brown seaweeds and some dinoflagellates	Jeffrey and Wright 1994
Lutein	Red seaweed, green algae, higher plants	Hager and Stransky 1970
Peridinin	Autotrophic dinoflagellates	Jeffrey et al 1975

4.2.5 Total lipids, quantification and reproducibility

Total lipids were extracted according to Chapter 2 (section 2.2.5; 2.2.5.1). Quantification was also carried out using X-Calibur (Finnigan Corporation, version 1.0) software through comparison with authentic standards and information from the literature (Table 4.4). The limitations, constraints and specificity of biomarkers have also been previously discussed in Chapter 2. The argements for and against the use of biomarkers remain true for this chapter.

Table 4.4 Source/Indicator lipids used as biomarkers and the literature they originate from.

Lipid biomarker	Source/Indicator	Literature
Diatoms	$C_{16:1}$(n-7)	Budge and Parrish (1998), Reuss and Poulson (2002)
	$C_{20:5}$(n-3) (EPA)	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006)
	$C_{16:1}/C_{16:0}$	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006), Neto (2002)
Dinoflagellates	$C_{20:4}$(n-6)	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006)
	$C_{22:6}$ (DHA)	Budge and Parrish (1998), Reuss and Poulson (2002), Fang et al (2006)
	DHA/EPA	Budge and Parrish (1998), Reuss and Poulson (2002)
Bacteria	$C_{18:1}$(n-7)	Budge and Parrish (1998)
	Branched *iso* and *anteiso* C_{15} and C_{17}	Gillan and Johns (1986), Kaneda (1991)
Zooplankton	$C_{20:1}$	Sargent and Henderson (1986), Kattner et al (2003)
	$C_{22:1}$	Sargent and Henderson (1986), Kattner et al (2003)
	$C_{20:1}$ and $C_{22:1}$ unsaturated alcohol	
General biomarkers	C_{14}-C_{22} saturated fatty acid – derived from phytoplankton	Viso and Marty (1993), Dunstan et al (1994)
	C_{24}-$_{32}$ saturated fatty acids – derived from vascular plants	Harvey (1994), Santos et al (1994)
Labile diatombiomarker	$C_{16:1}$(n-7)/$C_{16:0}$	Najdek (1996), Neto (2002)
Dinoflagellate and prymnesiophyte to diatom ratio	Total C_{18}/Total C_{16}	Najdek (1996), Neto (2002)
Invertebrate reworking of organic matter	C_{27}/C_{29} sterols ratio	Neto (2002)

4.2.6 Stable isotope analyses

The stable isotopic compostion ($\delta^{13}C$ and $\delta^{15}N$) of benthic and pelagic specimens and of suspended POM collected on GF/F filters were determined according to the methodology described in Chapter 2 (section 2.2.7). Benthic and pelagic animals and suspended POM were treated as in Chapter 3 (section 3.2.5).

4.2.7 Community structure and statistical analyses

Benthic and pelagic communities were sampled according to Chapter 3 (section 3.2.2). Having identified pelagic and benthic fauna before and after the wind-driven mixing event, species abundances were calculated and displayed as percentage composition and standardised per vertical water column haul and per benthic grab. The Shannon-Wiener diversity index (H') was calculated for each study site using multivariate statistical software package, PRIMER 6 (Clarke and Gorley 2006), which allows calculation of these biodiversity indices from quantitative species lists. This index of diversity is a commonly used measure of species diversity as it accounts for the number of species in a community (richness) and the relative abundance of species (evenness). The minimum value for the Shannon-Wiener index is 0, which would indicate a community with a single species. A high diversity index shows that species richness and evenness within a community is also high (Molles 1995).

Statistical analyses were then carried out using SigmaPlot (version 11). Statistical significance was tested between before and after the wind-induced mixing events for the following variables; pelagic community structure, benthic community structure, pigment biomarker abundance, lipid biomarker abundance and phytoplankton count data. Shapiro-Wilks tests were employed in order to test normality of the data. T-tests and one-way ANOVA tests were carried out on normally distributed data and Mann-Whitney Rank Sum tests or Kruskal-Wallis tests were carried out on non-normal data. Statistical analyses were also carried out on pigment biomarker abundance, lipid biomarker abundance and phytoplankton count data for the incubation experiment data.

4.3 Results and discussion

4.3.1 Pre- and post-storm observations

4.3.1.1 Physical water column structure

CTD profiles before, during and after the wind-induced mixing event (Figure 4.1) show chlorophyll (mg m^{-2}), salinity (pss), temperature (^{o}C) and transmission (%) measurements at CS1. The impacts of wind-driven mixing events and stratification on biological communities have been discussed previously (Pingree et al 1978; Rothschild and Osborn 1989; Visser et al 2001). During the storm at CS1, the wind stress was not sufficient for complete breakdown of stratification to occur (Williams, Pers. Comm.). However, a decrease in chlorophyll *a* concentration of approximately 1mg m^{-3} was observed at the SCM, which returned to pre-wind event levels after the storm had passed. It appears that the effect of the storm may have caused phytoplankton to be dissipated through the water column due to small scale oscillations having a diluting effect across the SCM (Sharples et al 2001). However, caution has to be taken when making this suggestion as natural variability within the water column may be contributing to the differences observed. Vertical variability of zooplankton, through migration processes, were mitigated against by sampling at the same time of day and by conducting replicate samples (3 vertical net hauls) so variability was reduced.

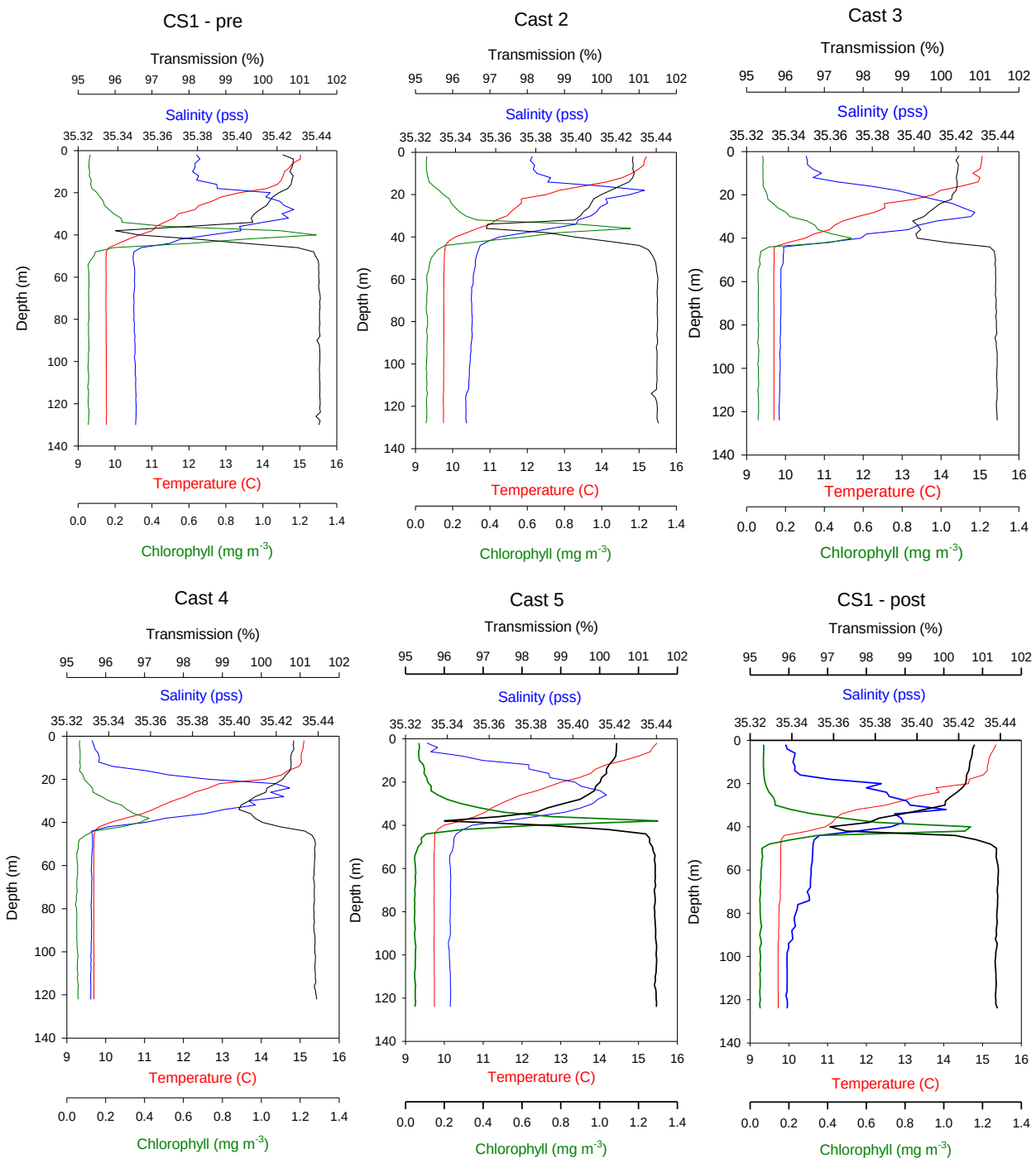

Figure 4.1 Chlorophyll (mg m^{-3}), salinity (pss), temperature (°C) and transmission (%) measurements from CTD casts. CS1-pre and CS1-post show profiles from which biological and chemical samples were taken. Casts 2 to 5 show intermediate profiles during the wind event.

4.3.1.2 Phytoplankton, zooplankton and benthic community structure

A total of 33 and 26 invertebrate and vertebrate taxa (pelagic and benthic) were identified prior to and post the wind-induced mixing event, respectively. Sampling for both pelagic and benthic communities was conducted in replicate. Compositional data are presented as

mean percentages per zooplankton net haul and Day grab (Table 4.5). Full tabulated data for individual species can be found in Appendix 4.7; Table 4.10; Table 4.11).

Table 4.5 Summary of pelagic and benthic species data, grouped by phyla. Pelagic data if shown from upper and lower water column horizons alongside full water column net hauls. Note upper and lower water column hauls may not equal full water column values as separate hauls were taken, leading to slightly different compositions.

	CS1-pre			CS2-post		
Pelagic summary	**Portion of the water column**			**Portion of the water column**		
Phyla	**Full**	**Upper**	**Lower**	**Full**	**Upper**	**Lower**
Annelida	0	0	0	0.07 6.6 (± 0.2)	0	0.05
Arthropoda	95.91 3209 (± 8.9)	85.1 2839 (± 11)	16.11	96.7 12854 (± 156)	47.42 6296 (± 56)	49.3
Chaetognatha	1.2 42 (± 4.4)	0.9 30 (± 5.7)	0.3	0.8 110 (± 5.4)	0.3 36 (± 0.3)	0.6
Chordata	0	0	0	0.03 2 (± 0.5)	0	0.01
Ctenophora	0.01 0.3 (± 0.1)	0	0.01	0	0	0
Echinodermata	2.3 77 (± 1.7)	3.3 109 (± 2)	0	2.3 300 (± 6)	3 399 (± 7.9)	0.02
Mollusca	0.3 11 (± 1.5)	0.1 5 (± 0.3)	0.2	0.1 14 (± 0.6)	0.1 22 (± 0.8)	0.05
Total % composition	**100 3340 (± 52)**	**89.4**	**16.6**	**100 13287 (± 214)**	**50.5**	**50**

Benthic summary		
Phyla	**CS1-pre**	**CS1-post**
Annelida	45.1 64 (± 5)	31.25 16 (± 0.71)
Arthropoda	8.74 5 (± 0)	14.72 4 (± 0)
Cnidaria	1.75 2 (± 2.2)	12.9 7 (± 3.5)
Echinodermata	14.69 25 (± 3.5)	9.19 5 (± 2.1)
Mollusca	61.7 25 (± 0.38)	31.99 16 (± 0.6)
Total % composition	**100 121 (± 8.9)**	**100 39 (±6.9)**

The Shannon-Wiener Index is an inclusive index, taking species richness and evenness into account. Benthic communities are clearly more diverse than pelagic communities (Figure 4.2). In both the pelagic and benthic communities diversity decreased after the wind-induced mixing event.

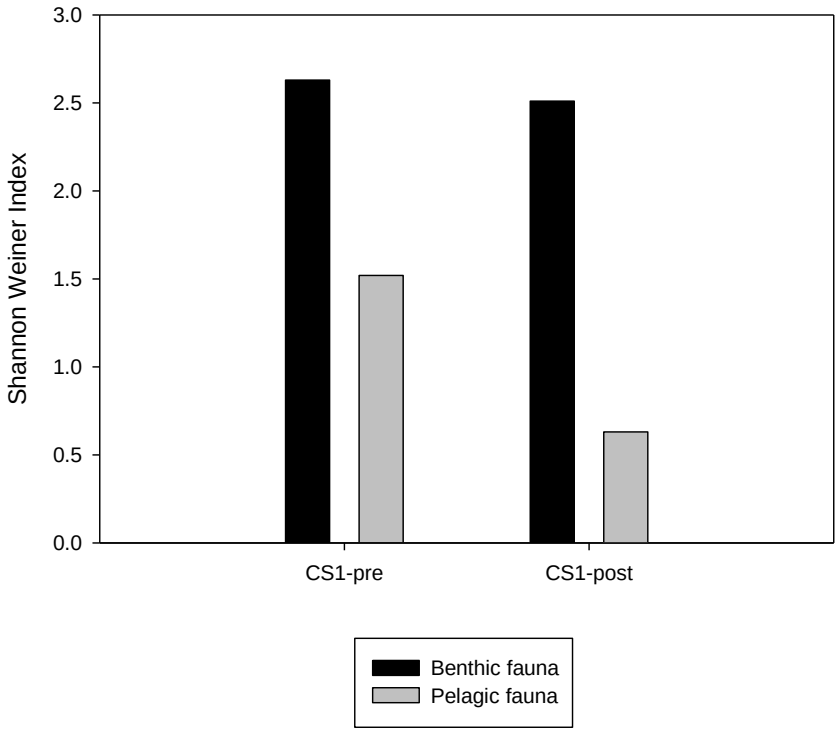

Figure 4.2 Shannon-Weiner Index before and after the wind induced mixing event, pelagic species represented by grey bars and benthic species represented by black bars.

Prior to the wind-induced mixing event, phytoplankton community counts showed fairly similar compositions at the two sampling depths in the water column (Figure 4.3). The plankton community (analysed through microscopy) comprised mainly of diatoms, dinoflagellates and ciliates. A slight increase in dinoflagellate abundance was observed with increasing water depth, the inverse was true with respect to diatoms. However, following the mixing event, ciliates were only observed in surface waters; dinoflagellates were present throughout the water column, while diatoms were present only in small numbers in surface waters and increased in abundance with depth. Microzooplankton were also present in phytoplankton samples in small numbers and were observed throughout the water column. The phytoplankter, *Chattonella* spp., often associated with toxic algal blooms (Smayda

1997), was ubiquitous. Previous studies (e.g. Lagadeuc et al 1997) indicated that during wind-induced mixing, phytoplankton become more evenly distributed throughout the water column. At CS1 there was some homogenisation through the water column as previously discussed, but it should be noted that with these types of measurements, it is difficult to rule out natural variability over the sampling time period and changes in the community structure resulting from horizontal advection.

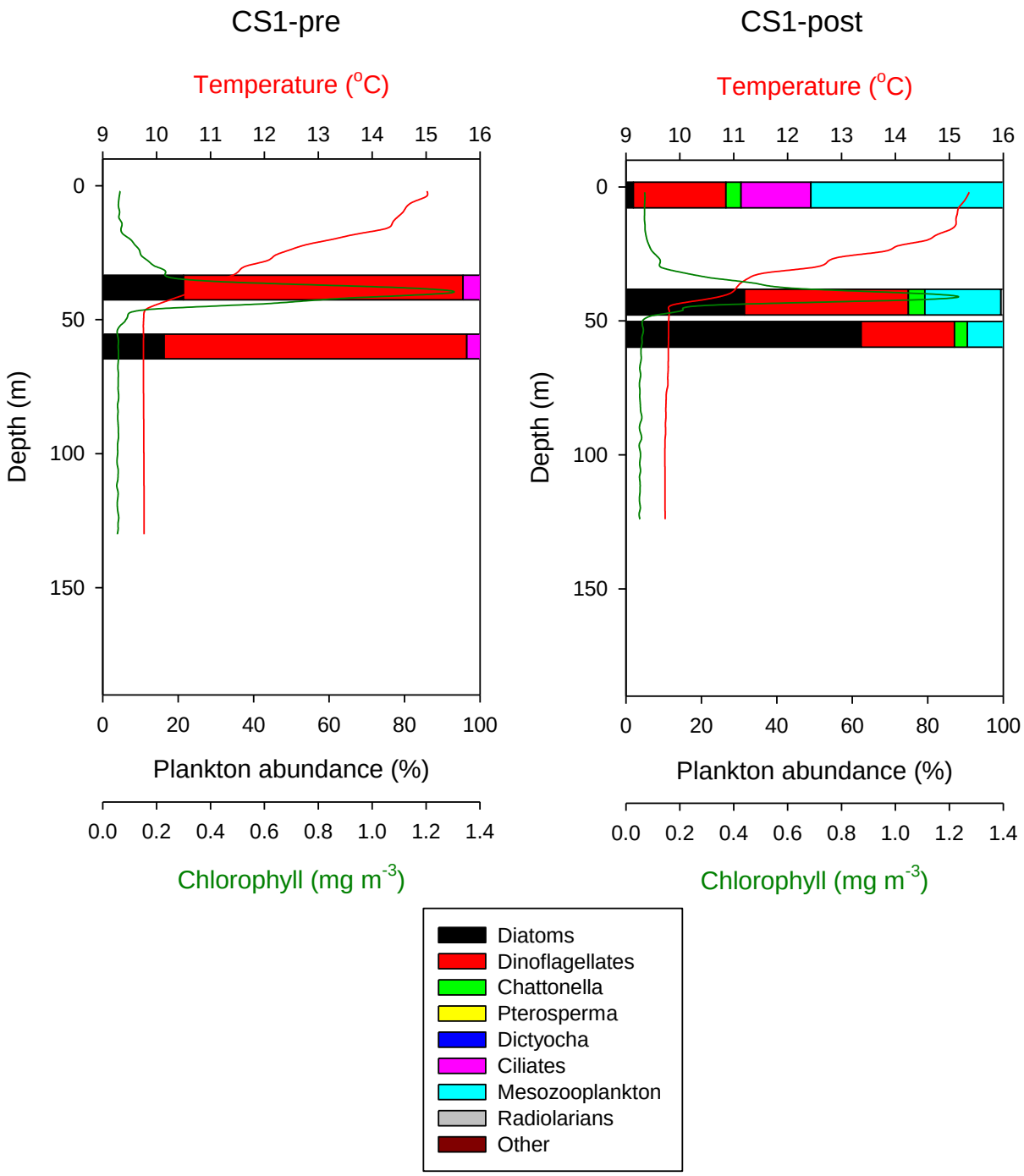

Figure 4.3 Plankton and phytoplankton count data (collected from niskin bottles) from before and after the wind induced mixing event shown as plankton abundance (%) for each depth horizon sampled. Temperature (°C) and chlorophyll (mg m^{-3}) profiles have been shown in the plots for reference purposes.

Zooplankton community structures before and after the wind-induced mixing event were similar (Figure 4.4). On both sampling occasions, Arthropoda were the dominant phylum, while a decrease of the phylum Mollusca was observed after the storm. The most noticeable change was in the distribution of organisms though the water column. There have been relatively few studies of the impact of the erosion of the SCM on the distribution of zooplankton within the water column. However, there is evidence of zooplankton distributions changing, depending on the water column structure. For instance, under mixed conditions a more homogeneous distribution has been reported compared with stratified conditions, where uneven distributions of zooplankton have been observed (Turner and Dagg 1983; Haury et al 1990; Checkley et al 1992). At CS1, pre-storm, 80% of pelagic individuals inhabited the upper part of the water column. However, post-storm, the zooplankton were more evenly distributed though the water column (Figure 4.4). This homogenisation of zooplankton through the water column following a wind-induced mixing event has been observed previously by Lagadeuc et al (1997). Furthermore, during storms, copepods may actively descend to deep layers of lower chlorophyll *a* concentrations (Mackas et al 1993). In contrast, Mullin et al (1985) reported that zooplankton stratification remained similar before and after wind-induced mixing. The distribution of zooplankton could also be linked to the distribution of phytoplankton and for some species, to their inability to swim against strong turbulent mixing.

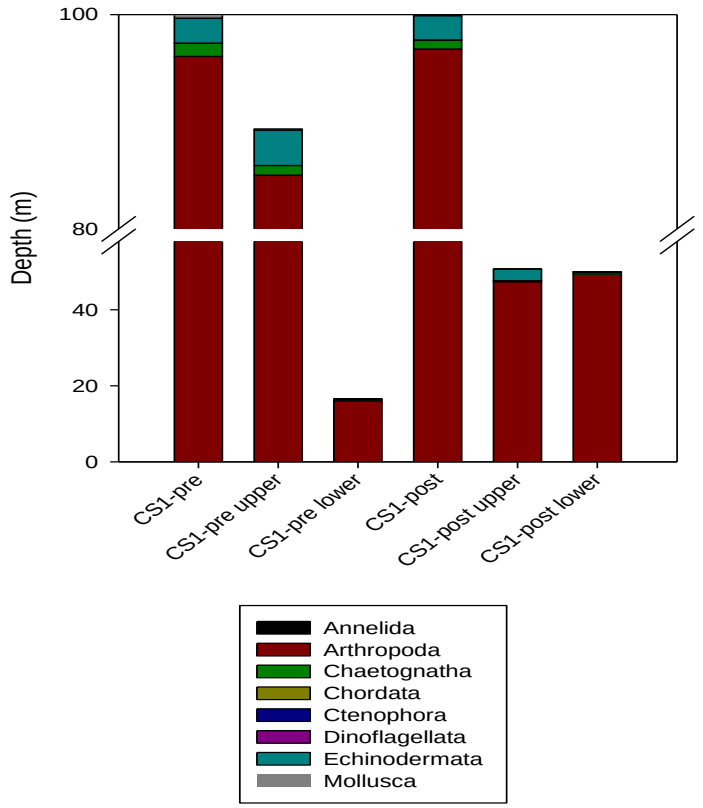

Figure 4.4 Pelagic community data, grouped by phyla, and displayed as relative abundance by % composition. Data are shown for pre and post wind induced mixing event for full water column, upper water column (from the base of the SCM to the surface) and the lower water column (base of the SCM to the benthos).

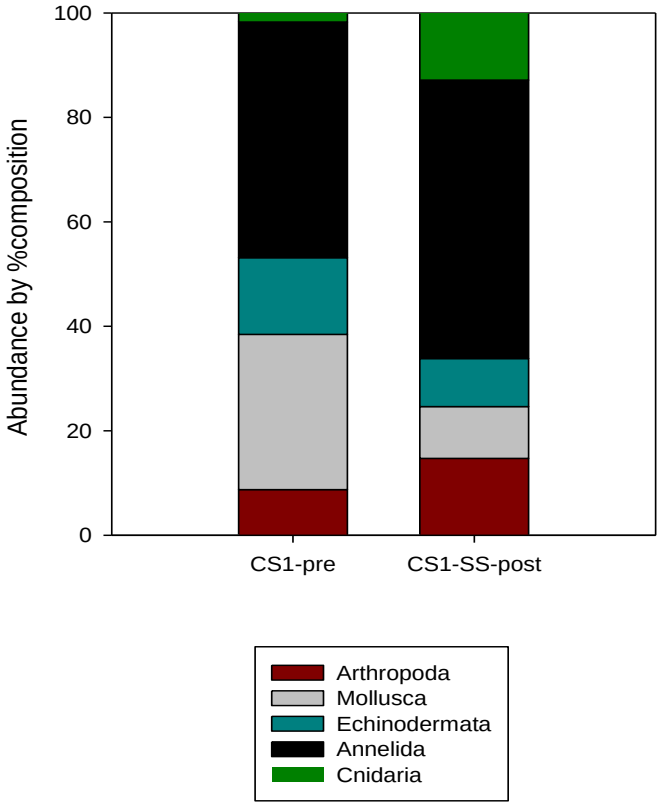

Figure 4.5 Benthic community data grouped by phyla and displayed as relative abundance by % composition.

Changes in benthic community structure following a mixing event have been documented and frequently wave action is responsible for effecting sediment characteristics, organisms' activity and erosion (Grant 1983; Brey 1991). At CS1, the benthic community structure changed between pre- and post-storm (Figure 4.5). Annelida were the dominant phylum before and after the mixing event. Echonodermata and Mollusca were observed to decrease in abundance by ~50%, following the storm, but Arthropoda and Cnidaria increased in abundance. Larval benthic polychaetes and gastropods were present in the pelagic zone after the storm. Resuspension from the mixing could be a possible cause, but it is unlikely in this case that mixing would reach deep into the water column (approximately 135m). However, it has been necessary to adopt a cautious approach in interpreting changes in pelagic and benthic communities, due to the snapshot nature of sampling and the uncertainty in replication (*i.e.* in exact station locations pre- and post-storm) and the likelihood of natural patchiness.

The pelagic and benthic community structures before and after the wind-induced mixing event were subtly different in a qualitative sense. The distribution of pelagic species appears to become more evenly distributed through the water column and the benthos also changed. However, statistical analyses on relative abundances of both communities (t-test) revealed no significant differences between the communities pre- and post-storm (pelagic species $t = -0.90$, $P = 0.37$; benthic species $t = 1.38$, $P = 0.17$). This could reflect the dominant groups remaining before and after the wind event. For example in the pelagic samples, Arthropoda dominated both pre- and post-storm, but there is an increase of Mollusca, possibly due to resuspension from the benthos (Figure 4.4). In the benthic community Annelida dominate pre- and post-storm, but Mollusca are depleted post-storm (Figure 4.5).

4.3.1.3 Particulate organic matter – pigment and lipid biomarkers

Wind induced mixing affects suspended POM and processes within the water column for example by increasing turbidity, nutrient availability and recycling. Wind-driven bed shear-stress induced by sea breezes and storm events has been shown to increase suspension of POM within the water column (Verspecht and Pattiaratchi 2010).

Absolute concentrations of pigments from HPLC analysis before and after the wind event showed similar patterns of pigment distribution through the water column. However, greater concentrations were observed after the wind event (Table 4.6). Greater concentrations were generally observed deeper in the water column with the exception of pigment chlorophyll C3 and chlorophyll b, which prior to the storm had greater concentration in the SCM but after the wind event greater concentration was observed at depth. The compositions of pigments were further examined through percentage composition analysis. Plankton communities through this method have previously been discussed (Figure 4.3) and are referred back to here.

Table 4.6 Table showing the pigment components prior to and after the wind induced mixing event and at each depth, data are shown as μg L^{-1}.

	CS1-pre	CS1-post
Total identifiable pigments		
Surface	n.d	n.d
SCM	1.137	1.781
BML	1.008	10.020
Chlorophyll C3		
Surface	n.d	n.d
SCM	0.226	0.131
BML	0.141	1.335
Peridinin		
Surface	n.d	n.d
SCM	0.000	0.026
BML	0.000	0.000
19-Butanoyloxyfucoxanthin		
Surface	n.d	n.d
SCM	0.125	0.269
BML	0.098	1.275
Fucoxanthin		
Surface	n.d	n.d
SCM	0.309	0.466
BML	0.255	3.214
19-Hexanoyloxyfucoxanthin		
Surface	n.d	n.d
SCM	0.000	0.183
BML	0.152	0.659
Alloxanthin		
Surface	n.d	n.d
SCM	0.063	0.068
	0.052	0.363
Lutein/Zeaxanthin		
Surface	n.d	n.d
SCM	0.000	0.056
BML	0.046	0.246
Chlorophyll b		
Surface	n.d	n.d
SCM	0.056	0.043
BML	0.015	0.268
Chlorophyll a		
Surface	n.d	n.d
SCM	0.358	0.540
BML	0.249	2.659

Pre wind event: At the SCM, a greater proportion of diatom biomarkers (fucoxanthin; Jeffery and Wright 1994) were present compared to the lower water column (Figure 4.6); this trend was also observed in microscopy phytoplankton counts and absolute pigment

concentrations (Table 4.6). The base of the water column had a greater contribution from coccolith biomarkers (19-hexanoyloxyfucoxanthin; Arpin et al 1976, Jeffery and Wright 1994, 19-butanoyloxyfucoxanthin; Vesk and Jeffery 1987, Jeffery and Wright 1994 and lutein; Hager and Stransky 1970) that were not present in the SCM.

Post-wind event: Greater relative concentration of diatom biomarkers were observed in the lower water column (fucoxanthin; Jeffery and Wright 1994) (Figure 4.6); this trend was also observed in microscopy phytoplankton counts and absolute pigment concentrations (Table 4.6). Dinoflagellate biomarkers were evident at the SCM. Coccolith and algal biomarkers (19-hexanoyloxyfucoxanthin; Arpin et al 1976, Jeffery and Wright 1994, 19-butanoyloxyfucoxanthin; Vesk and Jeffery 1987, Jeffery and Wright 1994 and lutein; Hager and Stransky 1970) were present at both depth horizons sampled.

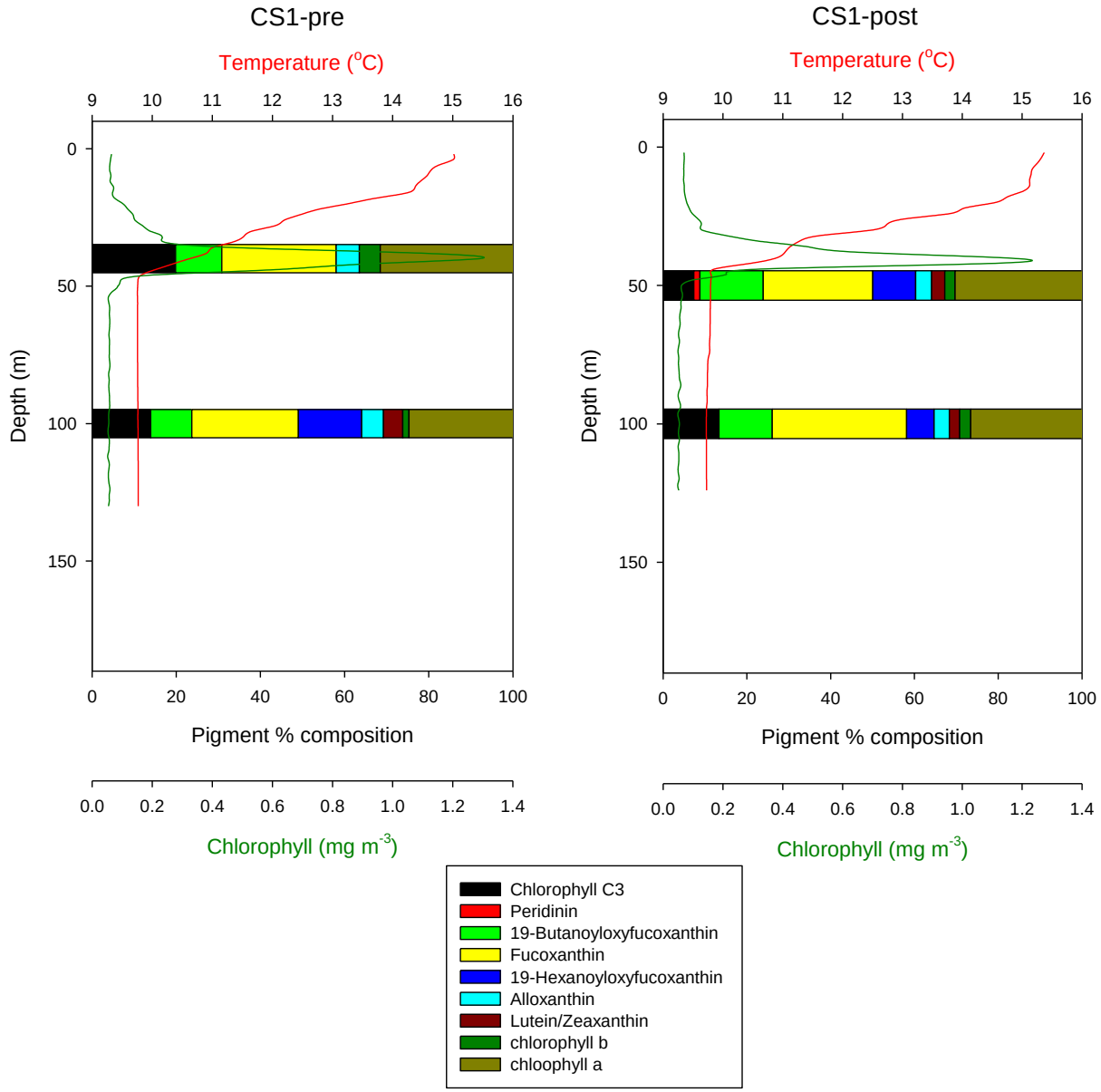

Figure 4.6 Pigment analysis for the survey site before and after the wind induced mixing event shown as pigment % composition for each depth horizon sampled. Temperature (°C) and chlorophyll (mg m^{-3}) profiles have been shown in the plots for reference purposes.

At CS1, phytoplankton pigment biomarkers showed a more homogenous distribution through the water column post-storm. Furthermore, phytoplankton communities based on pigment analyses of suspended POM (Figure 4.6) and a one-way ANOVA test showed there to be a significant difference pre- and post-storm ($P = 0.018$).

As previously described in Chapter 2, lipids when used as molecular indicators have a useful role in tracing the source, transformation and fate of organic matter (Hedges et al 2001; Wakeham 2002). As in Chapter 2, the lipid composition of suspended POM has been examined prior to and after the wind induced mixing event.

Absolute concentrations of lipids before when compared to after the wind event (Table 4.7). However, after the wind event did show a more homogenous distribution of lipids though the water column. Distribution of individual compounds though the water column was more variable after the wind event than before, where greater abundance was generally observed lower in the water column. The composition of POM through the water column was further examined though percentage composition analysis.

Table 4.7 Table showing the lipid components prior to and after the wind induced mixing event and at each depth, data are shown as μg L^{-1}.

	CS1-pre	CS1-post
Total identifiable lipids		
Surface	nd	4407
SCM	12839	5767
BML	37254	3545
Fatty Acids		
Surface	nd	912
SCM	1583	2419
BML	2991	382
Mono-unsaturated Fatty Acids		
Surface	nd	2812
SCM	1460	1818
BML	2461	401
Poly-unsaturated Fatty Acids		
Surface	nd	314
SCM	1216	342
BML	3173	2570
Alcohols		
Surface	nd	209
SCM	2597	847
BML	4834	58
Sterols		
Surface	nd	158
SCM	5982	340
BML	23795	133

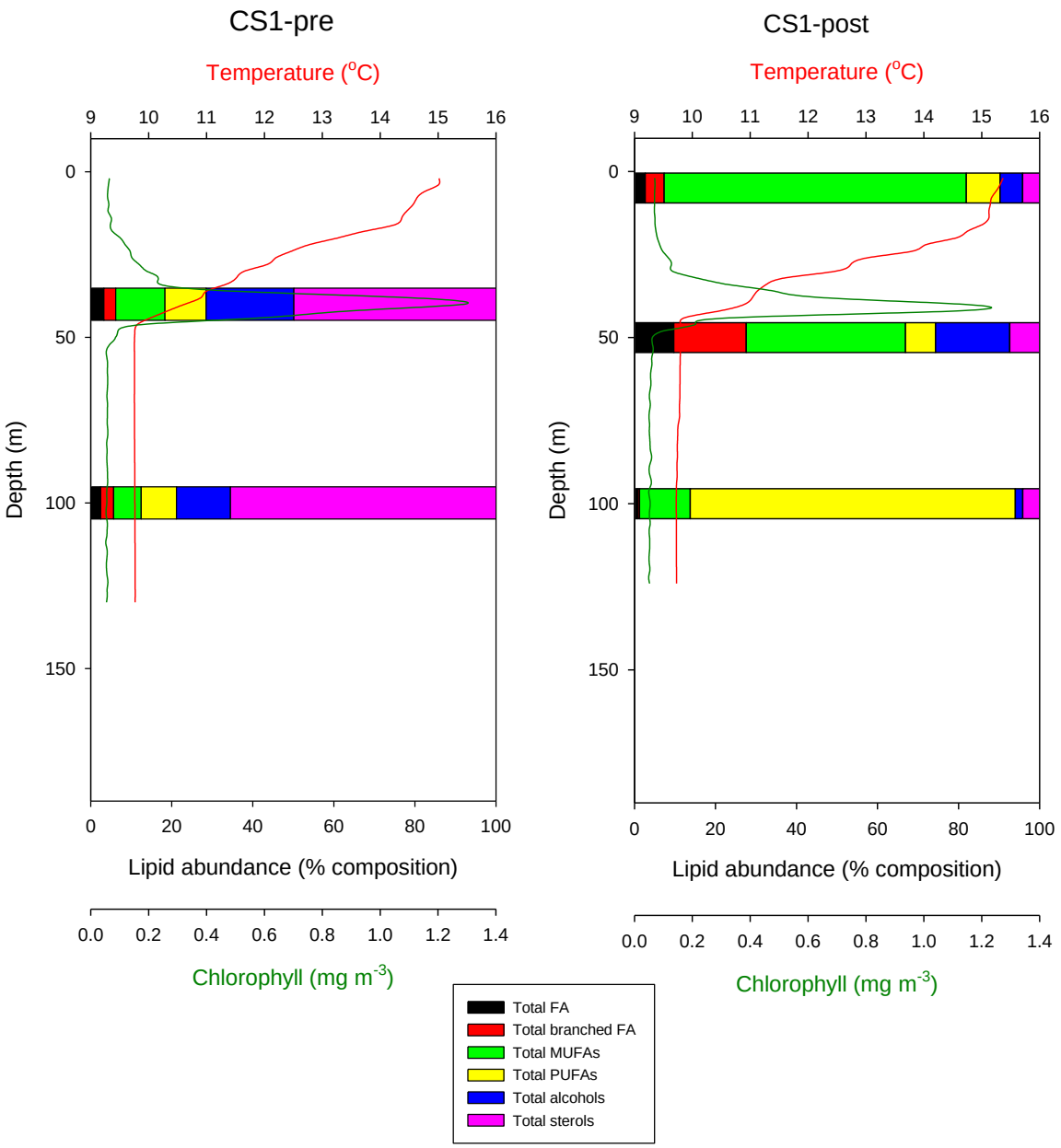

Figure 4.7 Lipid analysis from before and after the wind induced mixing event shown as percentage lipid abundance for each depth horizon sampled. Temperature (°C) and chlorophyll (mg m^{-3}) profiles have been shown in the plots for reference purposes.

Pre wind event: Phytoplankton- (C_{14}, C_{16}, C_{18} saturated and $C_{16:1}$ and $C_{18:1}$ mono-unsaturated fatty acids; Viso and Marty 1993, Dunstan et al 1994) and bacterially-derived biomarkers (branched fatty acids; Gillan and Johns 1986, Kaneda 1991) had similar compositions at the SCM and in bottom waters. Zooplankton biomarkers ($C_{20:1}$ and $C_{22:1}$ unsaturated alcohols; Sargent and Henderson 1986, Kattner et al 2003) were present in bottom waters but not at the SCM.

Post wind event: An increase in relative concentration of bacterial biomarkers (branched fatty acids; Gillan and Johns 1986, Kaneda 1991) was observed just below the SCM. Zooplankton biomarkers ($C_{20:1}$ and $C_{22:1}$ unsaturated alcohols; Sargent and Henderson 1986, Kattner et al 2003) were now evenly distributed throughout the water column. MUFAs decreased in relative concentration with depth. However, a large increase in the relative concentrations of HUFAs (particularly of the diatom biomarker $C_{20:5}$ EPA; Budge and Parrish 1998, Reuss and Poulson 2002, Fang et al 2006) was observed in the lower layer of the water column.

Lipid composition of sPOM pre- and post-storm was examined at each depth horizon (Figure 4.7). A one-way ANOVA test showed there to be a significant difference in sPOM collected before and after the storm ($P = 0.041$).

Other investigations undertaken at the same time as the present study, show that the integrated chlorophyll *a* concentration (over the euphotic zone) decreased by almost 10 mg m^{-2} at the SCM, post storm (Panton 2012). Increased mixing in the SCM causes fluxes of phytoplankton up and down through the water column, resulting in a potential loss of phytoplankton across the thermocline equivalent to up to 35% of total SCM chlorophyll (Sharples et al 2001). Evidence of this apparent phytoplankton "loss" from the SCM and transfer of cells to deeper water was also apparent from the increase in relative (and absolute) concentrations of highly labile (HUFAs) compounds in the bottom mixed layer post storm. This was confirmed both by phytoplankton count data and pigment analyses, which also indicated an increase in diatom concentration with water depth. Hence, diatom communities sinking though the water column may be primarily responsible for the "loss" of chlorophyll *a* from the SCM. Reprocessing of POM by microbial remineralisation and heterotrophic grazing is an important link in the transfer of carbon from the upper layers of the water column to the sediment, termed the biological pump (Honjo et al 2008). This potential movement of labile particles from the SCM into the deeper water column could impact on the biological pump and communities lower in the water column. Redistribution of labile material and "loss" from the SCM to deeper layers may fuel these processes, further increasing the reprocessing speed of the biological pump and providing nutrition to support deeper communities including the benthos.

As well as the observed decrease of primary production at the SCM, an increase of bacterial production was observed both from lipid biomarker distributions and bacterial production measurements (Panton 2012). As previously noted from counts, pigments and lipid data, zooplankton and phytoplankton appear to be more evenly distributed though the water column following mixing. However, from the phytoplankton count data, ciliates were only observed in surface waters; this distribution may alleviate grazing pressure on free-living bacteria at the SCM. As larger zooplankton, which may incidentally feed on bacteria attached to POM, appear to be distributed more homogonously though the water column after the mixing event this may further reduce grazing pressure on the bacterial population (Verity 1991; Azńa et al 2003).

4.3.1.4 Trophic dynamics – natural stable isotopes

The stable isotopic composition of nitrogen and carbon (samples with $\delta^{13}C$ values of less than -10‰ were removed from the data set; section 2.2.5) were determined in 25 individual taxa pre-storm (CS1-pre) (Figure 4.8). Summary isotope data for all phyla are presented here (Table 4.8) and full tabulated data can be found in Appendix 4.7; Table 4.11.

Table 4.8 Summary nitrogen and carbon isotope data for before and after the wind induced mixing event with standard deviation for each phylum.

	CS1-pre			CS2-post		
Phyla/Species	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**Number of observations**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**Number of observations**
POM	7.3 (± 0.4)	-18.3 (± 0.4)	2	8.19 (± 0.1)	-19.5 (±0.8)	2
Annelida	10.1 (± 0.42)	-16.43 (± 0.5)	5	9.7 (± 0.21)	-22.1 (± 0.64)	8
Arthropoda	7.94 (± 0.12)	-24.03 (± 0.79)	7	6.6 (± 0.22)	-22.2 (± 0.6)	8
Chaetognatha	10.54 (± 0.05)	-17.3 (± 0.7)	1	12.4 (± 0.7)	-21.41 (± 0.52)	1
Chordata	n.d	n.d	n.d	5.8	-22.5	1
Echinodermata	2.41 (± 0.22)	-25.86 (± 0.51)	3	6 (± 0.22)	-23.09 (± 0.62)	1
Mollusca	6.04 (± 0.32)	-24.4 (± 0.2)	9	5.7 (± 0.51)	-22.7 (± 0.3)	3

Nitrogen isotopic compositions ranged over 13.02‰ with a mean (± standard deviation) of 7.13 ± 0.2‰. While the carbon isotopic range was 15.04‰ with a mean (± standard deviation) of -22.4 ± 0.5‰. Post-storm (CS1-post), carbon and nitrogen isotope values were determined in 22 different taxa. Nitrogen isotopic compositions ranged over 13.97‰ with a

mean (± standard deviation) of 7.8 ± 0.4‰. While the carbon isotopic range was 7.27‰ with a mean (± standard deviation) of -22.25 ± 0.5‰. Differences between nitrogen and carbon isotope values before and after wind-induced mixing were not statistically significant (Nitrogen isotopes t-test = 665, P = 0.45; Carbon isotopes t-test = 528, P = 0.61). However, the range of carbon isotopes was much decreased after the wind induced mixing event (Figure 4.8). Changes in water column dynamics have been shown to affect the behaviour of copepods and can influence encounter rates, filtration currents and feeding behaviour (Rothschild and Osborn 1988; Kiørboe and Saiz 1995). This was observed by Saiz and Kiørboe (1995) in the species *Acartia tonsa*, which was shown to switch from filter feeding to ambush feeding during periods of water column mixing. During periods of greater turbulence, filter feeding copepods can experience difficulty in feeding due to erosion of the feeding current which is vital for them ingesting food (Kiørboe and Saiz 1995). This suggests that following the mixing event and a change in the physical water column dynamics, organisms adapted to different nutrient supplies due to nutrient fluxes, enhanced primary production and benthic resuspension. Some species may also have experienced difficulty in feeding due to increased turbulence decreasing grazing pressure. From summary and tabulated data (Table 4.8; Appendix 4.7; Table 4.11) it is also clear that different benthic species were collected in these samples and this could account for the difference in carbon isotope values. As a result, some caution should be taken in attributing the entirety of carbon isotope change solely on adaptation to physical dynamics.

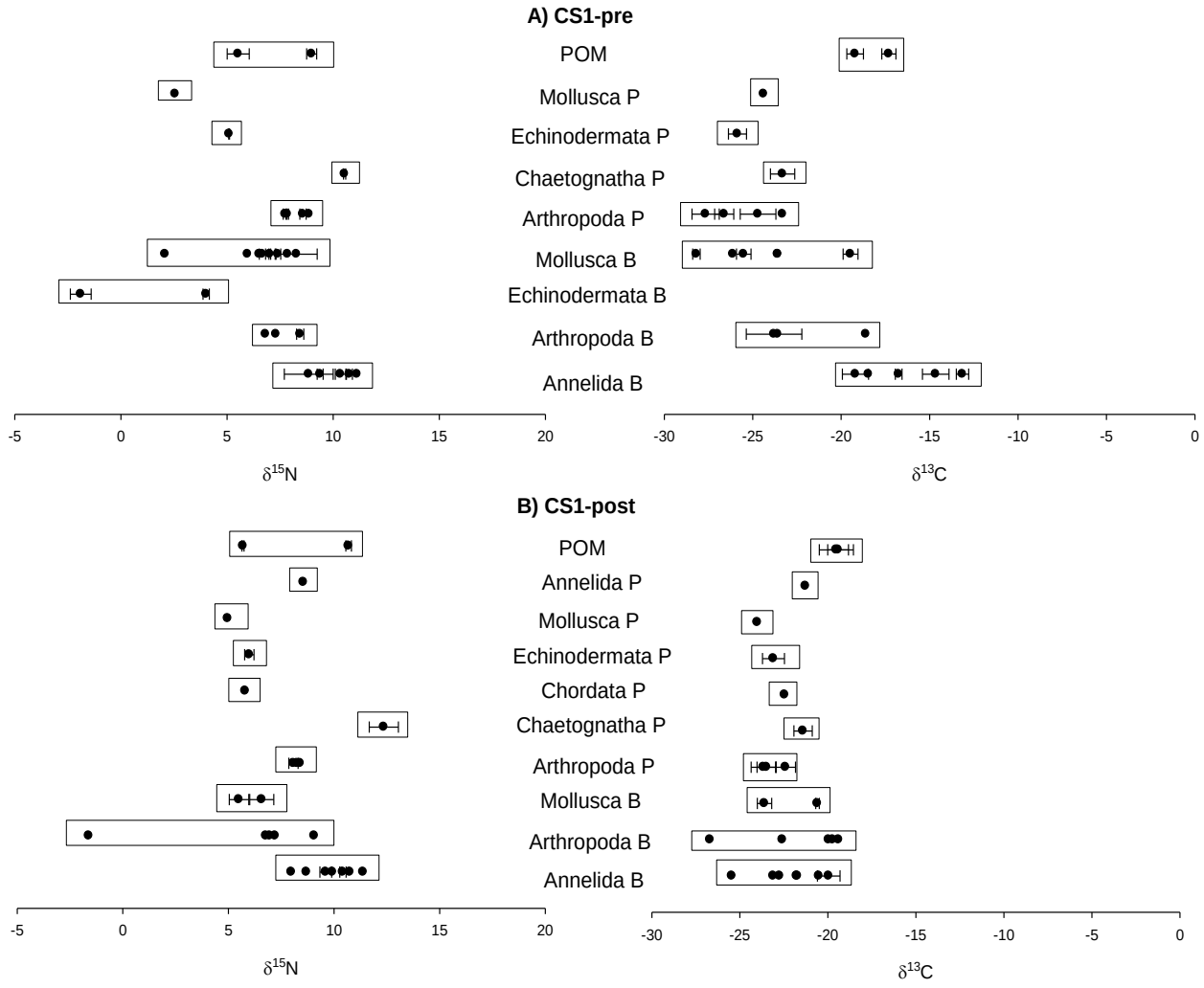

Figure 4.8 Individual $\delta^{15}N$ and $\delta^{13}C$ showing pelagic and benthic species, grouped by phyla, with error bars from triplicate analysis of each organism.

Nitrogen and carbon isotopes were cross-plotted in order to evaluate linearity in the trophic assemblages pre- and post-storm (Figure 4.9). A statistically significant, but very weak association between carbon and nitrogen isotopes was observed pre-storm ($r^2 = 0.180$, $P = 0.04$), which disappeared after the event, ($r^2 = 0.00$, $P = 0.96$). Prior to mixing, pelagic species seemed depleted in $\delta^{13}C$ compared to benthic species, but this was not apparent after mixing when pelagic and benthic organisms grouped together. Examination of trophic linearity by correlating $\delta^{15}N$ and $\delta^{13}C$ has provided some clues as to how pathways within the food chain can be altered due to changes in physical dynamics. Pre-storm, the weak but positive correlation between nitrogen and carbon isotopes suggests that the food web structure originates from a single photosynthetic source from primary production at the SCM (Polunin et al 2001; Drazen et al 2008). Post-storm, this relationship disappeared,

indicating a disrupted, more complex food web dynamic and perhaps more than one trophic pathway. For example, a change in the linearity of the food web dynamic may originate from resuspension of material from the benthos or recycling of photodetritus through grazing processes (Reid 2012).

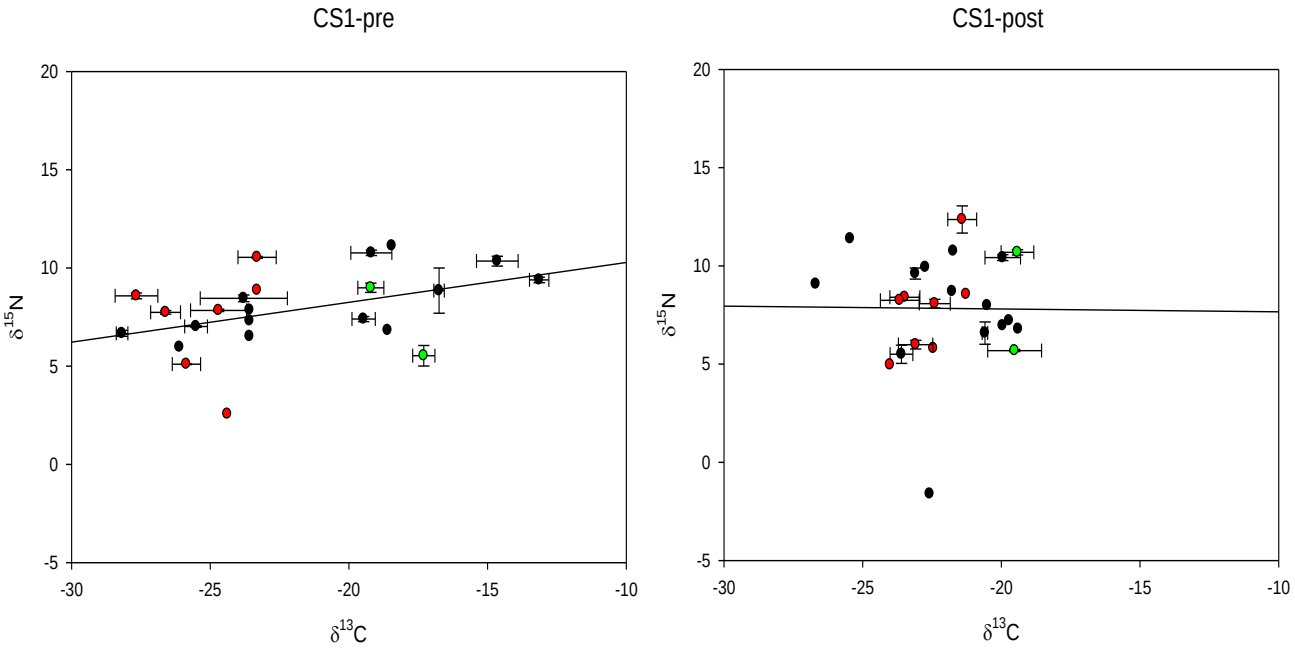

Figure 4.9 $\delta^{15}N$ and $\delta^{13}C$ bi-plots showing isotope values before and after the wind induced mixing event. Each data point represents individual organisms with error bars from triplicate analysis.

4.3.2 Incubation experiments

Pigment concentration, phytoplankton counts and lipid abundance through the incubation experiment were observed to change over time. Here, results are presented from the isolation experiment (non-nutrient addition scenario) and treatment experiments (nutrient addition scenario) (Figure 4.10 and Figure 4.11, respectively).

4.3.3 Isolation experiment - non-nutrient addition

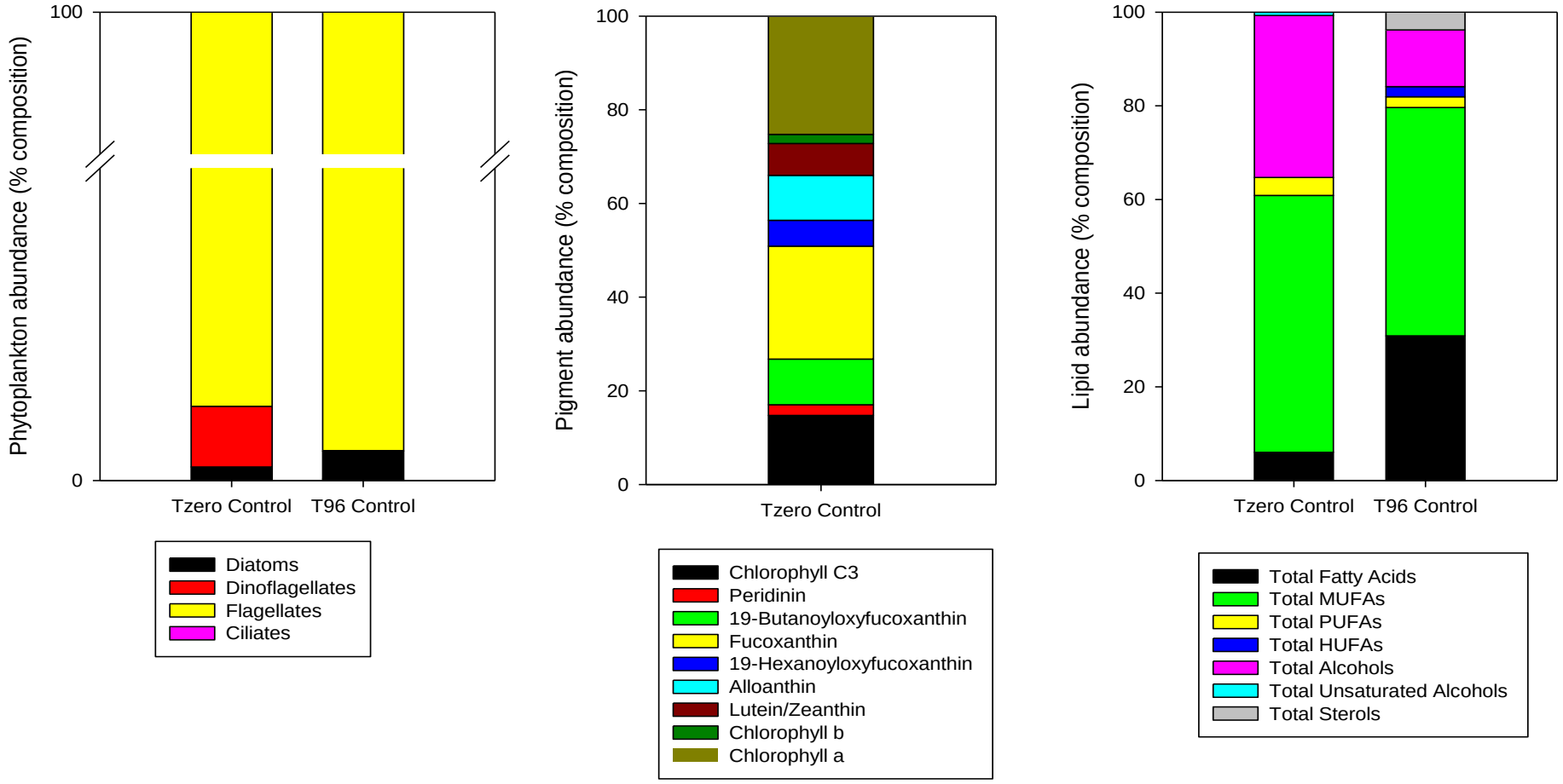

Figure 4.10 Phytoplankton, pigment and lipid abundance (% composition) for the control experiments.

The control incubations reflected a non-nutrient addition scenario (i.e. a non wind event situation). The compositions of phytoplankton and lipids changed over the incubation period (96h) (Figure 4.10). The phytoplankton community remained dominated by flagellates over the incubation while a decrease of dinoflagellates was observed between T_0 and T_{96}; these may have been grazed by microzooplankton populations and limited by nutrient availability. Between T_0 and T_{96} there was no statistical difference in the phytoplankton community structure ($P = 0.548$). The presence of the dinoflagellate biomarker, peridinin, at the start of the incubation was also notable. At T_0, lipid composition was mainly dominated by phytoplankton biomarkers ($C_{16:1}$ and $C_{18:1}$ mono-unsaturated fatty acids; Viso and Marty 1993, Dunstan et al 1994) and alcohols. Zooplankton biomarkers ($C_{20:1}$ and $C_{22:1}$ unsaturated alcohols; Sargent amd Henderson 1986) were present at the beginning of the incubation, albeit in small concentrations. After the incubation period, phytoplankton biomarkers (C_{14}, C_{16}, $C_{16:1}$ and $C_{18:1}$) increased in concentration. An increase in the relative concentration of $C_{20:5}$ (HUFA diatom biomarker; Budge and Parrish 1998, Reuss and Poulson 2002, Fang et al 2006) was observed over the 96h incubation period. Between T_0 and T_{96} there was no statistical difference in lipid concentration ($P = 0.318$). Thus, in the control where no nutrients were added, there was nonetheless sufficient for the community to continue to grow and develop, but there was no significant change in the community structure, nor in POM composition.

4.3.4 *Treatment experiment - nutrient addition*

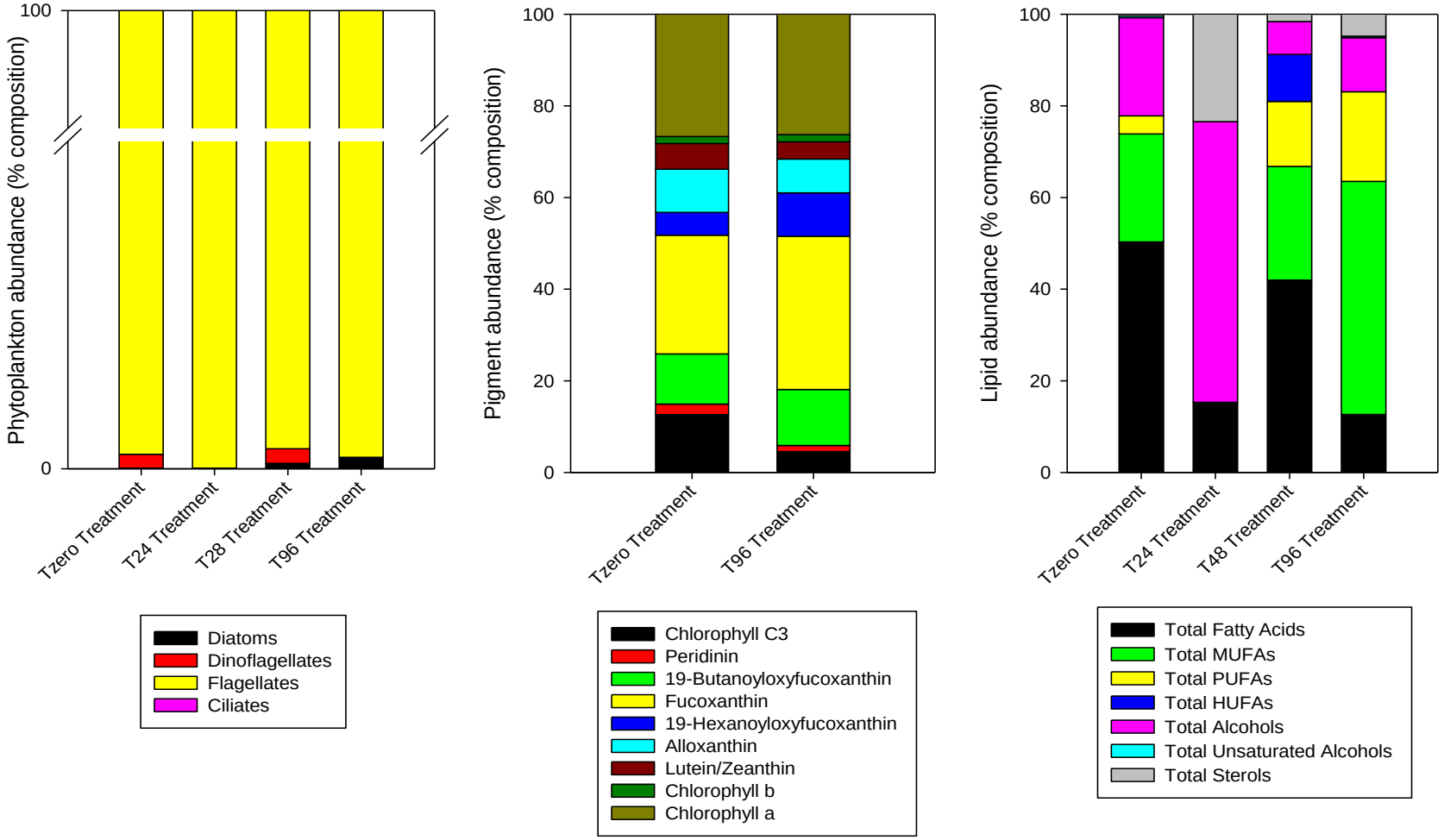

Figure 4.11 Phytoplankton, pigment and lipid abundance (% composition) for the treatment experiments, sampling took place at the onset of the experiment, after 24h, 28h and 96h.

Experiments simulated vertical mixing of deep nutrient rich bottom waters into the base of the SCM (Figure 4.11). The compositions of phytoplankton, pigments and lipids changed over the incubation period. Phytoplankton composition and lipid concentration were determined through the experiment, while pigments were analysed at T_0 and T_{96} only (Figure 4.11). As with the control incubations, the phytoplankton community was dominated by flagellates. Dinoflagellate concentration throughout the experiment was observed to oscillate between presence and absence; this could have been due to predator prey dynamics within the incubation. At T_0 and T_{48} dinoflagellates were present but at T_{24} and T_{96} they were not. Diatoms increased in concentration slightly throughout the incubation. Statistically significant differences over the incubation period were not apparent in the phytoplankton community structure ($P = 0.846$). Pigment biomarker analyses showed that the overall composition of pigments remained fairly similar between T_0 and T_{96}. However, the individual percentage composition of some pigment groups changed. The dinoflagellate biomarker, peridinin (Jeffrey et al 1975) was present both at T_0 and T_{96}. Diatom biomarkers (fucoxanthin; Jeffrey and Wright 1994) contributed ~25% to overall pigment composition at T_0, which increased to more than 30% over the course of the incubation. An increase in the relative concentrations of the coccolith biomarkers (19-hexanoyloxanthin; Arpin et al 1976, Jeffrey and Wright 1994, and 19-butanoyloxyfucoxanthin; Vesk and Jeffrey 1987, Jeffrey and Wright 1994) from ~5% to ~10% was also noted. The composition of lipids varied between the four time points of the incubation. A Mann-Whitney Rank Sum test revealed statistical differences over the incubation period of 96 h for phytopigment biomarkers ($P = 0.001$). At T_0, the community was dominated by saturated fatty acid phytoplankton biomarkers (C_{14}, C_{16} and C_{18}; Viso and Marty 1993, Dunstan et al 1994) this was also the case at T_{48}, but not at T_{24} and T_{96}. At T_{24} there was a change in the lipid composition, with a decrease in the relative concentration of saturated fatty acid biomarkers and an increase of alcohols and sterols. At T_{48} an increase in the relative concentration of HUFAs, similar to that observed post-storm during *in situ* sampling, was observed as was an increase in the relative concentration of diatom biomarkers. This increase of HUFAs (particularly EPA $C_{20:5}$ biomarker; Budge and Parrish 1998, Reuss and Poulson 2002, Fang et al 2006) is consistent with diatom growth, as observed in post-storm samples. At the end of the incubation period, mono-unsaturated fatty acid phytoplankton biomarkers ($C_{16:1}$ and $C_{18:1}$) dominated lipid composition. Sterols

and poly-unsaturated fatty acids also increased in relative concentration between T_{48} and T_{96}. A Kruskal-Wallis test revealed statistical differences over the incubation period in lipid concentration ($P = 0.045$).

4.4 Synthesis

In this Chapter, changes in pelagic and benthic communities were observed in conjunction with changes in organic matter composition through the water column following a storm event. These variations appear to be closely linked to changes in water column structure. The results emphasise the plasticity of the shelf-sea environment and ability of organisms to adapt to changing physical conditions. An experimental simulation of a wind-induced mixing event was also carried out and parallels between this and *in situ* observations were clear. Here, syntheses of the biological and chemical observations are presented in schematic form (Figure 4.12) highlighting the original hypotheses and whether these were confirmed:

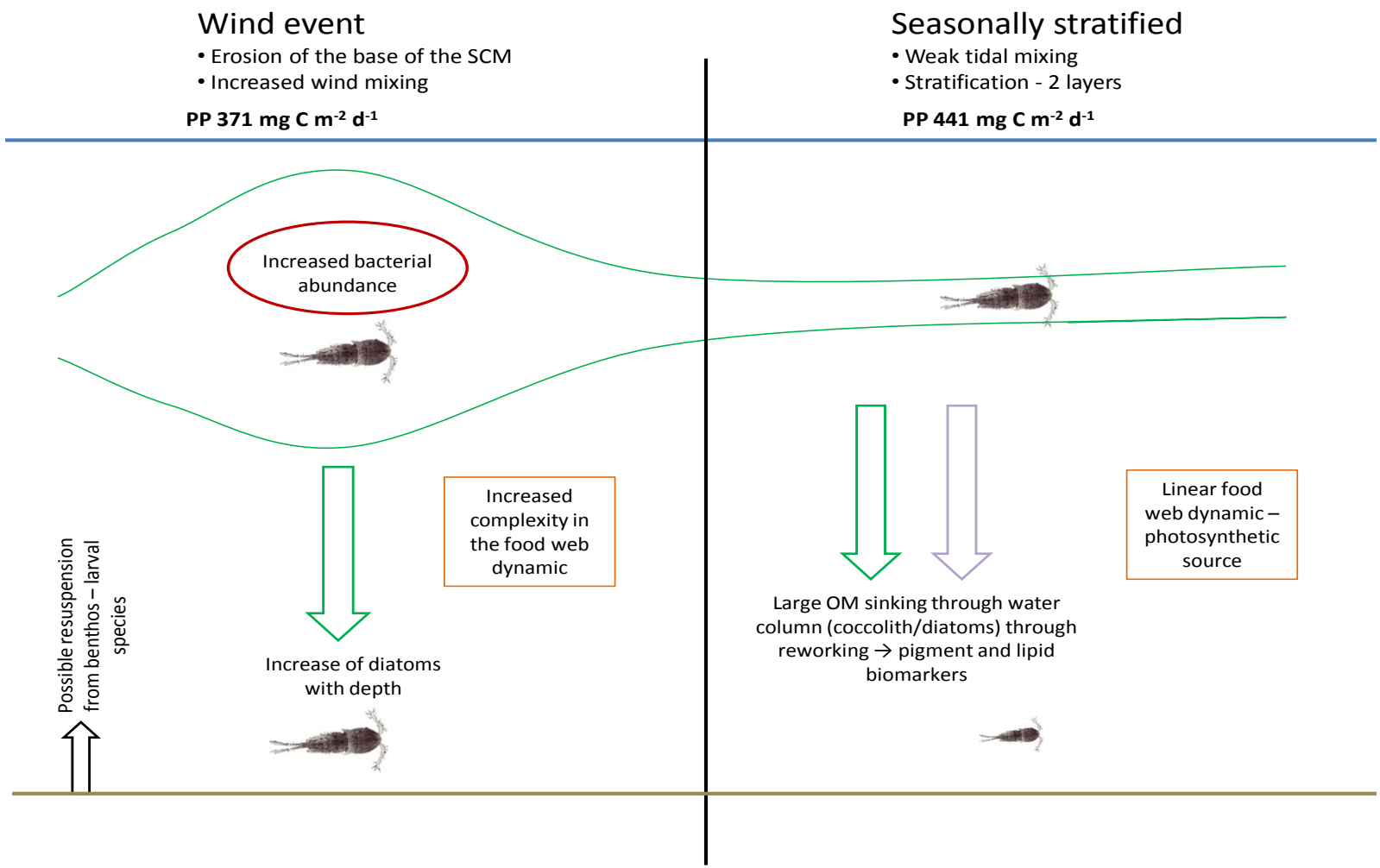

Figure 4.12 Schematic diagram of observations made in the water column following a wind induced mixing event. Black arrows represent physical dynamics within the water column. Purple and green arrows represent phytoplankton and zooplankton community dynamics inferred from biomarker analyses. Red circle represents bacterial community dynamics inferred from lipid analysis. Food web dynamics are depicted though orange boxes.

1) In the event of mixing within a stratified water column (even if there is only minimal erosion of the DCM and the thermocline remains intact) the distribution of POM becomes dispersed differently through the water column. This is apparent through the composition of POM and biomarker evidence.

In this study a wind induced mixing event slightly affected the physical dynamics of the water column. As shown in the schematic diagram (Figure 4.12) prior to the wind event there was clear SCM and thermocline denoting seasonal stratification. After the wind event, the thermocline remained intact but there was broadening of the SCM vertically within the water column.

The impact that the wind induced mixing event had on a specific region of seasonally stratified shelf sea has been depicted in Figure 4.12 through a schematic diagram. Prior to the wind event, pigment concentrations were greatest at the SCM the influence of small scale internal mixing appears to show downward movement of material downwards through the water column. Total lipid abundances showed homogenisation through the water column following the wind induced mixing event. Caution must be expressed when linking these changes in POM distribution and composition purely to the wind event as natural variability and horizontal advection may be contributing to these apparent changes. However, differences in POM composition from biomarker evidence before and after the wind event were statistically significant. A way to confirm these observations would be repeat sampling over several different wind events and sample the community throughout the wind event on a small temporal scale to try and identify variation.

2) During a mixing event within the stratified region of the shelf sea, chlorophyll is distributed more evenly thorough the water column. As a result of this chlorophyll homogenisation through the water column pelagic organisms are also observed to orient themselves differently optimising food intake and ultimately growth and reproduction.

In this study, it has been observed that chlorophyll becomes more homogenisised over the course of the wind event through the water column, this is depicted in Figure 4.12. Evidence of the apparent "loss" of chlorophyll *a* from the SCM has been shown from other investigations carried out simultaneously to this study. A decrease in chlorophyll *a* of 10 mg m^{-2} at the SCM after the wind event was observed (Panton 2012). Evidence of how this

chlorophyll *a* may be distributed through the water column is provided by PUFAs and Chlorophyll *a* biomarkers which support the sinking of photosynthetic organisms (like diatoms) through the water column. As in Chapter 2, an increase of internal mixing within the water column can aid the sinking of labile material down through the water column (Neto 2002; Huisman et al 2004).

As a consequence of the re-distribution of chlorophyll, differences in the biomarkers were also apparent suggesting that motile organisms (heterotrophic zooplankton) orient themselves within the water column to optimise food intake. From lipid zooplankton biomarkers ($C_{20:1}$, $C_{22:1}$ fatty acids and unsaturated alcohols) and plankton count data, it has been observed that zooplankton communities are distributed through the water column after the wind event, whereas prior to it they were in upper layers of the water column. Correlations of $\delta^{13}C$ and $\delta^{15}N$ also indicate that following the wind event the food web was possibly more complex as a reduction in the correlation was observed. This also suggests that following the wind event recycling of photodetritus through grazing processes was observed (Reid 2012). Despite this being speculation, it may be possible as the concentration of food resources at the SCM became diluted over a greater depth therefore heterotrophs have to utilise the available food sources by dispersing themselves throughout the water column.

The simulation of wind induced mixing events proved to be successful in demonstrating changes in POM with an influx of nutrients as parallels between the experiments and *in situ* measurements were apparent. Communities did not change significantly in the control experiments, but once nutrients were added to simulate the storm event, the changes in biomarkers were similar to those changes observed *in situ* scenarios. The most noticeable change was an increase of PUFAs after 48h of incubation. The increase of these compounds was also observed in the natural environment after the wind induced mixing event. It is possible to suggest that this may be an increase of labile material (possibly diatoms) responding to increased nutrients within the water column and incubation assays.

This Chapter has focussed on two “snapshots” before and after a wind-induced mixing event in the seasonally stratified region of the NW European Continental Shelf Sea. Disruption of

the water column structure in this region has rarely been studied and the response of the biological community was investigated. All samples were collected in replicate, however sampling resolution was coarse and observations made were “snapshots” in time, therefore it is not possible to consider changes over short time scales (including processes such as horizontal advection). Despite these limitations, results are promising and provide evidence that diapycnal mixing events include a response in the biological community.

In other studies the significance of wind induced mixing events has been highlighted and the potential impact on organic matter processing, supply and storage discussed (Sanchez-Vidal et al 2012; Marcos et al 2011; Somot et al 2006). It has been suggested that with global changes in climate patterns there will be an increase in the severity and number of wind events throughout the world’s oceans (Young et al 2011). With these investigations in other parts of the world’s oceans it is possible to hypothesise about climate change implications to the study sites investigated here. As has been observed in this study, an increase of mixing within a stratified water column increases turbulence within the water column enhancing sinking of labile material towards the benthos. Sanchez-Vidal et al (2012) have reported the effects of a coastal storm event in the Western Mediterranean Sea. Sanchez-Vidal et al (2012) reported that storm events in shallow waters had an important effect on deep-sea ecosystems through the arrival of organic carbon to deep sediments increasing sequestration. Processes similar to this may occur on the North West European Continental shelf, increasing the amount of organic carbon sequested at depth potentially increasing the amount of atmospheric CO_2 fixed by phytoplankton and then reprocessed by grazing and microbial processes and ultimately buried in of shelf sediments.

In order to investigate these interesting processes, estimation of fluxes through the water column and movement of organic matter off the continental shelf during a storm event would be beneficial in estimating sequestration fluctuations during a storm event. In order to capture these changes, sampling before during and after a storm event would be essential in assessing natural variation and stability of communities in response to these disturbance events.

4.5 References

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4.6 Appendix

Table 4.9 Complete pelagic species list expressed as percentage composition per vertical net haul. Data shown for upper and lower depth horizons alongside full water column haul.

Phyla/Species	CS1-pre Portion of the water column			CS2-post Portion of the water column		
	Full	**Upper**	**Lower**	**Full**	**Upper**	**Lower**
Annelida						
Polychaete larvae	0.0	0.0	0.0	0.03	0.0	0.02
Tomopteris spp	0.0	0.0	0.0	0.04	0.0	0.03
Arthropoda						
Podon spp	0.01	0.0	0.01	0.0	0.0	0.0
Mysid Parathemisto	0.1	0.1	0.1	0.2	0.1	0.1
Mysid	0.3	0.1	0.2	0.1	0.02	0.1
Pseudocalanus spp	59.9	64.9	0.0	80.7	40.7	40.0
Paracalanus spp	35.6	20.0	15.8	15.7	6.6	9.1
Chaetognatha						
Sagitta spp	1.2	0.9	0.3	0.8	0.3	0.6
Chordata						
Fish eggs	0.0	0.0	0.0	0.03	0.0	0.01
Ctenophora						
Ctenophore	0.01	0.0	0.01	0.0	0.0	0.0
Echinodermata						
Pluteus	2.2	2.9	0.0	2.0	2.8	0.0
Echinocyamus pusillus	0.1	0.4	0.0	0.3	0.2	0.02
Mollusca						
Gastropod larvae	0.3	0.1	0.2	0.1	0.1	0.05
Total	**100**	**89.4**	**16.6**	**100**	**50.5**	**50**

Table 4.10 Complete benthic species list expressed as percentage composition per Day grab.

Phyla/Species	CS1-pre	CS2-post
Annelida		
Lumbrineris laterilli	3.50	0.00
Lumbrineris spp	0.00	3.68
Hubrechtella dubia	5.24	7.35
Glycera tridactyla	2.62	5.51
Nephtys cirrosa	1.75	3.68
Nephtys hombergi	0.00	3.68
Fabricola spp	0.00	7.35
Arthropoda		
Bathyporeia guilliamsoniana	3.50	0.00
Phoxocephalus spp	0.00	3.68
Carcinus maenas	1.75	0.00
Pagurus bernhardus	0.00	3.68
Eurydice spp	0.00	3.68
Pseudoparatanais spp	3.50	3.68
Cnidaria		
Sea anemone	1.75	12.9
Echinodermata		
Echinocyanms pusillus	9.97	9.19
Ophiura affinis	4.72	0.00
Mollusca		
Glycymeris glycymeris	0.00	0.00
Epitonium spp	1.75	3.68
Turritella communis	3.50	6.25
Astarte sulcata	0.00	0.00
Retusa obtusa	1.75	0.00
Odostomia spp	1.75	0.00
Aporrhain pespelecani	1.75	0.00
Lunatia catena	6.99	0.00
Boreotrophon truncatus	1.75	0.00
Colus islandicus	1.75	0.00
Palliolum tigerinum	1.75	0.00
Arctica islandica	1.75	0.00
Parvicardium scabrum	1.75	0.00
Lutraria lutraria	1.75	0.00
Margarites helicinus	1.75	0.00
Scaphopod tubes	31.99	22.06
Porifera		
Alcyonium digitatum	0.00	0.00
Sycon ciliatum	0.00	0.00

Table 4.11 Complete pelagic and benthic species list for carbon and nitrogen stable isotope data, expressed with standard error.

	CS1-pre		CS2-post	
Phyla/Species	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**
POM				
POM SCM	5.53 (± 0.52)	-17.3 (± 0.4)	5.69 (± 0.05)	-19.52 (± 0.97)
POM Bottom	8.99 (± 0.24)	-19.21 (± 0.47)	10.68 (± 0.13)	-19.42 (± 0.59)
Annelida				
Glycera tridactyla	10.77 (± 0.14)	-19.19 (± 0.74)	10.42 (± 0.15)	-19.95 (± 0.64)
Hubrechetella dubia	10.35 (± 0.25)	-14.65 (± 0.75)	9.93	-22.74
Lumbrineris latreilli	8.85 (± 1.15)	-16.75 (± 0.19)		
Lumbrineris spp			8.7	-21.77
Nephtys cirrosa	11.13	-18.45	10.75	-21.73
Nephtys hombergi			9.61 (± 0.28)	-23.1 (± 0)
Unknown bristle worm	9.39 (± 0.14)	-13.14 (± 0.35)		
Polycheate larvae			8.55	-21.27
Arthropoda				
Bathyporeia guilliamsoniana	7.31	-23.58		
Carcinus maenas	8.45 (± 0.17)	-23.79 (± 1.57)		
Eurydice spp			7.21	-19.72
Pagurus bernhardus			6.78	-19.39
Psedoparatanais spp	6.82	-18.6	6.78	-19.39
Urothe spp	8.87	-23.31		
Copepod	8.58 (± 0.15)	-27.66 (± 0.77)		
Copepod large			8.25	-23.66
Copepod pink	7.74 (± 0.09)	-26.61 (± 0.54)		
Copepod small			8.25	-23.66 (± 0.7)
Crab larvae			-1.61	-22.58
Evadue spp	7.84 (± 0.06)	-24.7 (± 1.01)		
Mysid			8.08 (±0.22)	-22.4 (± 0.56)
Chaetognatha				
Arrow worm	10.54 (± 0.5)	-23.31 (± 0.69)	12.36 (± 0.69)	-21.41 (± 0.52)
Chordata				
Fish egg			5.8	-22.45
Fish larvae				
Echinodermata				
Echinocyanms pusillus	-1.89	-6.89		
Ophiura affinis	4.02 (± 0.15)	-7.64 (± 0.6)		
Pleutus	5.1 (± 0.02)	-25.86 (± 0.51)	5.99 (± 0.22)	-23.09 (± 0.62)
Mollusca				
Aporrhais pespelecani	7.4 (± 0.13)	-19.47 (± 0.42)		
Arctica islandica	7.02 (± 0.04)	-25.51 (± 0.41)		
Brachystomia scalaris	2.08	-5.57		
Lutraria lutraria	5.97	-26.11		
Palliolum tigerinum	7.89	-23.58		
Phaxas pellucidus			5.5 (± 0.46)	-23.6 (± 0.41)
Retusa obtusa	8.28 (± 0.97)	-6.66 (± 1.53)		
Turritella communis	6.67 (± 0.15)	-28.18 (± 0.21)	6.58 (± 0.57)	-20.59 (± 0.1)
Gastropod larvae	2.56		4.97	-24.01

For full nomenclature and chemical structures of lipid compunds, see Appendix 2.6; Chapter 2.

Chapter 5

Synthesis

5.1 *The shelf sea environment*

The biological and economic importance of shelf seas is clear. They account for less than 10% surface area of the ocean, but 40% of the world's population lives within 100km of the coast and they are considered to be 3-4 times more productive than open ocean systems (Simpson and Sharples 2012). Additionally, they are very diverse regions of ocean and create ideal habitats for many different organisms. The biodiversity and success of species within shelf seas is due to close relationships and interactions between competition, predation and mutualism at different trophic levels throughout the complex food web (Suchanek 1994). As a result of their productivity and biodiversity, shelf seas are hugely important for fishing activity and up to 90% of global catches originate from on-shelf stocks (Pauly et al 2002). The United Nations Fisheries and Aquaculture Department estimated that the fish catch landed by the UK fleet had a value of £546 million in 2002. Shelf seas are also a vital link for ocean carbon sequestration and current estimates suggest that up to 40% of this occurs in these regions (Chen and Borges 2009). However, the world's climate is changing and there are increasing concerns about impacts on the oceans. Young et al (2011) suggested that there will be an increase in the frequency and severity of storm events. As shelf seas are small and close to anthropogenic influence, the impact on them is predicted to be large and if the delicate balance of carbon fixation, drawdown and remineralisation is affected, this could impact higher trophic levels and ultimately vital fish stocks.

As has been observed in this study, shelf seas exhibit distinct physical dynamics. Thus, coastal areas have shown to be influenced by tidal forces and mixing creating uniform physical dynamics through the water column (Simpson and Sharples 2012). In the middle of the continental shelf, tidal forces are weaker and seasonal summer stratification is induced through solar heating (Pingree et al 1976; Sharples and Holligan 2006). At the edge of the continental shelf, where the landmass falls away into the deep ocean, pulses of nutrients are pushed onto the shelf from the deep ocean and enhanced mixing is observed (Sharples et al 2007). Previous studies have concentrated on physical estuarine dynamics, tidal fronts, mixing regions and marginal dynamics. There have also been studies investigating the impacts of anthropogenic activity on biological communities (fishing and pollution) but rarely are studies conducted that incorporate physics, biology and biogeochemistry.

5.2 This study

This study was part of a wider investigation into the physical dynamics encountered across the shelf-sea environment, with particular focus on the impact of wind driven mixing across the thermocline. Here, the opportunity was taken to observe benthic, pelagic and suspended POM communities across four distinct hydrodynamic areas of the shelf. The distinct regions included: vertically mixed coastal, seasonally stratified mid-shelf, continental margin (shelf break) and a seasonally stratified region. The impact of a wind-induced mixing event on the seasonally stratified region was also investigated through *in situ* observations and incubation experiments. The influence of these different hydrodynamic regimes on biology and the biological pump was assessed through a sampling campaign incorporating biogeochemical, biological and physical measurements and analyses. It was hypothesised that in regions of different physical characteristics, biological communities would show different characteristics, distributing themselves to maximise food uptake, growth and reproduction. Until now, evidence to support this has been largely absent. Here each of the distinct regions are summarised through the use of a schematic diagram and the initial questions posed at the beginning of the study are re-assessed (Figure 5.1).

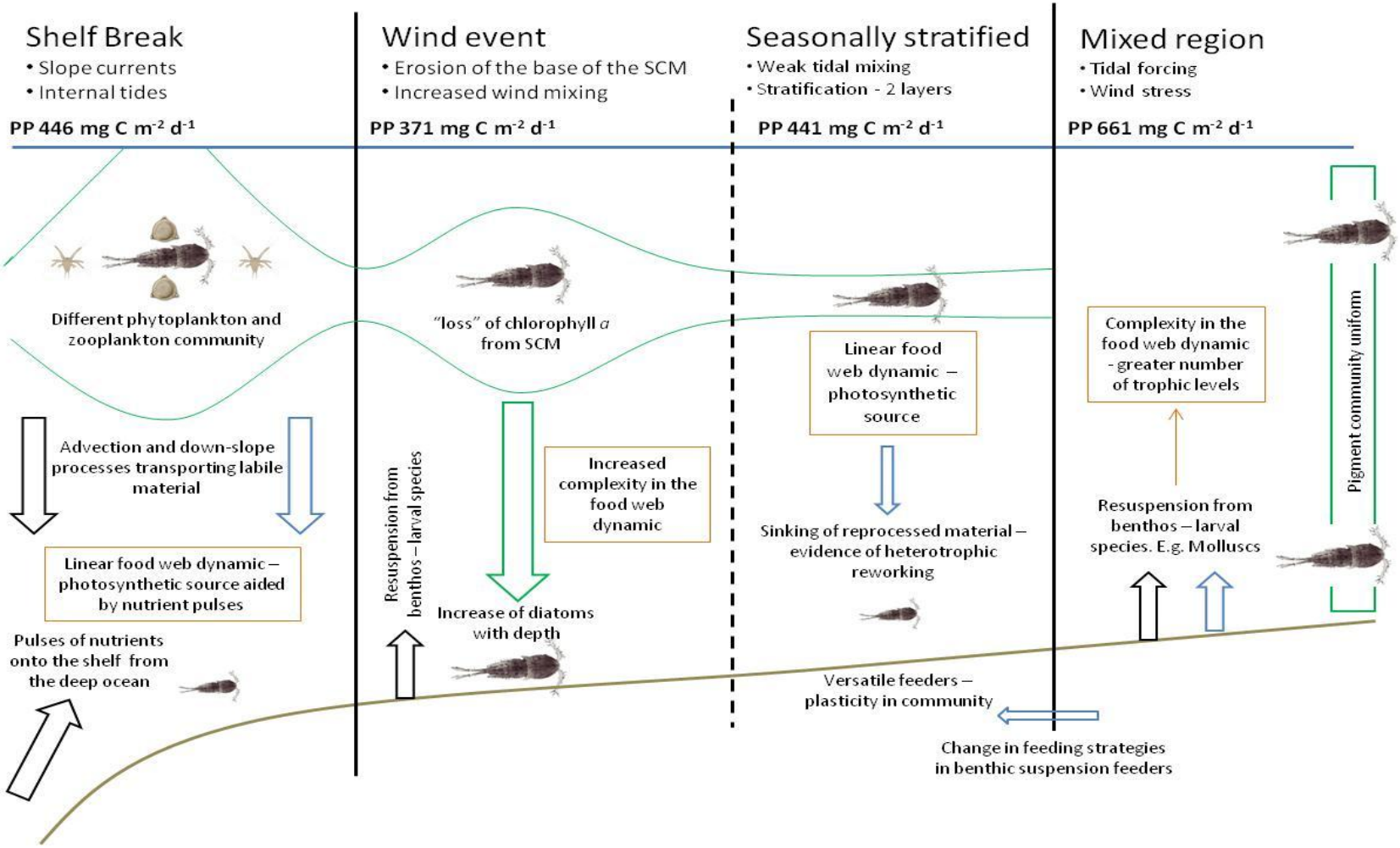

Figure 5.1 Schematic diagram showing the four distinct physical dynamics sampled. The major processes and observations are summarised. Green lines, boxes and arrows represent observations of chlorophyll *a* and phytoplankton pigment abundances. Orange boxes and arrows describe food web dynamics. Black arrows represent physical forces. Blue arrows represent biogeochemical dynamics.

5.2.1 Vertically mixed regions

Vertically mixed coastal regions display uniformity in physical water column structure (Simpson and Sharples 2012). As part of the wider study carried out during *RRS Discovery* 352, the vertically mixed stations (LB-M and IS-M) were found to be the most productive in terms of phytoplankton production with a mean value of 661 mg C m^{-2} d^{-1} (Panton 2012). It is possible that due to the shallow water column depth (LB-M = 48m, IS-M = 104m) light penetrates deep enough into the water column to increase the potential for carbon fixation due to elevated nutrient concentrations (Panton 2012).

As depicted in Figure 5.1 uniformity within the water column was observed in pigment and zooplankton biomarker profiles, where similar abundances of specific compounds were observed at the surface and base of the water column. It has previously been suggested that turbulence aids the sinking of labile material and that this, coupled with resuspension of benthic material supports production and the potential for grazing throughout the water column (Neto 2002; Huisman et al 2004). At both sites sampled with mixed water column structures, evidence of increased pelagic and benthic coupling was observed, particularly in larval life stages of benthic organisms. For example, larvae of the benthic phyla Mollusca and Annelida were frequently observed suspended in the water column; this distribution may be a consequence of the vigorous mixing and turbulent conditions.

Food web web dynamics were studied using carbon and nitrogen stable isotopes. Through correlation of carbon and nitrogen isotopes the linearity of the trophic assemblages was assessed and the number of estimated trophic levels was found to be greatest in the regions of mixed water column structure. Elsewhere, it has been suggested that a higher number of trophic levels may lead to high productivity and energy transfer between organisms (Oksanen et al 1981; Dickman 2008). In this The Celtic Sea, this certainly seems to be the case.

5.2.2 Seasonally stratified regions

The mid-region of the Celtic Sea is stratified in the summer. The stratification develops due to weak tidal forcing and increased solar heating, leading to surface waters depleted in nitrate and the development of a SCM (Pingree et al 1976; Fasham et al 1983; Sharples and Holligan 2006).

In the seasonally stratified seas, primary productivity was less than regions of mixed water column structure with a mean production of 441 mg C m^{-2} d^{-1}. As depicted in Figure 5.1 evidence from vertical zooplankton hauls showed there to be greater abundance of species at the SCM. Biomarker concentration (lipid and pigment) was also found to be greatest at this depth horizon. This suggests that the SCM is an optimum horizon for respiration, feeding and reproduction constrained by the physical dynamics of the water column.

Deeper in the water column fewer pelagic organisms were observed but there was evidence of larger aggregates of organic material possibly originating from a heterotrophic source. As this material is processed by grazers higher in the water column, it forms large aggregates that sink towards the benthos. Organisms inhabiting the benthos displayed more versatile feeding strategies, occupying feeding guilds capable of utilising different feeding methods. When the food web structure was examined through stable isotope analyses, fewer trophic levels and a more linear food web originating from a photosynthetic source at the SCM was observed than in the fully mixed shelf sea. As the physical dynamics at this region of the continental shelf change seasonally, it may be that benthic organisms capable of responding to different amounts of food resources orientate themselves in this region. This versatility has been observed in fish species where generalist feeders dominate the Celtic Sea as they are able to respond to changing food resources throughout the seasonal cycle (Pinnegar et al 2003; Trenkel et al 2005).

5.2.3 Shelf Break

The shelf break is the boundary region between the continental shelf and slope; here the hydrodynamic setting is different compared to the rest of the shelf. Pulses of nutrients from the deep ocean up onto the shelf and increased internal mixing fuel a community change at the base of the food web (Sharples et al 2009). As depicted in Figure 5.1, primary production is higher than in the seasonally stratified mid-shelf region. but lower than the regions with mixed water column structure.

Sharples et al (2009) showed that the biomass of phytoplankton at the shelf edge did not change, however there was a shift in the community structure. Here, it was also clearly evident that the pelagic community structure differed from the rest of the continental shelf. Larger copepod species, some oceanic species, and greater abundances of nauplii and fish larvae were observed. It is possible to hypothise that these changes are influenced by the different hydrodynamic setting and the mixing of continental shelf and deep ocean communities.

As has been previously observed by Sharples et al (2009), the hydrodynamic setting is different at the shelf break and there are advective and down-slope processes that may transport labile organic material down through the water column. Biomarker evidence suggests that the physical processes in this region are acting in a similar way to those in the mixed regions aiding transport of material through the water column.

From analysis of the carbon and nitrogen isotopes linearity of the food web was similar that that of the seasonally stratified region suggesting that inputs were largely from a photosynthetic source in the upper region of the water column. The number of estimated trophic levels was greater than the seasonally stratified region possibly indicating greater energy transfer and production in this region than the mid-shelf.

5.2.4 Wind induced mixing

Periodic wind events in the seasonally stratified mid-shelf region of the NW European Continental Shelf Sea have been shown to increase mixing of the water column (Burchard and Rippeth 2009). Here, erosion of the base of the SCM (but not complete breakdown of stratification) was observed following a wind event (Williams, Pers. Comm.).

As depicted in Figure 5.1 homogenisation of biogeochemical and biological parameters was apparent after the wind event in the pelagic community. Zooplankton community analysis showed their abundance to be evenly distributed between the upper and lower water column. In this case, the mixing within the water column was fairly weak, so this homogenisation was possibly due to organisms attempting to optimise feeding potential over a dissipated organic matter source rather than involuntary movement due to mixing forces. Physical evidence showed that as the SCM was eroded, chlorophyll *a* was mixed down though the water column and there was a decrease of 10 mg m^{-2} at the SCM (Panton 2012). This apparent "loss" from the SCM could be responsible for the decrease in primary production, 371 mg C m^{-2} d^{-1}, when compared to the rest of the continental shelf. Evidence of phytoplankton sinking through the water column was apparent in the lipid biomarkers at the base of the water column where fresh labile markers (possibly diatoms) were observed in much greater quantities than before the wind event.

After the wind event, there was an increased abundance of benthic organisms observed in the pelagic region (much like that of the mixed water column sites). This is could be a consequence of the mixing and resuspension from the benthos. However, as the event was small and stratification was not completely disrupted there is some doubt that this is the sole cause. It is more probable that this observation was due to natural variation.

When comparing linearity of the food web structure before and after the wind event it is clear that mixing appears to increase the complexity of the food web dynamic. Prior to the wind event the dynamic was linear probably originating from a photosynthetic source while after there appears to be more complexity with similarity to vertically mixed rather than stratified regions of the shelf.

5.3 Consequences of changing physical dynamics

As I have shown, the biological and biogeochemical properties of the NW European Continental Shelf Sea are influenced by physical dynamics and this is particularly reflected in the adaptations and patchiness of benthic and pelagic organisms. Each of the data chapters focused on different communities and processes observed across the continental shelf, in each of the chapters the initial hypotheses posed have been addressed but here those finding will be summarised.

Chapter 2

This chapter focused on how the different physical regions of the Irish and Celtic Seas impact on POM transport, reprocessing and cycling. The following key points were observed:

- The position of phytoplankton, examinied through pigment analysis, appears to occur at optimal positions for growth and respiration through the water column responding to physical dynamics.
- Biomarkers showed that reworking of POM occurred at different depths across the shelf possibly due to zooplankton seeking optimal resources within the water column.
- Increased mixing within the water column was found to promote the sinking of labile material. This was observed at sites with mixed water columns, at the shelf break and following a wind induced mixing event.

Chapter 3

This chapter focused on the differences in benthic and pelagic community structure between the distinct physical regions of the Irish and Celtic Seas. The following key points were observed:

- Physical structure of the water column influenced the distribution of pelagic organisms through the water column. Organisms appeared to orient themselves to optimise feeding potential.

- Benthic communities showed a greater degree of patchiness across the shelf but in seasonally stratified regions, where physical dynamics regularly change, organisms were observed to occupy feeding guilds with greater versatility in feeding.
- Trophic behaviour appears to change across the shelf with more complex dynamics in mixed regions and at the shelf break. Higher primary production also appears to be linked with greater numbers of trophic levels within a system possibly indicating more energy transfer between organisms.

Chapter 4

This chapter focused on a change of hydrodynamic setting within the water column and how this impacts on the biological community and processing of POM. The following key points were observed:

- From *in situ* observations measurements of chlorophyll *a* showed an apparent "loss" from the SCM and redistribution through the water column following a wind induced mixing event.
- Sinking of labile material through the water column was observed through biomarker evidence following the wind event.
- Zooplankton communities were observed to be more evenly distributed though the water column which was possibly in response to the distribution of food over a wider depth range.

As this study has shown, biological communities across the North West Continental Shelf respond to the different physical dynamics. It appears that mixing and turbulence increases the amount of labile material moving through the water column and distributes heterotrophic communities more evenly. Other studies have suggested that as the world's climate is changing and the frequency and severity of storm events increase there may be an impact on the processing, storage and supply of organic matter (Sanchez-Vidal et al 2012; Marcos et al 2011; Young et al 2011; Somot et al 2006). Results in this study appear to show that wind events may increase the complexity of the food web and increase the number of trophic levels leading to greater processing of organic matter. This could increase the amount of organic matter processed through the biological pump and eventual export and

burial of organic matter in sediments. Shelf sea environments are responsible for up to 40% of global carbon sequestration (Chen et al 2009) and the apparent plasticity in the communities in response to wind events may enhance this further in the future. Shelf Seas could play an even greater role in counteracting the increase of atmospheric CO_2.

5.4 Future work and developments

Four contrasting regions of the NW European Continental Shelf Sea were investigated in detail. Biological and physical interactions over a three week period during summer 2010 were studied. An important and beneficial development to this study would be to assess these interactions over a seasonal scale. Seasonality in this region affects the physical water column structure; in the winter much of the continental shelf is vertically mixed aided by increased wind stress, tidal forcing and periodic storm events. An understanding of seasonality would provide further insight into the adaptations of biological and biogeochemical processes undertaken across the continental shelf.

Patchiness within the biological communities is an important feature across the shelf sea. It has been suggested that this is due to competitive strategies and adaptations to the physical environment. Time and equipment restraints sometimes impaired the amount of replication that feasibly could be carried out, however, where possible replicates were taken. In some cases, for example *in situ* SAPs measurements, there was no replication. If this work, or similar, were to be carried out in the future some suggested improvements to equipment choice would be made. In conjunction with sampling suspended POM column, the use of sediment traps would be useful in more accurately assessing the fluxes, quality and composition of sinking POM. Lipid, amino acid and isotopic analyses could be conducted on this sinking sediment further elucidating reworking processes through the water column. The use of equipment that enables *in situ* replication would also benefit the statistical robustness of the study. A multi-coring device which simultaneously collects up to 10 core samples at a time would provide a clearer picture of benthic community structure at each of the sampling sites. Caution would have to be taken to ensure that true replication of sampling was conducted. If just one drop of the coring device was made, despite multiple cores taken, this would not be regarded as true replication. Therefore, multiple drops ideally would be conducted. Caution using this coring device would also have to be made if

sampling sandy sediments as in these conditions samples can be difficult to take. However, if this method was used in conjunction with Day grab samples it would provide a rigorous assessment of the benthic community structure. For the pelagic community structure, a MultiNet sampling device would allow more precise sampling of different depth horizons of the water column. This device incorporates multiple nets which are remotely opened and closed in order to sample distinct water depth ranges as the net is pulled vertically though the water column.

In addition to the broad range of analytical techniques used in this study, further analyses could be carried out to elucidate biogeochemical properties further. Compound specific isotope analysis is a useful development in stable isotope analysis and can be used to determine specific food sources, for example in assessing carbon sources and lineation of food web structure. This could be coupled with analysis of amino acids from pelagic and benthic species to further investigate food web linkages and how they are affected by changing physical dynamics.

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