**DOCTOR OF PHILOSOPHY**

**Multivariate statistical analyses in lipid biomarker studies**

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# Multivariate Statistical Analyses in Lipid Biomarker Studies

Thesis submitted for the degree of Doctor of Philosophy

By

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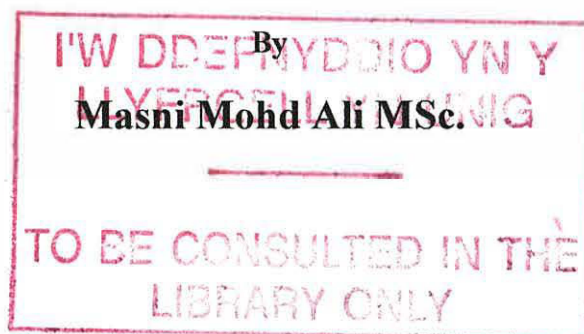
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Dedicated to

my lovely daughter

Nur Syakirah

# Abstract

Fatty acids, fatty alcohols and sterols were quantified in surface sediment samples taken from the Mawddach Estuary and from the Clyde Sea to assess the spatial variability. These biomarkers were also quantified in sediment cores from the Conwy Estuary and Loch Riddon to investigate their temporal variability. These bi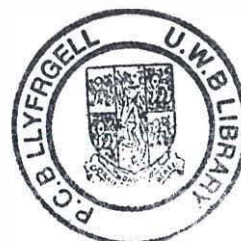omarkers are valuable tracers to assess organic matter sources in different marine environments. Traditional biomarker approaches such as Alcohol Source Indices (ASI) and Sterol Source Indices (SSI) based on the ratio between terrestrial and marine fatty alcohols and sterols were developed to see the influence of terrestrial organic matter within marine sediments. Other ratios based on short/long, odd/even and coprostanol/cholesterol highlighted the bacterial/sewage derived organic matter and their distribution. This approach also shows the degradation of marine derived lipids with time as seen in the core samples.

Principal Component Analysis (PCA), factor analysis and cluster analysis were used to classify these biomarkers according to their primary source such as terrestrial, marine, bacteria and sewage. PCA based on the proportion data rather than raw data clearly shows the compound separations according to their geochemical sources. PCA of fatty alcohols and fatty acids also provide more information on secondary processes. However, in the Conwy core, PCA was not able to separate the marine and bacterial derived markers. Cluster analysis with Ward's method differentiated the Clyde Sea sediment samples and the Loch Riddon core into two regions: 1) Samples that were influenced by marine and bacterial organic matter, and 2) Samples that were dominated by terrestrial inputs. Two and three factors were extracted from factor analysis conducted on the Mawddach Estuary and the Loch Riddon core. Three significant sources in the Mawddach were marine/bacterial, sewage and terrestrial. In the Loch Riddon core, bacterial/marine and terrestrial became the significant sources. To quantify the contribution or transportation of organic matter in these sampling sites, Partial Least Squares (PLS) path model were developed. PLS successfully quantified the decrease of organic matter down the core, as well as the contribution of organic matter due to current movements, river runoff and tidal exchange.

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# Contents

**Chapter 3: Surface samples from Mawddach Estuary**

Appendices

## List of Figures

**Chapter 4**

**Chapter 5**

**Chapter 6**

**Chapter 7**

**List of tables**

**CHAPTER 1: INTRODUCTION**

**1.1 Introduction**

The organic matter in the ocean is one of the larger reactive reservoirs of carbon on the earth's surface. Knowledge of the chemical nature and quantity of organic components and their interactions with other chemical and biological systems is important in the understanding and modelling of the carbon cycle. The global view of carbon cycling considers the ocean as an entity interacting with the atmosphere, the continent, the sediment and all form of biota at different time and space scales through complex interfaces (Saliot *et al.*, 1991). Since the majority of organic carbon in the oceans is held within the sediments, and approximately 80% of sediments on earth are trapped in the continental margins, the processes occurring in coastal areas are of vital importance in the global carbon cycle (Grimalt and Albaiges, 1990). Coastal margins are dynamic regions where carbon inputs are typically derived from both terrestrial and marine sources. A river estuary and its adjoining shelf constitute a complex mixing zone and receives organic matter from diverse sources such as continental plants, phytoplankton, zooplankton, bacteria, anthropogenic, atmosphere fallout *etc*. The study of the organic inputs to these systems both in bulk terms and at the molecular level is important to the distribution and fate of organic material during their incorporation into marine sediments, and it provides a better understanding to the cycle of organic carbon in coastal areas.

**1.2 The molecular biomarker approach**

Molecular biomarkers, *i.e.* organic compounds detected in the geosphere with structures suggesting an unambiguous link with known natural products, are specific indicator compounds that can be utilised for source inputs (Aboul-Kassim and Simoneit, 1996). Such molecules are characterised by their restricted occurrence, source specificity, molecular stability and suitable concentration for analytical detection (Brassell and Eglinton, 1986). Biomarkers have wide spread applications in organic matter source identifications in both recent and ancient sediments (Lajat *et al.*, 1990; Rowland and Robson, 1990; Hedges and Prahl, 1993).

Most work has been conducted on the lipid fractions extracted by organic solvent from organisms and sediments. Such lipid biomarkers can be expected to reflect both the source and biogeochemical processes involved (Eglinton *et al.*, 1993). Lipid biomarkers are powerful indicators of organic matter production, because of the specificity of their biosynthesis with regard to terrestrial and marine organisms, the adaptation of biosynthetic pathways to environmental parameters such as temperature, and their stability in recent sedimentary environments (Smith *et al.*, 1983; Saliot *et al.*, 1991; Laureillard and Saliot. 1993).

Individual biomarker molecules synthesised by living organisms provide the starting point for the interpretation of biomarkers. These parent compounds then undergo diagenesis which gives rise to derived marker compounds exhibiting major or minor changes in the original molecular structure (Eglinton *et al.*, 1993). Thus, the presence of single biomarker compounds can provide useful information, for example some measure of input from a particular organism. This concept has proved to be very useful in petroleum exploration and palaeoenvironmental geochemistry where organic biomarkers are used as tracers of the originally sedimented organic materials (Grimalt and Olive, 1993).

Unambiguous markers are necessary to identify accurately sources of carbon input but unfortunately most molecular markers are common to several types of living organisms, both terrestrial and marine (Saliot *et al.*, 1991, Colombo *et al.*, 1997). Biomarkers that always have been used as indicators of source materials are sterols, fatty alcohols, fatty acids and hydrocarbons. Synthetic compounds (anthropogenic) such as linear alkyl-benzene sulphonate and alkyl-phenols can also be used to trace sources (Killops and Killops, 1993). However these compounds are only appropriate for recent years and can only be used to trace anthropogenic inputs such as sewage.

### 1.2.1 Sterols

Sterols are found in both free and bound form (*e.g.* steryl esters and glycosides) in organisms. The term sterol is commonly used to denote the steroidal alcohols (Killops and Killops, 1993) or more specifically, stanols denote saturated stenols, whilst stenols contain an unsaturated C=C bond within the ring system. Sterols are a very popular series of

Cholesterol

Brassicasterol

Dinosterol

Campesterol

β-sitosterol

Ergosterol

Stigmasterol

Coprostanol
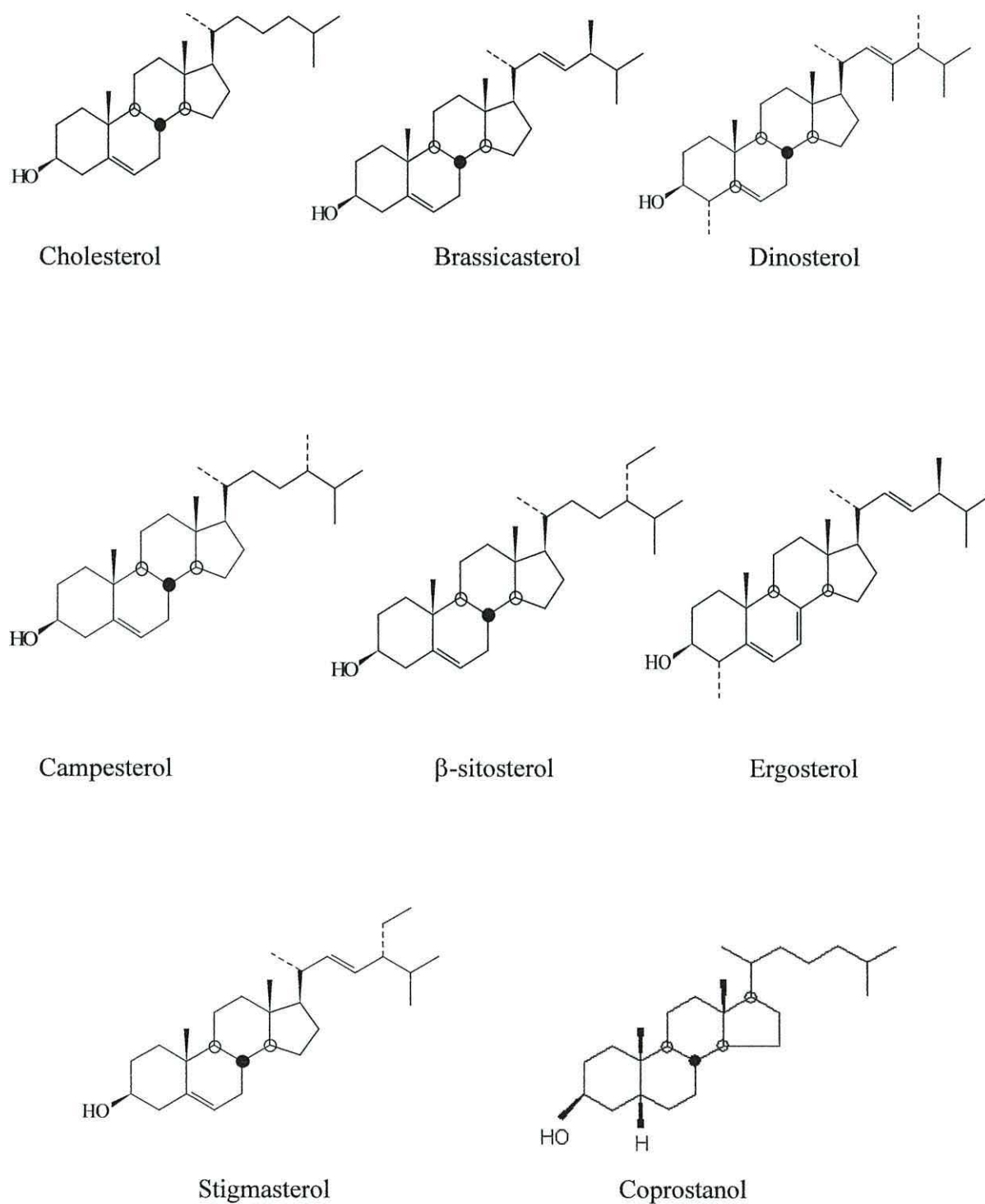
Figure 1.1: Some geochemically important sterols

biogeochemical markers and they are ubiquitous constituents of the living world. Sterols are relatively stable structural components and are found in the marine environment and geological record allowing detection and source elucidation at a later date (Volkman, 1986). Some of the geochemically important sterols are illustrated in Figure 1.1. They have been successfully used as tracers of inputs from various species of marine and terrestrial plants and animals as well as from sewage discharges (Volkman, 1986; Saliot *et al.*, 1991; Nichols and Espey, 1991; Mudge and Norris, 1997).

Sterols occur in high concentrations in most recent sediment. Like other lipids, sterols are better preserved in sedimentary environments than most other biological products such as carbohydrates and amino acids (Saliot *et al.*, 1991) because they are neither quickly degraded under reducing conditions nor completely catabolized. They also possess structural features, such as positions of double bonds and patterns of side-chain alkylation, which are restricted to a few group of organisms (Volkman, 1986). Thus, sterol fingerprints can record plant and animal organic matter input. Sterols of geochemical significance are mainly $C_{27}$ to $C_{30}$ compounds with a $\beta$-hydroxy group at C-3 and a side chain on the D-ring (Killops and Killops, 1993). Sterols present a great structural diversity, and are often specific to various types of living organisms (Volkman, 1986; Volkman *et al.*, 1987; Lajat *et al.*, 1990; Hedges and Prahl, 1993). Over the past two decades, extensive research has been carried out on sterol distributions in sediments and seawater to investigate their potential as ecological markers for the contribution from terrestrial and marine organisms to sediments (Volkman, 1986; Volkman *et al.*, 1987). Generally the sterol distributions found in lake sediments tend to be dominated by $C_{29}$ sterols, which are major constituents of higher plants; they differ significantly from the distributions observed in plankton and marine sediments, where $C_{27}$ and $C_{28}$ sterols are abundant (Huang and Meinschen, 1976).

**1.2.1.1 Sterol distributions and associated sterol signatures**
Table 1.1 shows the source allocation for sterols. The plankton in general contains $C_{27}$ and $C_{28}$ sterols. Phytoplankton usually contains abundant $C_{28}$ sterols (although diatoms can contain approximately equal amounts of $C_{27}$, $C_{28}$ and $C_{29}$ sterols). Zooplankton often contains abundant $C_{27}$ sterols, particularly cholesterol (cholest-5-en-3β-ol) (Killops and

Killops, 1993). Chlorophyceae are known to accumulate sterol esters. High amounts of these sterols have been found in the sterol esters from the Krka Estuary waters (Laureillard and Saliot, 1993), confirming the occurrence of Chlorophyceae and the relationship between the presence of Chlorophyceae and 24-ethylcholesta-5,22-dien-3β-ol. Several $C_{27}$, $C_{28}$ and $C_{29}$ sterols were identified in the samples from the rivers of the Orinoco Basin (Jaffe *et al.*, 1995) showing that they probably arose from an autochthonous planktonic input. Cholesterol occurs in variable amounts in most phytoplankton (including cyanobacteria) and is sometimes a major component. In most species of diatom group, apart from the cholesterol, the major sterol found is generally 24-methylcholesta-5,22-dien-3β-ol (brassicasterol) (Laureillard and Saliot, 1993; Colombo *et al.*, 1997). Some species also contain large amounts of cholesterol or 24-methylcholesta-5,24(28)-dien-3β-ol. Dinoflagellates are the most important source of 4-methylsterols in the sediments (*e.g.* the $C_{30}$ compound 4α,23,24-trimethyl-5α(H)-cholest-22-en-3β-ol also known as dinosterol). Many reports of dinosterol in sediments are well correlated with the presence of dinoflagellate remains. Dinosterol is a significant constituent of the sterol distributions isolated from sediments from the Peru upwelling region (Smith *et al.*, 1983; Volkman *et al.*, 1987). Dinoflagellates occur in the phytoplankton in this region and thus are a possible source of dinosterol (Volkman *et al.*, 1987) but Smith *et al.*, (1983) also found significant amounts of dinosterol in sediments, which lacked recognisable dinoflagellate remains or cysts. These sediments contain abundant organic matter from diatoms, and most of the lipids were attributable to this source (Smith *et al.*, 1983; Volkman *et al.*, 1987), so there is a possibility that some of the dinosterol present could have been contributed from diatoms rather than dinoflagellates. Although dinosterol is not a unique marker for organic matter from dinoflagellates, it is equally true that dinoflagellates are the most likely source of this sterol in many marine environments.

Three sterols are often found in large amounts in terrestrial higher plants; β-sitosterol (24-ethyl-cholest-5-en-3β-ol), stigmasterol (24-ethylcholesta-5,22E-dien-3β-ol) and campesterol (24-methylcholest-5-en-3β-ol). Thus, these compounds are commonly used as tracers of continentally derived organic matter inputs into marine system although inferences drawn from sterol distributions regarding terrigenous *vs.* marine sources of organic matter must be made within caution (Volkman, 1986). Very low concentrations of

cholesterol and brassicasterol may be present in some of the higher plants. Calculations based on the ratio of $C_{27}$ to $C_{29}$ sterols were proposed by Huang and Meinschein (1976) to determine the contributions of terrigenous and autochthonous organic material in marine and lacustrine sediments. Triangular diagrams (Figure 1.2) plotting $C_{27}$, $C_{28}$ and $C_{29}$ sterol contents of reference materials, such as marine phytoplankton and higher terrestrial plants, were proposed and used extensively up to the 1980's to discriminate between input sources and characterise ecological systems (Saliot *et al.*, 1991). This approach to sterol
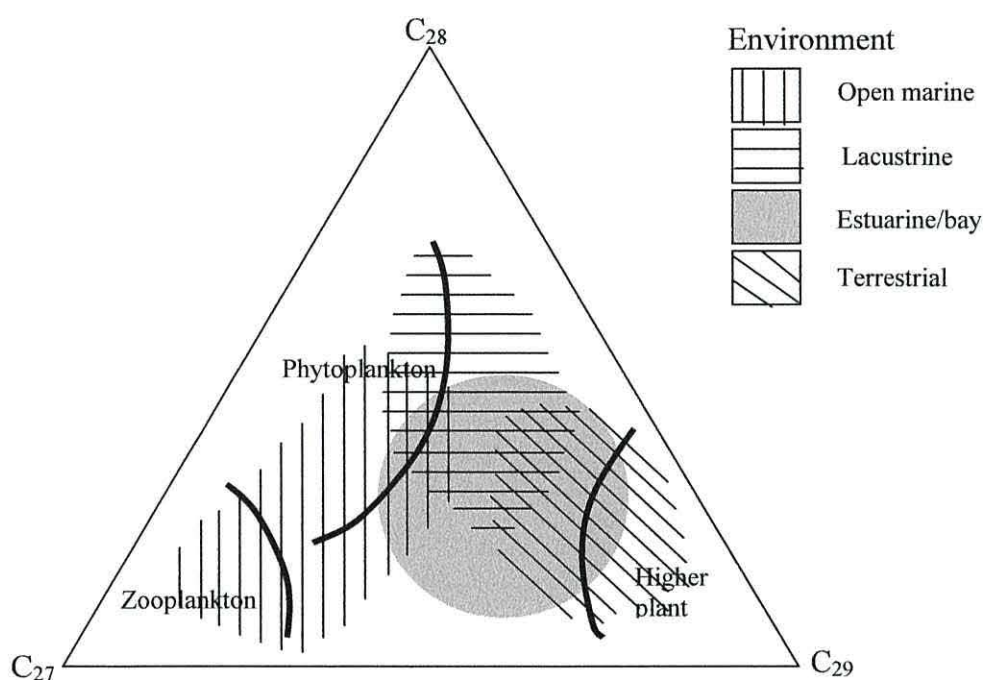


Figure 1.2: Sterol distributions (expressed as relative amounts of $C_{27}$, $C_{28}$ and $C_{29}$ compounds) in relation to source organisms and environments (From Killops and Killops, 1993, After Huang and Meinschein, 1979)

distribution is very simplistic and may not always provide accurate indications of contributing organism groups. It can be difficult to distinguish between marine phytoplankton and zooplankton on the basis of carbon number. Marine phytoplankton can also contain $C_{28}$ and $C_{29}$ sterols with similar structure to higher plant sterols (Killops and Killops, 1993). For example, the $C_{29}$ compound 24-ethylcholest-5-en-3$\beta$-ol is found in higher plants and many unicellular algae and it is also often dominant among the sterols of cyanobacteria. Stigmasterol is also common in Prymnesiophycean algae, diatoms and

dinoflagellates (Huang and Meinschein, 1979; Volkman, 1986; Volkman *et al.*, 1998). Clearly there are many environments where high concentrations of 24-ethylcholest-5-en-3β-ol could be derived from sources other than vascular plants. Therefore, further studies are required on the validity of $C_{29}$ sterol as a terrestrial marker in sediments. A better approach is to compare inferences drawn from the sterol data with information derived from other lipid classes. The presence of organic matter from terrestrial plants can be confirmed from such biomarkers as long-chain alkanes showing a high proportion of odd chain-lengths, saturated even-chain $C_{40}$-$C_{60}$ wax esters (Cranwell and Volkman, 1981) and hydroxy fatty acids.

Coprostanol (5β-cholestan-3β-ol) is the principal sterol in human and higher animal faeces (Walker *et al.*, 1982). Several papers have demonstrated that its concentration can be used for a quantitative measurement of faecal pollution in aquatic environments (Venkatesan and Kaplan, 1990). Consequently, it has been used as an indicator of sewage contamination. Recently, it has been suggested that coprostanol may be an unambiguous indicator of faecal contamination in seawater and marine sediments (Jeng and Han, 1994; Mannino and Harvey, 1999; Mudge and Lintern, 1999; Peng *et al.*, 2002). Coprostanol is produced mainly in the intestines of mammals (including man) by enteric microbial reduction of cholesterol, the main steroid found in the tissues of vertebrates (Escalona *et al.*, 1980). The presence of this compound in natural environments is considered a reliable indicator of mammalian faecal contamination because this process is the only known source of coprostanol (Yde *et al.*, 1982). Some marine mammals have also been shown to contain a high percentage of epicoprostanol (Venkatesan and Santiago, 1989). Human waste, however, contains only the 3β isomer (coprostanol) not epicoprostanol. A chronological study of coprostanol concentrations in a dated sediment core has been shown to reflect the true sewage inputs over 160 years. Coprostanol will degrade during aerobic wastewater treatment processes (McCalley *et al.*, 1981). However, coprostanol, along with cholesterol (cholest-5-en-3β-ol) and cholestanol (5α-cholestan-3β-ol) is quite persistent in anoxic sedimentary environments (Readman *et al.*, 1986; Bartlett, 1987; Jeng and Han, 1996). Coprostanol also has been shown to be a reliable marker of sewage pollution when coliform bacteria may have been destroyed due to high temperatures or the presence of toxic substances (Yde *et al.*, 1982, Dureth *et al.*, 1986).

Table 1.1: Source allocation for sterols

| Compound | Source | Reference |
|---|---|---|
| Cholesterol | Algae | Volkman, 1986, Barrett *et al.*, 1995 |
| | Fish, sharks, seal | Borchjensen and Mollerup, 1996 |
| | Domestic sewage | Grimalt *et al.*, 1990; Nichols *et al.*, 1993a; Conte *et al.*, 1994; Mudge and Seguel, 1997 |
| β-Sitosterol | Terrestrial plants | Goad and Goodwin, 1972 |
| | Phytoplankton | Volkman, 1986; Mudge *et al.*, 1999 |
| 5β-Coprostanol | Sewage | Walker *et al.*, 1982; Yde *et al.*, 1982; Dureth *et al.*, 1986; Grimalt *et al.*, 1990; Venkatesan and Kaplan, 1990; Nichols and Espey, 1991; Nichols *et al.*, 1993a; Jeng and Han, 1994; Mudge and Bebianno, 1997; Mannino and Harvey, 1999; Mudge and Lintern, 1999; Peng *et al.*, 2002 |
| Epicoprostanol | Sewage | Mc Calley *et al.*, 1981; Mudge and Bebianno, 1997 |
| Ergosterol | Eumycetic fungi | Newell and Fell, 1992 |
| | Vascular plant | Mudge and Norris, 1997 |
| 24norcholesta-5,22(E)-dien-3β-ol and 24norcholesta-22(E)- en-3β-ol | Marine organisms such as sponges and marine worm | Bohlin *et al.*, 1982; Volkman, 1986; Ponomarenko *et al.*, 1995; Yunker *et al.*, 1995 |
| Cholesta 5,22-dien-3β-ol | Diatoms | Volkman, 1986; Nichols *et al.*, 1993b; Skerratt *et al.*, 1995 |
| Brassicasterol | Algae especially diatom | Volkman, 1986; Laureillard and Saliot, 1993; Falcão, 1996; Colombo *et al.*, 1997 |
| Fucosterol | Fucoid seaweed | Piovetti *et al.*, 1991; Falcão, 1996 |
| Dinosterol | Dinoflagellate | Volkman *et al.*, 1987; Piretti *et al.*, 1991 |
| Campesterol | Algae | Volkman, 1986 |
| | Terrestrial plants | Killops and Killops, 1993 |
| Stigmasterol | Algae, diatoms and dinoflagellates | Huang and Meinschein, 1979; Volkman, 1986; Volkman *et al.*, 1998 |
| | Terrestrial plants | Nichols *et al.*, 1982; Killops and Killops, 1993 |

Sherwin *et al.*, (1993) analysed coprostanol in sediments and mussels collected from 25 stations in the canals and lagoons of Venice, Italy to investigate dispersal and accumulation of untreated sewage. Concentrations of sedimentary coprostanol ranged from $0.2\mu g\ g^{-1}$ in a reference station in the northern Adriatic Sea to $41\mu g\ g^{-1}$ in an interior canal of Venice. These values made up 4% to 44% of the total sterols, and indicated which areas of the lagoon had the greatest contribution from sewage discharge. Areas with high sedimentary coprostanol concentrations are good indicator of sewage discharge in the lagoon. Areas with high sedimentary coprostanol concentrations either lacked mussels entirely or contained mussels with severe environmental stress. Their data suggested that sedimentary coprostanol concentrations appear to be a useful indicator of potential ecological problems due to the direct input of untreated waste. The different distribution of the sterols in the sediments can be a method to discriminate the different input of organic material. Nevertheless, the microbial transformations of these compounds under different environmental conditions, and the absence of specificity of single sterols in living material, make it difficult to achieve this aim. However, high levels of coprostanol (expressed both as a percentage of total sterols and as the cholesterol/coprostanol ratio) seems to be a good indicator of human faecal contamination and this is confirmed by the high levels of coprostanol detected in the sediment samples within Venice, Italy.

Leeming and Nichols (1998) collected 63 sediment samples from Derwent Estuary in Tasmania, Australia to analyse sterol biomarkers such as coprostanol and $\beta$-sitosterol. The estuary was divided into three parts; lower, mid and upper estuaries. Sewage is discharged at 13 points throughout the estuary. Wood fibre has been discharged from the paper mill in the upper estuary. Their study showed the influence that human inputs (sewage and pulp-mill effluent) have on sediment composition. Sediments of the mid Derwent Estuary and parts of the upper estuary are heavily polluted by sewage, whereas pulp-fibre pollution is most severe in the upper estuary, with diminishing amounts downstream. Sediments of the lower estuary have biomarker concentrations and distribution more typical of open marine systems. This study demonstrates that sterol biomarkers can be used to assess and monitor the effects of human derived inputs.

## 1.2.1.2 Diagenesis

In organic geochemistry, the term diagenesis is applied to the processes affecting the products of primary production that take place prior to deposition and during the early stages of burial under conditions of relatively low temperature and pressure (Killops and Killops, 1993). Microbial activity is one of the major agents altering sedimentary organic matter during early stages of diagenesis near the sediment-water interface. The more biochemically labile organic compounds are preferentially consumed, leaving behind the more biochemically stable material and a variety of alteration products. Simple and unbound organic compounds tend to become incorporated into a more complex and poorly characterised polymeric material (Wakeham, 1987; Wakeham and Ertel, 1988). Degradation of organic material begins in the water column and continues after sedimentation. Different compound classes are degraded at different rates. The quantity and quality of organic matter eventually preserved in sediments varies greatly depending on the nature of material delivered to the sediment and on the depositional environments (Wakeham and Ertel, 1988), Once at the sediment, the diagenesis of organic matter generally proceeds by aerobic decomposition in the upper few centimetres of the sediment followed by less efficient anaerobic processes below the depth at which all oxygen has been consumed (Wakeham and Ertel, 1988; Volkman *et al.*, 2000; Rütters *et al.*, 2002).

Two important factors controlling organic matter in the sediments are the quantity organic input (related to its source) and the microbial system where the organic matter is deposited (*e.g.* oxic, iron reducing, sulphate reducing, methanogenic, *etc.*). Other factors are its nutritional value to benthic organisms, its chemical and physical availability and the environmental conditions under which it was deposited (Canuel and Martens, 1996).

## 1.2.2 Fatty alcohols

Fatty alcohols or *n*-alkanols are straight-chain saturated alcohols. In marine sediments, they are primarily derived from wax esters. Wax esters are known to be widely present in the biosphere for example as external lipids of cuticular wax of land plants and as energy reserves for organisms. They are simple primarily esters of long-chain fatty alcohols and long-chain fatty acids. The chain lengths and the degree of unsaturation can vary in both the acid and alcohol moieties (Sargent *et al.*, 1977). Wax esters can be supplied to coastal

marine sediments from various kinds of organisms but the primary sources of wax esters in these environments are thought to be the marine zooplankton and terrestrial plants (Fukushima and Ishiwatari, 1984).

Large amounts of wax esters and their corresponding fatty alcohols have been identified in marine animals. The fatty alcohols of all marine wax esters are invariably either saturated, especially 16:0 or monounsaturated, such as 20:1 and 22:1 (Sargent *et al.*, 1977). An enormous quantity of wax esters produced and excreted by zooplankton such as copepods sometimes resulted in massive oil slicks on the surface of seawater. The major compounds in these natural oil slicks were monounsaturated fatty alcohols 20:1 and 22:1. The major fatty alcohols contained in the mixed zooplankton from the coastal upwelling area of Hyuga Nada in Japan, was 16:0, followed by the monounsaturates 20:1 and 16:1 (Rajendran *et al.*, 1991). Sargent *et al.*, (1977) found that *Calanus finmarchicus* from a Norwegian fjord contained 16:0, 20:1 and 22:1 fatty alcohols which accounted for 18%, 27% and 28% respectively of total fatty alcohols. Calanoid wax esters also usually contain small amounts of 18:1 units, both in the acid and alcohol moieties (Sargent *et al.*, 1981). The major fatty alcohol in wax esters from *Euphausiia crystallorophias* are 14:0, followed by 16:0 whereas 16:0 followed by 14:0 are major alcohols in wax esters from *Thysanoessa inermis* (Sargent *et al.*, 1981).

Wax esters are also produced by marine phytoplankton. For example, cryptomonad, *Chroomonas salina* grown photoheterotrophically in the presence of glycerol, produced large amounts of wax esters containing mainly 12:0, 14:0 and 16:0 alcohols (Antia *et al.*, 1974). It is believed that some phytoplankton species could contain substantial amounts of wax esters at least grown under restricting conditions. Boon *et al.* (1975) suggested wax esters present in diatomaceous ooze from Walvis Bay, S.W. Africa may originate from phytoplankton cells. In view of the abundant phytoplankton in Walvis Bay, and its periodic blooming, the nutrient deficient conditions, suggested necessary by Sargent *et al.*, (1977) are certainly possible. Volkman *et al.*, (1989) shown that diatom, *Navicula sp* contains a $C_{34}$ tetra-unsaturated alcohol. That was the first report of such a long-chain alcohol in diatoms, although $C_{30}$-$C_{32}$ mono- and di-unsaturated alcohols were recently identified in the acid hydrolised lipids from algae *Nannochloropsis* (Volkman *et al.*, 1992).

Another major source of wax esters and consequently fatty alcohols are terrestrial higher plants. Waxes mainly function as protective coatings, such as leaf cuticular waxes. These deposits are known to be complex mixtures of long chain alkanes, alcohols, ketones, aldehydes, acetals, esters and acids (Eglinton and Hamilton, 1967). The alcohol fraction of higher plant waxes may occur either free or esterified to long chain fatty acids in the form of wax esters. These may reach up to $C_{52}$, of which the compositional acids and alcohols are rich in straight-chain saturated species greater than $C_{24}$ (Fukushima and Ishiwatari, 1984). The size of leaf wax alcohols was found to vary between 20 and 31 atoms, with the series being dominated by an even number of carbon atoms. The most abundant chain lengths were found to be $C_{24}$, $C_{26}$ and $C_{28}$, both in free alcohols and those bound in wax esters. Waxes from the leaves of *Fagus sylvatica* L. (European beech tree) and *Hordeum vulgare* L. (barley) have been analysed by Reynhardt and Reinderer (1994). They found that the wax from *F. sylvatica* consisted mainly of *n*-alkanals, *n*-alkanes and *n*-alkanols, with chain lengths ranging from 20 to 52 carbon atoms. The wax from *H. vulgare* consisted mainly of *n*-alkanols, which were found to have chain lengths ranging from 20-50 carbon atoms. It shows that the fatty alcohols derived from the terrestrial higher plants consist comparative long, unsaturated carbon chains (> $C_{20}$), which may be free or esterified to fatty acids in the form of alkyl esters. Both aquatic organisms and terrestrial plants are the potential source of coastal marine sediment (Fukushima and Ishiwatari, 1984).

### 1.2.2.1 Distribution of fatty alcohols in marine sediments

Several studies have been conducted to establish the nature and source of lipids in intertidal sediments. Fatty alcohols are included in some multimarker studies of coastal sediments. Mudge and Norris (1997) analysed the fatty alcohols and sterols in the surface sediments from the Conwy Estuary in North Wales. Twenty-four (24) samples were collected; 4 marine samples, 13 estuarine samples and 7 freshwater samples. They found that marine samples were characterised by relatively high concentration of short chain fatty alcohols with the domination of $C_{16}$. Marine derived fatty alcohols are present in greater amounts than the terrestrially derived fatty alcohols in the lower estuary. The upper estuary is characterised by an increasing dominance of terrestrially derived materials, while the freshwater samples have greater concentrations of long chain fatty alcohols ($C_{22}$-$C_{26}$).

A sample of the sediment-water column interface, which lies on the continental shelf under the Peru upwelling regime, has been examined for their lipid distribution by Smith *et al.*, (1983). The major fatty alcohol in the sediment was phytol, which is assumed to be derived mainly from phytoplanktonic chlorophylls. The biological input to this region is thought to be from phytoplankton blooms. However phytol was also found to account for some 10% of the total alcohols in the wax esters of the euphausiid species *T. inermis* (Sargent *et al.*, 1981). Clearly phytol in such zooplankton species derives from the ingestion of phytoplanktonic material and its presence in the sediments may not reflect the direct input of phyplanktonic chlorophylls. The most abundant *n*-alkanol was $C_{18}$, having a concentration about six times greater than its homologues, which ranged from $C_{12}$-$C_{22}$. The absence of 20:1 and 22:1 alcohols appears to rule out an origin from copepods (Sargent *et al.*, 1977). Higher plant waxes tend to contain mainly longer chain ($>C_{22}$) saturated alcohols (Cranwell and Volkman, 1981). No such compounds were detected, further evidence that there is no significant higher plant input to these sediments. These results were totally contrasted with a study conducted by Volkman *et al.*, (1987). They also collected sediment samples from the Peru upwelling region and found acyclic alcohols consisted mainly of straight chain saturated alcohols from $C_{12}$ and $C_{32}$. The abundance of $C_{26}$ *n*-alkanol indicates a common source of higher plants. They also found unsaturated alcohols from $C_{14}$ to $C_{26}$.

Fukushima and Ishiwatari (1984) conducted a study to isolate wax esters from coastal marine sediment. The aim of the study was to obtain further insight into the distribution of wax esters from various sediments and to determine the early diagenesis of these esters. They collected surface and core samples from Tokyo Bay. The core analysis showed that alcohol compositions changed from the top to the bottom of the core. The concentration decreased towards the deeper section, which was due to decrease of the short-chain ($C_{14}$-$C_{18}$) alcohols. Wax esters with short-chain alcohols were found in the Tokyo Bay surface sediment from the core sample and seemed to resemble those of the esters from intertidal sediment (Volkman *et al.*, 1981). These wax esters originated from aquatic organisms such as zooplankton and marine invertebrates either as direct inputs or derived from the diet of these organisms. In contrast, the long-chain ($>C_{22}$) alcohol concentration varied little throughout the core and it is thought that the long-chain constituents should have been

preserved in the sediment with little deterioration (Fukushima and Ishiwatari, 1984). Not only source inputs of organic matter control the biomarker signatures in coastal sediments, but also the subsequent degradation of organic matter, and there appears to be preferential degradation of marine derived compounds in the sediment from this study.

Grimalt and Albaiges (1990) conducted a molecular study of the lipid fraction from the riverbed, coastal lagoons, bays and open sea environments of the Ebro Delta in the western Mediterranean. Their aim was to use the lipid markers to characterise the depositional environments. Two different distributions were recognised in the sediments of the study area. Short chain fatty alcohols predominate in the open sea samples, with $C_{18}$ and $C_{20}$ being the greatest amount of saturated components and 18:1 and 20:1 for the unsaturated components. It is suggested that $C_{18}$ originated from marine source such as zooplankton inputs either as intact animals, faecal matter or detritus (Smith *et al.*, 1983). The second type of distribution was found near the river mouth, comprises $C_{16}$-$C_{30}$ *n*-alcohols. The *n*-alkanols were analysed following hydrolyses of the sediment extracts. These alcohols are predominantly even carbon number homologue and include two main groups: one in the $C_{22}$-$C_{30}$ range, corresponding to the samples containing important terrestrial inputs (Volkman *et al.*, 1987), and the other composed of $C_{14}$-$C_{20}$ alcohols in samples with major marine contributions. Two main features influence the sedimentary systems of the Ebro Delta: the inputs of organic matter and the hydrogeographic conditions responsible for the dispersion, degradation or concentration of these organic contributions.

### 1.2.3 Fatty acids

Fatty acids are widely occurring compounds and fulfil a variety of roles, such as cellular membrane components, energy stores and protective coatings. They represent the major lipid constituents of many marine organisms and, therefore are the dominant lipids in particles and sediments (Saliot *et al.*, 1991; Volkman *et al.*, 1998). Nevertheless, they can be ambiguous source indicators since they are present in many organisms. Sources of fatty acids include bacteria, microalgae, higher plants and marine fauna (Table 1.2). They have great structural diversity and high biological specificity (Parkes, 1987). Therefore they have been used as taxonomic indicators such as in microalgae (Volkman *et al.*, 1998). Fatty acids have also been used in the characterisation of microbial populations in polluted

marine sediments (Parkes and Taylor, 1983) as well as in the assessment of reactivity of recently deposited organic matter (Canuel and Martens, 1996).

More than 500 fatty acids are known in plants and animals and most occur bound to other compounds through ester linkages (Killops and Killops, 1993). However, the specificity of some individuals or groups of acids has been used to assess the origins of organic matter in marine samples. Fatty acids are designated as total number of carbon atoms: number of double-bonds followed by the position of the double-bond from the $\omega$ (aliphatic) or $\Delta$ (carboxylic) end of the molecule.

Micro algae are a major source of fatty acids in most sedimentary environments. Short chain saturated fatty acids ($C_{14}$-$C_{22}$) are typical of phytoplankton (Claustre *et al.*, 1989) and have been reported in settling particles in marine environment (Reemtsma *et al.*, 1990; Colombo *et al.*, 1997; Carrie *et al.*, 1998). The saturated 14:0 acid is a major lipid component of phytoplankton, especially the Bacillariophyceae (diatoms) and Prymnesiophyceae (coccolithoporids) (Reitan *et al.*, 1994) and to a lesser extent in some of the Dinophyceae (dinoflagellates) (Mudge *et al.*, 1998). Saturated 16:0 is also a major fatty acid in phytoplankton and is ubiquitous in all marine organisms (Carrie *et al.*, 1998).

Recent fatty acid studies have shown algal species to be rich sources of polyunsaturated fatty acids (PUFAs) (Volkman *et al.*, 1998). Polyunsaturated fatty acids are biosynthesised from saturated fatty acids by the action of desaturase enzymes (Killops and Killops, 1993). Polyunsaturated fatty acids present in the sediments have been used to indicate "fresh" algal organic matter (Canuel and Martens, 1996; Carrie *et al.*, 1998). PUFAs are normally associated with phytoplankton where they have been used to distinguish between phytoplankton taxonomic classes using the position of double bonds. Some phytoplankton contains high concentration of certain PUFAs such as 20:5$\omega$3 and 22:6$\omega$3 (Volkman *et al.*, 1989). For example, marine eustigmatophytes such as *Nannochloropsis sp.* contain 20:5$\omega$3 but little 22:6$\omega$3, whereas haptophytes such as *Pavlova sp.* contain both 20:5$\omega$3 and 22:6$\omega$3 (Volkman *et al.*, 1998). Chlorophtes (green algae) have a predominant 18:0 PUFAs such as 18:2$\omega$6 and 18:3$\omega$3 (Volkman *et al.*, 1989; Volkman *et al.*, 1998). Dinoflagellates have high levels of 20:5$\omega$3 and 22:6$\omega$3 as well as $C_{18}$ fatty acids such as

18:3ω3 and 18:4ω3 (Reitan *et al.*, 1994). Diatom fatty acids are characterised by low concentrations of 18:0 fatty acids relative to 16:0 fatty acids and 20:5ω3 (Smith *et al.*, 1983; Colombo *et al.*, 1997).

The 18:1ω9 fatty acid is common in animals, higher plants and algae. 16:1 fatty acid is also often an abundant acid in sediments. 16:1 fatty acid is relatively common in marine algal species (Reitan *et al.*, 1994) although the 16:1ω5 isomer is less common and has been identified in some brown algae (Khotimchenko, 1995). 16:1ω7 is a major fatty acid of diatoms (Killops and Killops, 1993; Skerratt *et al.*, 1995).

Bacteria contain the most distinct fatty acid compositions with high proportions of 13:0 to 21:0 odd numbered fatty acids, often branched and with at most one unsaturation (Claustre *et al.*, 1989). Appreciable amounts of branched-chain fatty acids have been reported in several studies (Perry *et al.*, 1979; Parkes and Taylor, 1983). Branched acids can be source-specific and they are rarely unsaturated. *Iso* and *anteiso* saturated fatty acids are found in fungi, molluscs and phytoplankton, but they are generally in higher levels in bacteria and are often observed in the $C_{13}$-$C_{17}$ range (Killops and Killops, 1993). The 18:1ω7 fatty acid is particularly abundant in bacteria and is classically used as a biomarker for bacterial activity (Bradshaw *et al.*, 1991; Killops and Killops, 1993). Bacteria can also be a significant source of 16:1ω7 and 16:1ω9 has been found to predominate in several species of heterotrophic marine bacteria (Bertone *et al.*, 1996). Cyanobacteria exhibit 16:0, 16:1ω7 and 18:1ω9 fatty acids. The odd-numbered acids, 15:1 and 17:1 with ω6 or ω8 C=C bonds, are bacterial markers, produced by the anaerobic biosynthetic route (Sargent *et al.*, 1977).

A common feature of the fatty acid distributions in sediments is the presence of 20:0-30:0 saturated straight chain fatty acids that show a strong predominance of even chain lengths. In many types of sediment, particularly those from lacustrine environments, these fatty acids are probably derived from the surface waxes of higher plants (Fukushima and Ishiwatari, 1984; Volkman, 1986; Volkman *et al.*, 1998). Long-chain saturated fatty acids (generally $C_{20}$-$C_{30}$) and longer in sediments is characteristic of higher plant detritus (Killops and Killops, 1993). However, diatoms have been found to be responsible for the

accumulation of high molecular weight fatty acids (up to 26:0-28:0) in recent sediments (Volkman *et al.*, 1980; Nichols *et al.*, 1986).

Table 1.2: Source allocation for fatty acids

| Compound | Source | Reference |
|---|---|---|
| **14 carbon compounds** | | |
| Saturated 14:0 | Phytoplankton | Claustre *et al.*, 1989; Reemtsma *et al.*, 1990; Reitan *et al.*, 1994; Napolitano *et al.*, 1995 |
| 14:1 | Cyanobacteria | Caudales and Wells, 1992; Caudales *et al.*, 1993 |
| | Marine mammals | Kakela and Hyvarinen, 1993 |
| | Yeasts | Sajbidor *et al.*, 1994 |
| **16 carbon compounds** | | |
| Saturated 16:0 | Phytoplankton | Reitan *et al.*, 1994; Carrie *et al.*, 1998 |
| | Higher plants | Rao *et al.*, 1990 |
| | Sewage plants | Quemeneur and Marty, 1994; Mudge and Bebianno, 1997 |
| 16:1 | Marine algae | Reitan *et al.*, 1994; Berge *et al.*, 1995 |
| 16:1ω5 | Brown macro algae | Khotimchenko, 1995 |
| 16:1ω7 | Phytoplankton | Killops and Killops, 1993; Berge *et al.*, 1995; Skerratt *et al.*, 1995; Suzuki and Matsuyama, 1995 |
| 16:1ω9 | Heterotrophic bacteria | Bertone *et al.*, 1996 |
| 16:2 | Green algae | Wakeham and Canuel, 1990; Khotimchenko, 1993; Floreto *et al.*, 1996 |
| **18 carbon compounds** | | |
| Saturated 18:0 | Sewage | Newton, 1995 |
| 18:1ω7 | Bacteria | Currie and Johns, 1988; Bradshaw *et al.*, 1991; Killops and Killops, 1993; Kharlamenko *et al.*, 1995; Bertone *et al.*, 1996 |

| 18:2 | Waste water Sewage | Quemeneur and Marty, 1994 Nichols and Espey, 1991 |
|---|---|---|
| 18:3, 18:4 and 18:5 | Algae | Dunstan *et al.*, 1992 |
| 18:4ω3 | Phytoplankton | Hama and Handa, 1992; Reitan *et al.*, 1994 |
| | Diatoms | Berge *et al.*, 1995; Suzuki and Matsuyama, 1995 |
| 18:5ω3 | Coccolithophores Green micro algae | Pond and Harris, 1996 Dunstan *et al.*, 1992 |
| 20 carbon compounds<br><br>20:2 and 20:3 | Brown macro algae | Khotimchenko, 1991 |
| 20:5ω3 | Red and brown algae Diatoms Phytoplankton | Dembitsky *et al.*, 1991; Banaimoon, 1992 Fahl and Kattner, 1993 Volkman *et al.*, 1989 |
| 22 carbon compounds<br><br>22:1ω11 and 22:6ω3 | Phytoplankton<br><br>Copepods | Volkman *et al.*, 1989; Graeve *et al.*, 1994; Volkman *et al.*, 1989 Kattner *et al.*, 1994 |
| Long chain saturated compounds | Terrestrial plants | Fukushima and Ishiwatari, 1984; Volkman, 1986; Currie and Johns, 1988; Volkman *et al.*, 1998 |
| Odd chain length compounds | Bacteria | Rajendran *et al.*, 1997 |

## 1.2.3.1 Distribution of fatty acids in marine sediments

A number of studies have been conducted in an attempt to establish the nature and source of lipids in marine sediments. Few studies that been conducted concentrated on lipids and their diagenesis, though some recent work has included this group of lipids in a more multimarker approach in the analysis of coastal sediments as considerable amounts of saturated and unsaturated fatty acids are present in the surface sediments (Colombo *et al.*, 1997; Carrie *et al.*, 1998; Mudge *et al.*, 1998). The lipid composition of recently deposited sediments is the result of the input of biological material and its utilisation by

microorganisms, together with the direct contribution from microorganisms to the sediment biomass. The rapid alterations which lipids can undergo during the early stages of deposition, which are referred to as biological diagenesis, are considered to be brought about largely by microbial activity in the upper layers of recent sediments (Matsuda and Koyama, 1977). In general, individual and total fatty acids decrease in concentration with the increasing depth (Farrington and Quinn, 1973; Van Vleet and Quinn, 1979). Fatty acids are rapidly altered in marine sediments compared to other lipid groups. Polyunsaturated fatty acids degrade more rapidly than monounsaturated and saturated fatty acids. Within the saturated fatty acids, short chain fatty acids have higher rates of degradation than long chain fatty acids (Kawamura and Ishiwatari, 1984; Lajat *et al.*, 1990; Sun and Wakeham, 1994; Canuel and Martens, 1996).

Sediment samples in the Ebro Delta were collected by Grimalt and Albaiges (1990) including representative samples from the riverbed, coastal lagoons, bays and open sea environments. The sediment was dominated by a group of short chain (14:0-20:0) fatty acids with even carbon number preferences. Their occurrence is usually attributed to planktonic and bacterial inputs (Farrington and Quinn, 1973). Unsaturated fatty acids are particularly preserved in the lagoon, i.e. in the more reducing environments. A second group of straight-chain fatty acids forms a modal distribution of even carbon numbered 22:0-30:0 saturated homologs. These are constituents derived from cuticular waxes of higher plants, and their occurrence in coastal environments is associated with terrigenous inputs (Farrington and Quinn, 1973). Branched fatty acids, namely, *iso* and *anteiso* 15:0 and 17:0 acids were also present. These acids are common in cultures of bacterial species and in sedimentary microbial population (Volkman *et al.*, 1980). Thus, they are primarily indicators of microbial inputs. The greater amounts of autochthonous fatty acids in all sediments can be explained by the enhanced primary productivity stimulated by the nutrient supply or the river. The river is also an important contribution of allochthonous fatty acids (Gomez-Belinchon *et al.*, 1988), which are predominantly found in the water particulate phase. Sediment samples from the riverbed are characterised by more than 50% of long chain saturated fatty acids, thus defining an area of accumulation of fluvial allochthonous inputs. The coastal sands contain less total concentrations of fatty acids in the Ebro Delta compared to other environments, reflects low autochthonous and

allochthonous inputs. The polyunsaturated fatty acids are abundant in marine organisms, but they decrease rapidly to low values in marine sediments. The PUFAs composition is noted for a specific and preferential degradation. Due to the fact that unsaturated fatty acids are unstable relative to saturated fatty acids, it is possible to observe a decrease in the unsaturated fatty acids relative to the saturated fatty acids with increasing depths in recent sediments (Farrington and Quinn, 1973).

Smith *et al.* (1983) analysed the fatty acids from a sample of surficial sediment from the Peru Continental Shelf. This sample represents less than one year's accumulation but it provides information on the contemporary biological input and earliest stages of diagenesis. The total fatty acids show a distribution typical of a marine system, with no detectable fatty acids >24:0 indicating a lack of terrigenous input. The highly unsaturated fatty acids such as 20:4, 20:5 and the polyunsaturated 16:0 and 18:0 acids are characteristic of many marine planktonic algae. 22:6 is more typically associated with marine animals (Morris and Culkin, 1976) but has been identified in a variety of algae (Volkman *et al.*, 1981) and so could derive from phytoplankton. The presence of these polyunsaturated compounds is strong evidence that the lipids do represent the original input to the sediments, and has not undergone extensive degradation. *Iso* and *anteiso* 13:0, 15:0, 17:0 and 19:0 acids were also present. These compounds are usually taken as bacterial indicators (Boon *et al.*, 1975; Perry *et al.*, 1979). The presence of labile compounds such as polyunsaturated fatty acids (with two to six double bonds) implies that the sediment had undergone very little diagenetic alteration. The lipids are probably largely unchanged from the state in which they actually reached the sediment. They may therefore serve as a useful baseline in assessing diagenesis in older sediments, where diagenetic transformations are more advanced.

Lajat *et al.*, (1990) collected a sediment core of 55cm from the Santa Barbara Basin, off California. This basin is characterised by high phytoplankton production in surface waters and low oxygen content in bottom waters. The sediments show no bioturbation, and development of a bacterial mat community at the surface with predominance of sulphur-oxidising bacteria trapping particles, thus preventing redistribution of sediments. Free fatty acid concentrations showed a rapid decrease of concentration with depth for saturated

compounds, branched compounds and unsaturated fatty acids. Similar patterns have been reported by Boon *et al.*, (1975), Van Vleet and Quinn (1979) and Kawamura and Ishiwatari (1984). The decrease was particularly dramatic during the first two years, depending on the chemical structure and thus on the stability of the molecule: short-chain fatty acids degrade more rapidly than long-chain compounds, and polyunsaturated more than monounsaturated fatty acids. Ninety percent (90%) of the stock of polyunsaturated fatty acids is consumed within the first 2cm of the core.

Colombo *et al.*, (1997) collected a sediment core of 35cm from the Laurentian Trough. This site is characterised by high sedimentation rates. Sedimentary fatty acids show a decrease in the proportion of unsaturated and shorter chain fatty acids and a sharp increase of long chain terrestrial (>20:0) and bacterial branched fatty acids. This reflects the preferential utilisation of marine organic matter and of unsaturated and shorter chain fatty acids. These compounds are primary substrate for organisms, whereas higher molecular weight acids are not (Nishimura and Baker, 1987). The preservation of terrestrial fatty acids may also be enhanced by their inclusion resistant and microbially inaccessible higher plant matrices (Kawamura and Ishiwatari, 1984; Nishimura and Baker; 1987; Haddad *et al.*, 1992).

## 1.2.4 Other biomarkers

Other biomarkers that can be used to trace sources are lignins, photosynthetic pigments and stable isotopes. Lignins (phenolic polymers) are a major component of wood and occur in the cell walls of all vascular plant tissue (Readman *et al.*, 1986b; Dittmar and Lara, 2001). Their source, natural abundance, wide distribution and resistance to microbial degradation render them as good terrestrial markers (Readman *et al.*, 1986). Their phenolic aldehyde oxidation products can afford characterisation of their source material (Dittmar and Lara, 2001; Fisher *et al.*, 2003). Chemical oxidation and thermochemolysis of lignins and subsequent analyses of the resultant phenols are useful methods, since they provide information on specific vascular plant sources, *i.e.* gymnosperm *vs.* angiosperm and woody *vs.* non-woody (Hedges and Mann, 1979; Readman *et al.*, 1986; Gordon and Goñi, 2003). Furthermore, quantification of the phenols allows one to estimate the amount of lignin present (Ertel and Hedges, 1985). Therefore, the vascular plant fluxes to the sediments can

be estimated. Lignin has been used as a tracer of terrigenous organic matter in rivers (Hedges *et al.*, 1986; Goñi et al., 2000; Onstad et al., 2000), estuaries (Readman *et al.*, 1986b; Goñi and Thomas, 2000), and the coastal and open ocean (Hedges and Parker, 1976; Hedges and Mann, 1979; Prahl *et al.*, 1994; Goñi *et al.*, 1997, 1998).

Sedimentary records of photosynthetic pigments have been studied as more direct and general indicators of the phytoplankton population to elucidate the response of a lake phytoplankton system to environmental changes (Wetzel, 2001; Tani *et al.*, 2002). Chlorophyll, carotenoid and phycobilin pigments singly or in combination, have been used for chemosystematic identification of phytoplankton assemblages (Millie *et al.*, 1993). In the open ocean, chlorophyll *a* is widely used to estimate phytoplankton biomass and primary productivity within the water column as it is the most abundant pigment component of almost all phytoplankton species (Sun *et al.*, 1994). This algal pigment can be quickly photo-oxidised in the euphotic zone (Carpenter *et al.*, 1986) or further degraded by microorganisms during settling of particles (Furlong and Carpenter, 1988) or grazing (Leavitt and Carpenter, 1990; Strom *et al.*, 1998). Therefore, pigment content can be used to assess the source of the newly deposited material and its degree of "freshness" (Pinturier-Geiss, 2001). However, substantial amounts of chlorophyll *a* and its major degradation products, the phaeopigments, are delivered to the seafloor through sinking of intact cells, faecal pellets or other detrital materials (Méjanelle *et al.*, 1995). Depth profiles of carotenoids, the biomarkers of algal taxa, such as myxoxanthophyll or oscillaxanthin, unique to cyanobacteria are very useful in showing the development of eutrophy in lakes (Soma *et al.*, 1996). Although pigments may vary among cells within a taxon or between taxa, the abundances of these diagnostic pigments generally reflect the major distribution of the respective phytoplankton groups. Identification of algal phylogenetic groups through pigment signatures is limited to the division or class level.

Stable carbon and nitrogen isotopes and C/N elemental ratios have been used to evaluate the sources and fate of organic matter in estuarine and marine sediments (Thornton and McManus, 1994; Lee, 1994; Andrews *et al.*, 1998; Graham *et al.*, 2001). This is due to the fact that isotope fractionation happens in biogeochemical processes like nutrient and $CO_2$ assimilation or nitrate reduction. Therefore, the stable isotope ratios of bulk nitrogen and

organic carbon are widely used to distinguish between marine and terrestrial organic matter (Francois *et al.*, 1997; Voß and Struck, 1997; Onstad *et al.*, 2000; Graham *et al.*, 2001). It is generally assumed that the isotope ratio of bulk sedimentary nitrogen reflects mainly the organic source (Freudenthal *et al.*, 2001). The diagenetic shifts are also assumed to be constant or do not cause isotopic variations comparable in magnitude to variations of the primary sedimentary signal (Freudenthal *et al.*, 2001).

## 1.3 The use of chemometric techniques

The distribution of biological markers in sediments constitutes a reservoir of information about the operation of biogeochemical processes and how these processes responded to environmental change. More detailed characterisation of individual biomarkers regarding microbial and chemical modification and the origins of specific marker compounds are required in order to obtain accurate interpretation. It is essential that current and future studies utilise a multimarker approach in order to eliminate some of the problems associated with the interpretation of biogeochemical data. However, if these techniques are to be effective, they require complimentary analytical procedures. Chemometrics, the computer manipulation of large amounts of quantitative chemical data, combined with the multivariate statistical analysis techniques, may now prove to be the most effective method of analysing multimolecular data sets (Eglinton *et al.*, 1993). Recent literature of lipid biomarkers has highlighted the usefulness of multivariate statistical techniques in extracting the maximum information from complex mixtures of compounds (McCaffrey *et al.*, 1991; Yunker *et al.*, 1995; Mudge and Norris, 1997, Mudge *et al.*, 1998; Hinrichs *et al.*, 1999; Mudge *et al.*, 1999).

The sophistication of analytical techniques has developed for the investigation of environmental samples has led to large chemical databases. The number of compound classes investigated for every sample has increased, and the number of individual components has expanded, as structural elucidation becomes more and more precise. Therefore, subsequent handling and interpretation of growing data sets requires pattern recognition techniques such as multivariate statistics (Saliot *et al.*, 1991). To realise the potential inherent in a large suite of biomarkers from a large number of samples, multivariate chemometric techniques such as Principal Component Analysis (PCA) and

Partial Least Squares (PLS) path modelling are invaluable (Yunker *et al.*, 1995). The efficiency of a method for extracting information and optimising interpretation ultimately depends on the quality and relevance of the data.

Multivariate techniques are generally based on the concept of relationship or similarity existing between individual or groups of objects in a set of observations. The hypothesis is held that similar compounds showing similar statistical behaviour are likely to have the same or related sources. These techniques are robust, objective and are also the ideal adjunct to a multimarker approach (Yunker *et al.*, 1995). However, few organic geochemists have attempted to apply multivariate methods in the determination of sources of organic material, which is a major aspect of biogeochemical studies. The mathematical methods, which form the basis for multivariate analysis, have been developed for investigating sample point configuration in a two dimensional space with minor loss information. This greatly facilitates interpretation as the true representation of the sample is usually multidimensional and therefore, impossible to inspect visually. Although these techniques are likely to prove invaluable to future work, it is of importance to note that some fundamental questions, such as the chemical composition of sources and the specificity of many biomarker compounds still remain unanswered (Saliot *et al.*, 1991).

### 1.3.1 Principal component analysis (PCA)

Principal component analysis is used to reduce the dimensionality of multivariate data. It is an appropriate way to reduce data sets containing high numbers of variables. By reducing the number of original variables to a smaller number of independent variables, this approach highlights fundamental differences between groups of variables. It employs a mathematical transformation of the original data with no assumptions about the form of the covariance matrix. The aim of this procedure is to determine a few linear combinations of the original variables, which can be used to summarise the data set without losing much information (Meglen, 1992). PCA identifies dimensions of maximum variation in the original multidimensional data space, resulting in terms of vectors (principal components) that are linear combinations of the original variables.

Fifty-nine surface samples were collected from the Ria Formosa Lagoon, in Portugal by Mudge *et al*. (1998) to analyse the fatty acids distribution in this system. A total of 170 different fatty acids were quantified. Principal component analysis has been employed to extract and quantify individual sources from complex assemblages. Fifty-eight compounds were chosen by discarding those with the largest coefficients of variation. PCA was carried out with the proportion data. Principal component 1, which account for 33.5% of the variation in the data clearly separates the phytoplankton markers (such as polyunsaturated fatty acids and 16:1ω7) and bacterial or sewage markers (such as branched or odd chain length compounds). On the other hand, principal component 2 (12.9% of the variation) separates the short chain fatty acids from the long chain moieties, which are the terrestrial markers. PCA also separates the samples according to the input of organic matter such as phytoplankton, sewage and terrestrial inputs.

Yunker *et al*., (1995) used the integration of multivariate techniques and a multimarker approach to study the terrestrial and marine biomarkers in a seasonally ice covered Arctic estuary. In order to study seasonal variations in the sources, distribution and partitioning of organic matter in the Mackenzie estuary, they quantified a broad range of lipid compounds from both the Mackenzie River and shelf areas in the Beaufort Sea. The resulting matrix was analysed using PCA. PCA classifies biomarker variables according to their primary source (*e.g.* terrigenous, marine) and identifies compounds that covary. The application of PCA multivariate classification to sterols overcomes the limitation that few sterols by themselves are of sufficiently restricted distribution to provide unambiguous biomarkers. The PCA model separates marine and plant sterols. They found sterols such as 24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22(E)-dien-3β-ol, cholesta-5,24-dien-3β-ol and cholesterol to have principally marine sources on the shelf. While the plant sterols are campesterol, stigmasterol and β-sitosterol. The PCA also distinguishes a class of terrigenous biomarkers that project between the plant sterols and the hydrocarbons. PCA and correlation analysis suggest that the vascular plant sterols campesterol, stigmasterol and β-sitosterol are primarily terrigenous in origin on the Mackenzie Shelf. In PCA models, the 20:1 and 22:1 fatty alcohols are the strongest indicators of marine input. These alkenols are produced abundantly by arctic zooplankton (Sargent *et al*., 1977; Graeve and Kattner, 1992). Due to their very high concentrations, particularly in sediment trap samples

from this area, 20:1 and 22:1 are much stronger marine indicators in PCA model than are sterols. Although this proved to be an important observation, the use of these individual markers may not be directly applicable to other environments as epipelagic calanoid species from temperate and tropical regions produce lesser amounts of wax esters than Arctic species (Lee and Hirota, 1976). A second marine origin but with a decreasing leverage in the PCA model may also be assigned to the shorter chain length alcohols 14:0, 18:1, 16:0 and 16:1. The remaining alcohol, 18:0, was a minor constituent in most samples and projected between the marine and terrestrial groups indicating its mixed origin on the Mackenzie Shelf. It was found that in sediment samples taken from the river and nearshore, the even number *n*-alkanols, exhibited a bell shaped distribution maximising at 22:0, which they attribute to a source in higher plant waxes.

McCaffrey *et al.*, (1991) attempted to assess the utility of sedimentary hydrocarbons and alcohols in short term changes in depositional conditions in the coastal Peruvian upwelling regime at 15°S. Since continental run-off from Peru increases during the El Nino Southern Oscillation (ENSO), it was hypothesised that a sedimentary record of terrigenous input to the Peru margin sediments has potential as an indicator of past ENSO events. Identification of a signature of higher plant contributions to these sediments is complicated by the fact that marine sources of many lipids are also abundant in higher plants. Also the magnitude to higher plant input to these sediments is small relative to marine inputs. They performed PCA of the concentrations of 35 lipids (*n*-alkanes, *n*-alkanols, hopanols, ketols, lycopane, phytol, stenols, stanols, sterenes and tetrahymanol) to assist in distinguishing the relative importance of marine and terrigenous sources of lipids in these sediments. Principal component 1 accounted for 35.5% of the variance in the data. It appeared to represent the input of lipids derived from marine organisms in the overlying water column, as many of the stenols with high loadings, such as cholesterol and dinosterol are known to have planktonic sources. On principal component 2, compounds with highest loadings were the odd carbon numbered *n*-alkanes and long-chain ($C_{24}$-$C_{28}$) even carbon numbered *n*-alkanols. These compounds are abundant in the epicuticular waxes of higher plants and in previous studies have been assumed to be directly indicative of terrigenous carbon sources. However, in contrast to other papers, McCaffrey *et al.*, (1991) recognised that odd carbon numbered *n*-alkanes and long chain even carbon numbered *n*-alkanols cannot be assumed *a*

*priori* to be markers for terrestrial input. In order to substantiate this inference and to eliminate any ambiguity, they compared the profiles of inorganic indicators of terrigenous input to the sediments. Concentrations of aluminium, titanium, zirconium and iron were measured in sections of a sediment core and their profiles were found to be well correlated with those of the biomarkers under consideration. This suggested that these lipid compounds represent primarily terrestrial input to the sediments and that the efficiency of preservation. The application of PCA to the data from the Peru surface sediments illustrates the usefulness of the technique in ascribing observed compounds to common sources.

### 1.3.2 Partial least squares (PLS) path modelling

Partial Least Squares approach to path modelling by latent variables has been developed by Wold (1966). PLS is ideally suited for charting and simplifying complex, multivariate relationships between groups of samples when large numbers of interrelated variables are involved. With this method a very large number of variables are replaced by a small number of latent variables, which are related to one another by a logical path, predetermined by the user. The latent variables are found by an iterative procedure involving simple and multiple regression analysis so that they simultaneously and optimally represent the measured features and provide the best fit to the path model (Gerlach *et al.*, 1979). The method includes principal component analysis, multiple regression analysis and canonical correlation analysis as special cases.

The PLS techniques need two blocks of data; those signatures representing the 100% condition (the *X*-block variables) and the data to be characterised (the *Y*-block data). The technique works best if there is a clearly identified path from the 100% (the signatures) to 0%. For example, in the estuarine system, the marine and terrestrial end members can be used as signatures.

The logical path used by Yunker *et al.*, (1995) in their study was the geochemical or oceanographic relationship. By apportioning samples into river and shelf groups or blocks, a PLS path model can be constructed which quantifies the contribution that suspended particulates from the Mackenzie River at freshnet make to samples of Mackenzie shelf

suspended particulates and sediments. In performing the PLS regression the 10 river freshnet samples were used to characterise the riverine biomarker signature (the $X$-block). The PLS program then uses the multivariate relationships between the 67 hydrocarbon and oxygenated lipid variables to quantify the amount of riverine material in each of the 54 samples of Beaufort Sea suspended particulates and sediments (the $Y$-block). The model showed that latent variable 1 primarily reflects the variation between lithic and higher plant materials, while latent variable 2 reflects the variation in the contribution of riverine and marine organic matter. Using the regression between these two latent variables, they can estimate the relative contribution of the $X$-block (100% river) to the rest of the samples ($Y$-blocks). The model predictions are consistent with transport of terrestrial material on particulates. They found that PLS tracks the seasonally varying balance between the trends of increasing input of terrigenous markers with increased river flow and the reduction in terrigenous contents as autochthonous production increases with increasing day length.

Mudge *et al.* (1999) collected 59 suface samples across the Ria Formosa Lagoon and identified 26 different sterols. They used the sites, which are rich with phytoplankton, as signature sites ($X$-block variables) in a PLS model. Ninety-five percent (95%) of the $X$-block variation was explained by the first two components. They found that the $Y$-block variables were clustered around the mean position of the $X$-block data in a plot of scores. This clustering of sites highlighted the important contribution that the phytoplankton made to the organic matter in the lagoon. They also found that the opposite end of the path were sites principally affected by sewage. Within the lagoon system, there were no clear end members that could be used as signatures to run a PLS analysis.

### 1.3.3 Factor analysis

Factor analysis is a mathematical tool, which can be used to examine a wide range of data sets. It has been used in disciplines such as chemistry, sociology, economics and psychology. The main applications of factor analysis are to reduce the number of variables and to detect structure in the relationships between variables. Therefore, factor analysis is applied as a data reduction or structure detection method (Rapp, 1991).

Factor analysis has been used in several organic geochemical studies (Irwin and Meyer, 1989; Rapp, 1991; Grimalt and Olive, 1993; Aboul-Kassim and Simoneit, 1996). It is a statistical procedure that is used to find correlations (similarities) between measurable variables. Factor analysis attempts to explain the correlation among a large set of variables in terms of a small number underlying factors (Meglen, 1992). In this technique, the emphasis is on transforming the underlying factors to the observed variables, to enhance the interpretability of the data. These variables with a high degree of similarity that are presumed to be measuring the same concept and/or idea are then combined to form new composite variables. The results are usually plots of the factor loadings and/or factor scores of factor 1 *vs.* factor 2 (the two factors with the highest eigenvalues). The plot represents relationships (groupings) between samples and/or between variables (such as compounds, ratios) (Rapp, 1991). There are a lot of numbers of combinations of choices for input into a factor analysis such as data matrix (peak areas, peak heights, real concentrations, normalised concentrations and numerous scaling procedures, *e.g.* autoscaling), mode (*Q*-mode and *R*-mode), similarity metric (covariance, correlation, cosine theta, *etc.*), and rotation.

Grimalt and Olive (1993) collected 40 water particulate samples within the Ebro Delta. The Ebro Delta has high concentrations of lipids, pigments and pollutants in the river and channel system compared with the bay water levels. Most of the allochthonous sources and nutrients enter the area by the river and their associated irrigation channels. Grimalt and Olive (1993) evaluated the usefulness of factor analysis for source input elucidation in environmental studies using molecular markers for sample description. Nine factors were identified to estimate their data. They found that the application of this method result a direct correspondence between factor loadings and marker groups defining geochemically consistent organic matter distributions to the system. For example, factor 1 and factor 2 differentiate the irrigation and discharge channel samples while factor 1 and factor 4 differentiate between rivers and irrigation samples. Therefore factor 2 and factor 4 differentiate between the river and discharge channel samples. Factor 1 was loaded by a series of algal markers such as *n*-alkanes (*n*-Heptadecane), fatty acids (15:0, 17:0 and *iso* 17:0), phytol and chlorophyll. 24-methylcholesta-5,22(E)-dien-3β-ol and 24-methylcholesta-5,24(28)-dien-3β-ol, which are related to diatom inputs were also loaded

on factor 1. The correspondence of primary productivity indicators and diatom markers shows the dominance of this species in the delta waters. Factor 2 was loaded by several fatty alcohols and sterols such as 24-ethylcholest-5,22(E)-dien-3β-ol and 24-ethylcholesta-5,24(28Z)-dien-3β-ol corresponds to green algal input. Dinosterol, which is a known marker for dinoflagellates, was also loaded on factor 2. This factor suggests that flagellates and green algae are also organic matter producers in the Ebro Delta. Meanwhile, several known pollutants such as cholestanol and N-hexadecyl-N-methyl-N-octadecylamine, which can be related to sewage and detergents, were loaded on factor 4. From their analysis, they also found that smaller number of compounds did not improve the application of factor analysis showing that limited number of sources is responsible for the occurrence of lipid markers in the environments.

Aboul-Kassim and Simoneit (1996) used a *Q*-mode factor analysis based on a grouping a multivariate data set based on the data structure defined by the similarity between samples in the Alexandria coast (Egypt). The measure of similarity used is the cosine theta matrix, *i.e.* the matrix whose element is the cosine of the angles between all samples. The goal of Q-mode factor analysis is to determine the absolute abundance of the dominant components in a sediment sample. It provides a description of the multivariate data set in terms of a few end members (EM), which account for the variance within the data set. They obtained the two end member (EM I and EM II) in their study. They also used both the statistical and biomarker approaches in order to assign the origin (source) of the observed factors. EM I was dominated mainly by *n*-alkanes (82%), regular isoprenoid hydrocarbons (*i.e.* pristane and phytane, 4.4%), the hopane series (8.2%) with a predominance of 17α(H),21β(H)-hopane and the diasterane/sterane series. The EM II was dominated mainly by *n*-alkanes of a terrestrial origin and traces of isoprenoids (0.5%), hopanes (1.2%) and diasteranes/steranes (0.5%). Thus, based on the statistical findings, these two end members can be compared with the compositions of sewage, petroleum, terrestrial and marine sources. It was obvious that the sources of the lipids in the surficial sediments of the Alexandria coastal region were mainly petroleum pollution (anthropogenic) from ships and waste water (EM I), followed by secondary terrestrial input represent by EM II.

**1.3.4 Cluster analysis**

The main purpose of using cluster analysis is to reduce the mass of data, without significant loss of information, to a tractable size, but in a way that emission patterns can be easily discerned. Cluster analysis is a numerical technique for defining groups of related samples based on high similarity coefficients, computed between each pair of samples, which are then clustered. Thus, in most clustering procedures the nucleus of clusters (centroid) is formed by joining the samples with highest similarity and gradually admitting more samples as the similarity coefficient is lowered (Kaiser and Esterby, 1991).

During the step of agglomeration in cluster analysis, all cases are first considered separate clusters; there are as many clusters as cases. At the second step, two of the cases are combined into a single cluster. At the third step, either the third case is added to the cluster already containing two cases, or two additional cases are merged into a new cluster. At every step, either individual cases are added to clusters or already existing clusters are combined. The end point of this process is a dendogram, or tree diagram. The clusters specified at a particular level are generally non-overlapping, *i.e.* distinct clusters do not meet and every case belongs to only one cluster (Howard, 1991).

Aboul-Kassim and Simoneit (1996) collected the surficial sediments from Alexandria coast in Egypt and cluster analysis was employed to classify the study area into specific regions, each having definite characteristics. The samples were collected from six zones. Zone I (beaches) receives a significant amount of untreated sewage; zone II (Eastern Harbour) and III (Western Harbour), the main trading and fishing harbours of the city, receive waste water and untreated sewage; zone IV (Kayet Bay) receives domestic sewage from the main metropolitan pumping station; zone V (Mex Bay) receives various industrial waters from several outfalls such as agricultural and chlor-alkali plant; and zone VI (Agami) is regarded as the reference area receiving little low discharge. Two main groups were distinguished in the complete linkage method dendrograms based on the organic geochemical parameters for the sediments and the distance between. The first cluster group includes samples mainly located in Zone I to IV and V, while the second cluster group consists of samples located in zone V and VI. All samples within the two main cluster groups were combined at short distances showing great similarity among these samples.

Mudge *et al.* (1998) analysed fatty acids in surface sediments from 59 sites in the Ria Formosa Lagoon. Cluster analysis was one of the multivariate statistical techniques used to interpret the data. From cluster analysis of variables, distinct groups of marine, bacterial, marine and bacterial, terrestrial and phytoplankton derived fatty acids can be seen in the dendogram. The terrestrial cluster was comprised entirely by long chain saturated fatty acids. Meanwhile the phytoplankton cluster contains $16:1\omega7$ together with a series of polyunsaturated compounds. Two distinct clusters of marine and bacterial fatty acids were apparent in their data along with a cluster containing compounds of mixed origin. These three clusters link together at a similarity greater than 90%. Cluster analysis of observations was also carried out. However there was no clear cluster observed between the samples.

There is another multivariate statistical analysis that has been used in environmental organic chemistry called Multidimensional Scaling (MDS). Readman *et al.* (1986b) employed MDS in their study to resolve co-variability between members of different compound classes to investigate source and processes affecting fates of the compounds in estuarine environments. From a non-technical point of view, the purpose of multidimensional scaling is to provide a visual representation of the pattern of proximities *i.e.* similarities or distances (Kruskal and Wish, 1984). Multidimensional scaling provides the researchers with a spatial representation of data that can facilitate interpretation and reveal relationships. However, MDS is the most commonly used to detect ecological patterns (Minchin, 1987; Nekola, 2003). In this study, MDS is not carried out, as the study is more towards quantification of biomarkers, their sources and diagenetic processes affecting their stability.

## 1.4 Aim and objectives
### 1.4.1 Aim
The aim of this project is to improve source identification using a combination of mathematical and statistical techniques with lipid biomarkers.

## 1.4.2 Objectives

The specific objectives set to achieve the aim of the project were:

- To review the mathematical and statistical techniques used in relation to biomarker studies.

- To conduct a sampling and analysis programme of sediments along a simple spatial gradient regime (*e. g.* a local estuary) and use mathematical and statistical techniques for source identification.

- To conduct a sampling and analysis programme of sediments along a complex gradient (*e. g.* the Clyde Sea). Use mathematical and statistical techniques for characterisation.

- To conduct a sampling and analysis programme of core sediments with temporal variability and use mathematical and statistical techniques to apportion source magnitudes.

- To determine or develop the "best" mathematical technique and biomarker combination for future analyses.

## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Extraction

The samples were stored at 4°C before the extraction. Samples for the Mawddach Estuary and the Conwy core were extracted immediately after they were collected. Meanwhile, surface samples from the Clyde Sea and the Loch Riddon core were kept for up to one month before the extraction. The storage condition *i.e.* in the fridge is similar to the environmental condition. No change of colour was observed in related to the oxygen usage.

The same extraction methods were used for all samples in this research. Subsamples of the sediments were dried in a drying cabinet (60°C). They were reweighed daily, until a constant dry weight was recorded. Using sediment wet weights and dry weights; the dry weight of sediment extracted for each sample was calculated.

Extraction of sediment was carried out in three stages: sediment reflux, liquid-liquid separation and derivatisation. The methods used for extraction and purification are adapted from procedures found in the literatures (Mudge and Norris, 1997; Mudge *et al.*, 1998). Experiments with spiked reference materials have shown that these methods release >95% of the fatty acids, fatty alcohols and sterols from sediments and full recovery is assumed here (Mudge and Norris, 1997; Pereira, 1999).

### 2.1.1 Reflux

Approximately 30-40 g wet weight of sediment was hydrolysed with 50 ml of 6% *w/v* potassium hydroxide in methanol (Rathburns). The samples were refluxed for 3.5 hours which gave >95% recovery for Pereira (1999). This procedure enables the lipids to be released from their ester/ether linkages. The reflux samples were centrifuged (Hermle Z 230 A) at 2500 r.p.m. for 5 minutes. The supernatant was then funnelled into the separating flask.

## 2.1.2 Liquid-liquid separation

### 2.1.2.1 Sterols and fatty alcohols

Free, non-polar lipids were extracted from the supernatant by liquid-liquid separation. 20 ml of hexane and 10 ml of Elgastat UHQ water were added into the supernatant. The mixture was then shaken vigorously. The non-polar fraction was transferred into a florentine flask and the whole procedure was repeated to ensure a maximise extraction. Samples were evaporated at 40°C in a rotary evaporator (RE120 Buchi Rotavapor). Samples were redissolved in 2-3 ml of hexane, transferred to a 14 ml vial. Anhydrous sodium sulphate was added to remove any water and polar compounds left in the samples. The remaining solution was filtered through a Number 2 Whatman filter paper and blow dried under oxygen free nitrogen (OFN).

### 2.1.2.2 Fatty acids

The remaining aqueous-methanol phase contained fatty acids and was acidified using the 10% hydrochloric acid (HCl) and transferred into the separating funnel. Twenty ml of dichloromethane:hexane (1:4) solution and 10 ml of UHQ water were added to separate the fatty acids fraction. The mixture was shaken and the lower phase was removed. The procedure was repeated once again. The collected phase was then roto-evaporated and transferred into a 14 ml vial.

## 2.1.3 Derivatisation

Samples derivatisation had to be done in order to permit analysis of compounds with the Gas Chromatograph (GC).

### 2.1.3.1 Sterols and fatty alcohols

2-3 drops of bis-(trimethylsilyl)triflouroacetamide (BSTFA) were added into the samples. BSTFA replaces active hydrogen atoms with a trimethylsilyl (TMS) group, $-Si(CH_3)_3$, making compounds more volatile and stable for the GC injection. Samples were heated in an aluminium block for 10 minutes at 60°C. They were evaporated to dryness under OFN, then redissolved in 1 ml of hexane. For quantitative purposes the lipids were transferred to a preweighed 1.4 ml vial, re-evaporated under OFN and reweighed. Samples were stored at -20°C until further analysis. If derivatisation was not complete, the underivatised sterols would have been present in the chromatogram and this is not happened in this study.

**2.1.3.2 Fatty acids**

Boron triflouride (BF$_3$) was used to derivatise the fatty acid samples. The chemical reaction involves is the hydrogen atom from the carboxyl end of the fatty acid is replaced by a methyl group forming a methyl ester.

The filtered samples were transferred into Reacti-vials. Two ml of BF$_3$ in methanol was added and backflushed with the OFN before the cap was screwed. The samples were heated to 100°C for 1 hour in aluminium block. Fatty acids then become fatty acids methyl esters (FAMEs). A water-pentane system was used to separate the FAMEs. The samples were transferred into a 14 ml vials. 1 part of water and 2 parts of pentane were added. The mixture was then shaken vigorously. The upper phase was removed and the whole process was repeated. The samples were then evaporated under OFN and the FAMEs were finally dissolved in hexane and transferred into the preweighed 1.4 ml vials.

**2.2 Data analysis**

**2.2.1 Gas Chromatography - Mass Spectrometry**

A computerised gas chromatography - mass spectrometry (GC-MS) (Fisions MD800) was used to analyse the fatty alcohols, sterols and fatty acids of the sediments with an on-column injection and secondary cooling. The compounds are carried through the column by helium carrier gas (99.996% pure with a flow rate 1ml min$^{-1}$ and column pressure of 50 kPascal) after the sample is vaporised. The compound retention time depends on their affinity for the column solid phase lining. The compounds are separated before going into the ionisation chamber. Characteristic ions were produced by the bombardment of compounds by electrons and they are separated according to their mass:charge (*m/z*) ratio. Masslab, which is a GC-MS program, was used to record and display the mass spectra. Table 2.1 shows the example of sterols identified by their retention time (not shown) and key masses from the sediment samples.

Table 2.1: Sterols identified from the sediment samples (QM = Quantitation Mass)

| Compound Name (TMS derivatives) | Trivial Name | Mol Wt | QM | Diagnostic Masses |
|---|---|---|---|---|
| 24 nor-cholesta-5, 22(E)-dien-3β-ol | | 442 | 352 | 97 129 255 313 442 |
| 24 nor-cholesta-22(E)-en-3β- ol | | 444 | 374 | 252 345 444 |
| 5β-cholestan-3β-ol | 5β- coprostanol | 460 | 370 | 75 215 257 355 |
| 5β-cholestan-3α-ol | epi-coprostanol | 460 | 370 | 215 257 |
| 27-nor-24-methylcholesta-5,22 dien-3β-ol | | 456 | 366 | 111 129 327 366 |
| Cholesta-5,22(E)-dien -3β-ol | | 456 | 366 | 69 111 129 327 366 |
| 5α-cholest-22(E)-en -3β-ol | | 458 | 374 | 237 345 374 |
| Cholest-5-en -3β-ol | cholesterol | 458 | 329 | 129 329 368 443 |
| 5α-cholestan-3β-ol | cholestanol | 460 | 370 | 215 305 384 403 445 |
| Cholest 5, 24 dien 3β – ol | | 472 | 343 | 129 366 456 |
| 24 - methylcholesta-5, 22- dien - 3β-ol | brassicasterol | 470 | 380 | 69 129 255 341 365 380 |
| 24-methyl-5α(H)-cholest–22–en-ol | | 472 | 257 | 257 345 374 457 |
| Ergostatrienol | | 468 | 337 | 325 363 |
| 5α (H)-cholest-7-en-3β-ol | | 458 | 458 | 300 353 398 443 456 |
| Ergosta-5,7,22(E)-trien-3β–ol | ergosterol | 468 | 337 | 337 363 |
| 24 -methylenecholest-5-en-3β-ol | | 470 | 386 | 129 281 296 341 343 380 386 |
| 24-ethyl coprostanol | | 488 | 398 | 215 383 |
| 24 -methylcholest-5-en-3β-ol | campesterol | 472 | 382 | 129 343 472 |
| 24 -methyl-5α(H)-cholestanol | | | 459 | 69 343 369 372 |
| 24-ethylcholesta-5,22(E)-dien-3β-ol | stigmasterol | 484 | 394 | 83 129 255 351 |
| 24 -ethyl-5(H)-cholest-22–en-ol | | 486 | 374 | 257 345 |
| 4α,24-dimethyl-5α cholesterol | | 488 | 388 | 379 456 |
| 23, 24-dimethylcholest-5-en-3β-ol | | 486 | 396 | 281 283 341 343 381 |
| 24 -ethylcholest-5-en-3β-ol | β-Sitosterol | 486 | 396 | 129 275 296 357 381 |
| 24 -ethyl-α(H)-cholestanol | | 488 | 75 | 215 296 383 386 398 431 473 |
| 24 dimethylcholestan-3β-ol | | 488 | 398 | 215 296 305 383 386 399 473 |
| 24 -ethylcholest-5,24(28)-dien-3β-ol | fucosterol | 484 | 386 | 129 296 371 386 |
| Trimethyl cholest-22(E)-en-3β– ol | | 500 | 359 | 69 269 271 388 |
| Dimethylcholest-dien-3β-ol | | 484 | 484 | 203 218 353 379 |
| Trimethylcholest-en-3β-ol | | 500 | 500 | 341 343 393 |
| 4α,23,24 trimethylcholest-22(E)-en-3β-ol | dinosterol | 500 | 388 | 359 388 |
| C30 dienol | | 498 | | 189 218 |

**2.2.1.1 Sterols and fatty alcohols**

The capillary column used for fatty alcohols and sterols was a non-polar, bonded phase BPX-5 (SGE) with 60 m length, 0.22 internal diameter and 0.25µm film thickness. The temperature program used started at 60°C, increasing at 15°C min$^{-1}$ to 300°C, then at 5°C min$^{-1}$ to a maximum of 350°C for 10 minutes. The MS was configured for electron impact ionisation of 70eV and a mass scan range of 45-585 *m/z* per second. Examples of GC chromatograms of sterols and fatty alcohols are shown in Figure 2.1 and Figure 2.2.

In the Loch Riddon core, some polycyclic aromatic hydrocarbons were present within the sterol fractions and quantified using the 202 *m/z*.

**2.2.1.2 Fatty acids**

A BPX-70 (SGE) column was used for fatty acid methyl esters. It is a very polar, bonded phase column with a 50 m length, 0.33 mm internal diameter and 0.25µm film thickness. The temperature program started at 80°C, increasing at 40°C min$^{-1}$ to 160°C, then at 0.5°C min$^{-1}$ to 170°C and finally at 10°C min$^{-1}$ to a maximum of 250°C for 8 minutes. Example of fatty acid chromatogram is shown in Figure 2.3. Meanwhile, Figure 2.4 shows the mass fragmentation patterns for example compounds quantified during these analyses (fatty acid 18:1ω7, C16 saturated alcohol and cholesterol).

**2.2.2 Calibration**

Calibration with standards of known concentration was carried out in order to quantify the peaks obtained during the analysis. Fatty alcohols were quantified using a C18-TMS fatty alcohol standard. Cholesterol-TMS was used to calibrate the sterols. Both of them were used as external standards and the calibration curves were obtained. Tricosanoic acid methyl ester (23:0) was used as an internal standard for the fatty acid methyl ester samples. The results are expressed in ng g$^{-1}$ dry weight (DW) and ng g$^{-1}$ wet weight (WW) in the case of the Mawddach sediments.

**2.2.3 Quality Assurance Procedures**

Standard method and techniques were adopted whenever possible during this work. In the laboratory, analyses were carried out in Decon-90 washed glassware. The efficiency of the
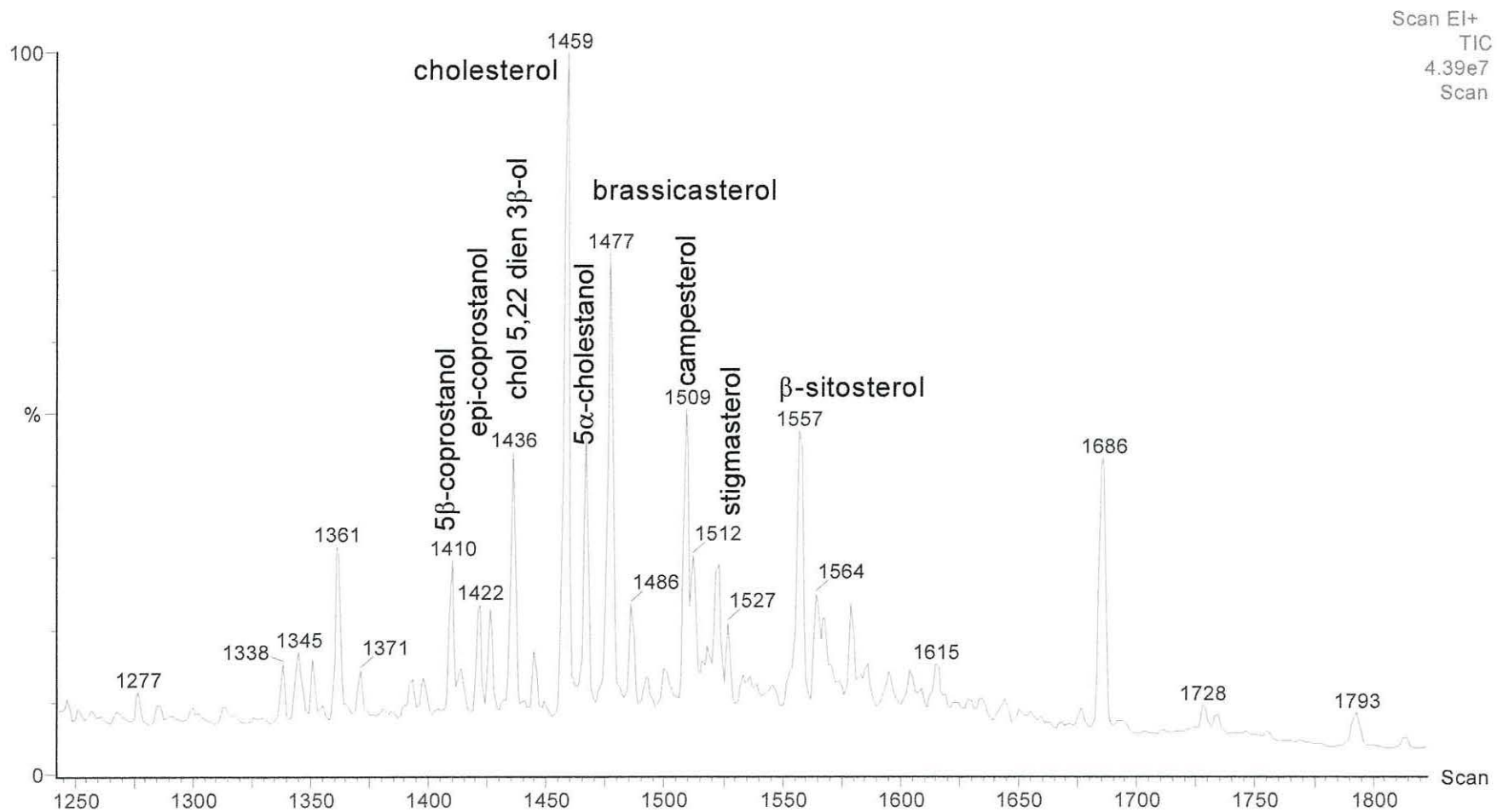
Figure 2.1: Example GC chromatogram of sterols as TMS ethers
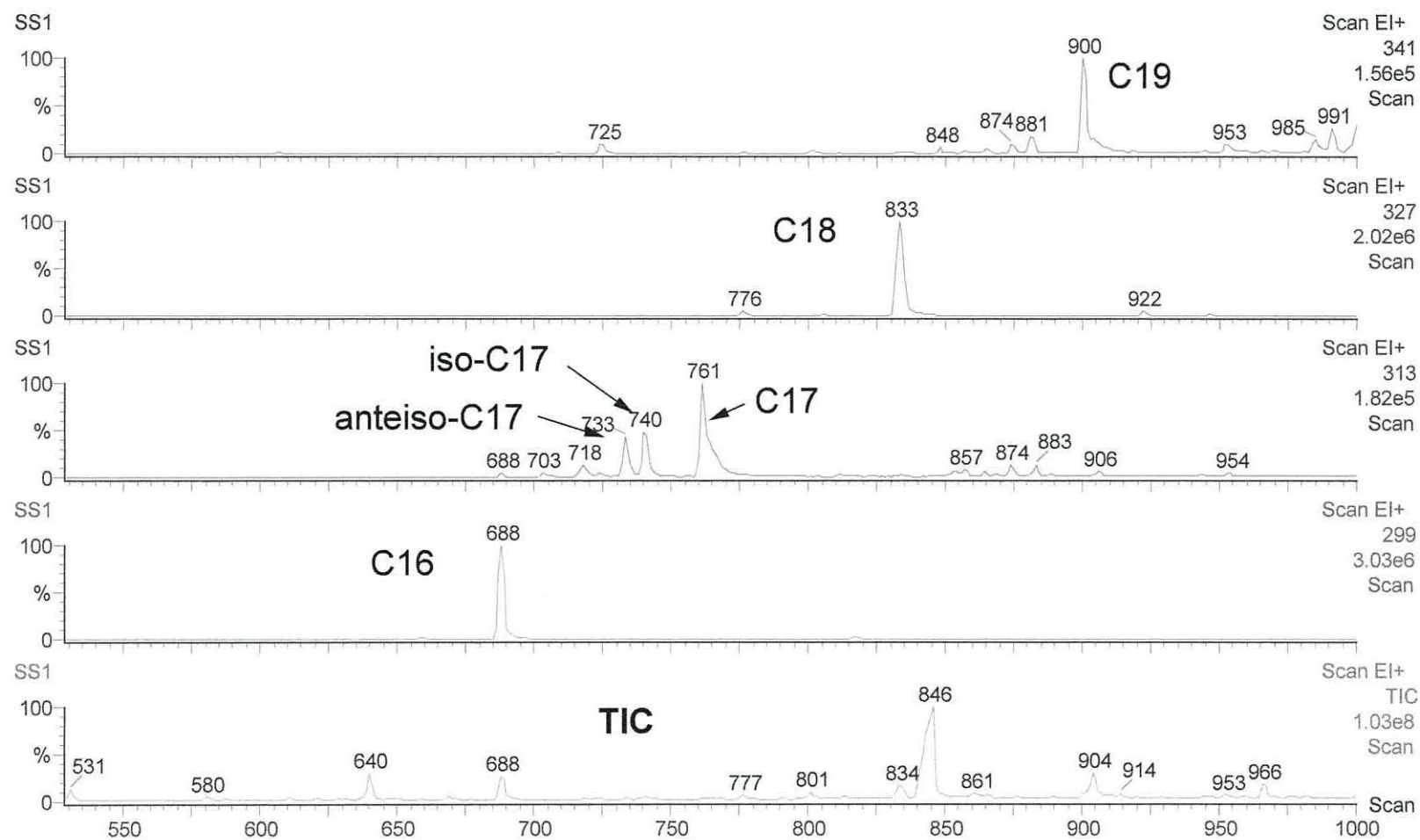
Figure 2.2: Example GC chromatogram of fatty alcohols as TMS ethers
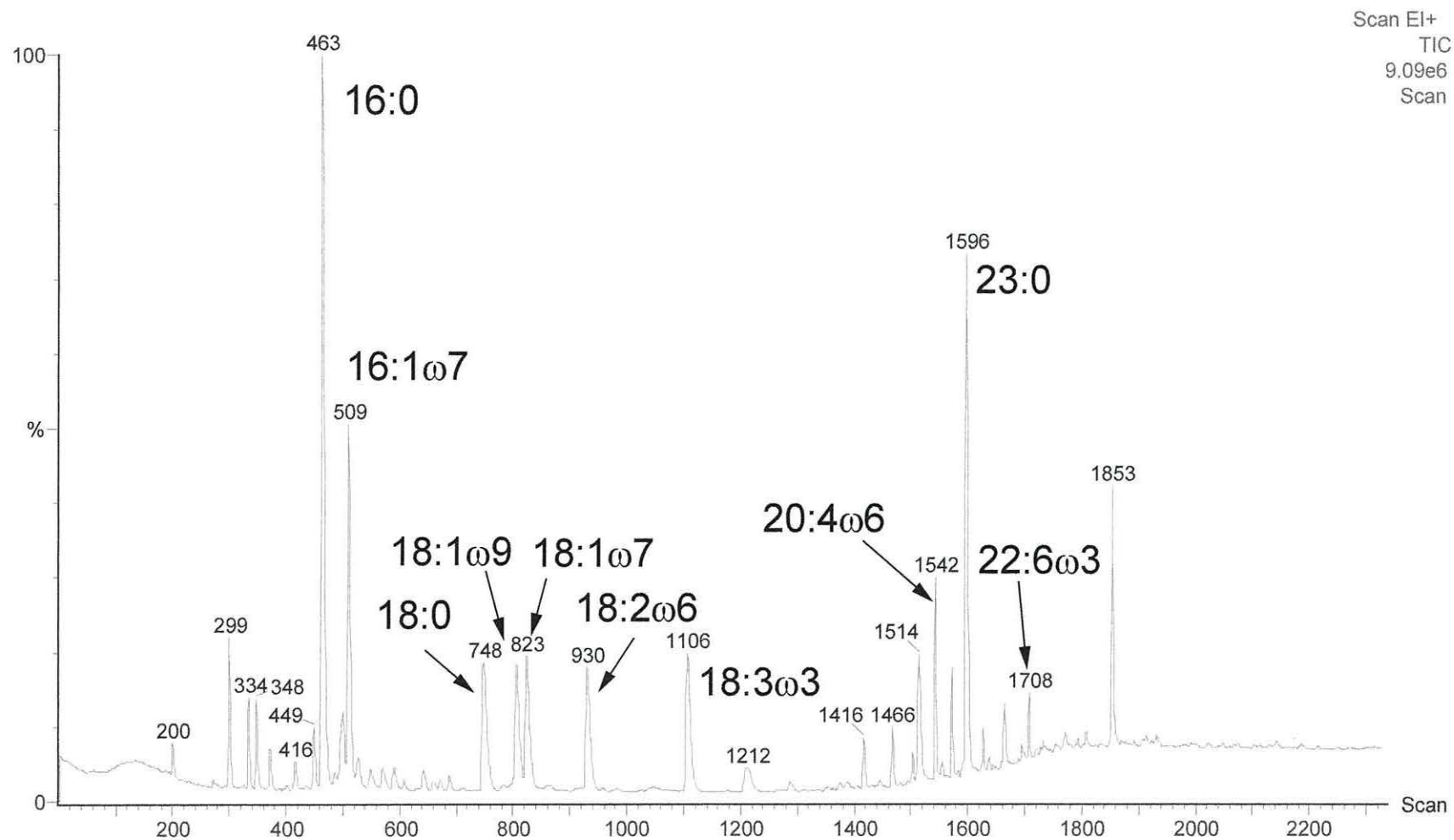
Figure 2.3: Example GC chromatogram of fatty acids as FAMES
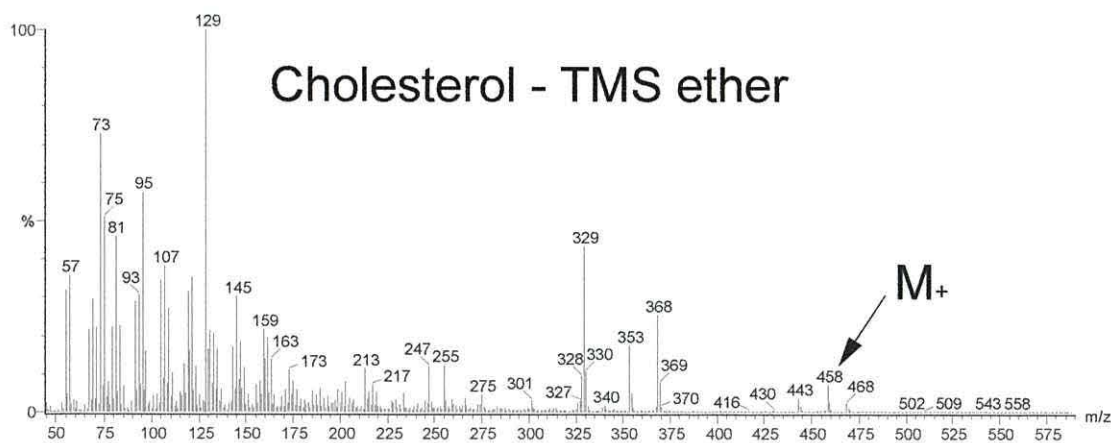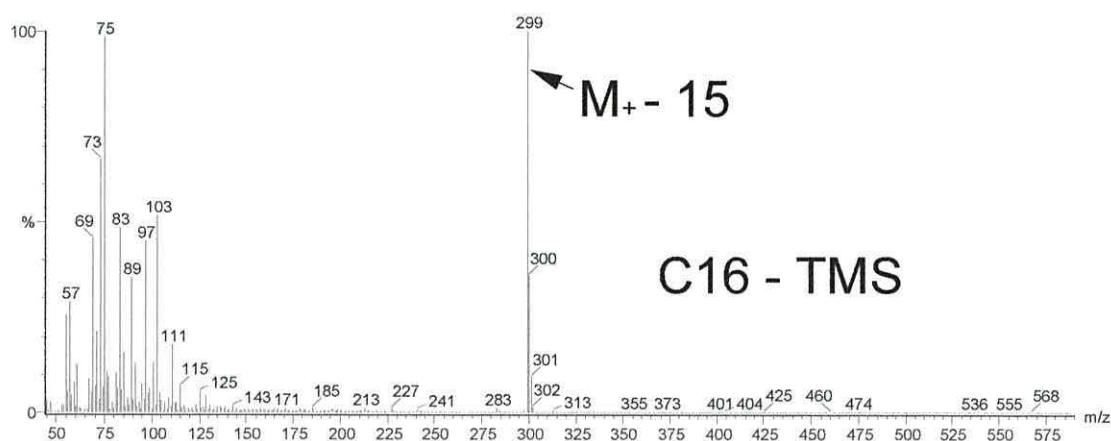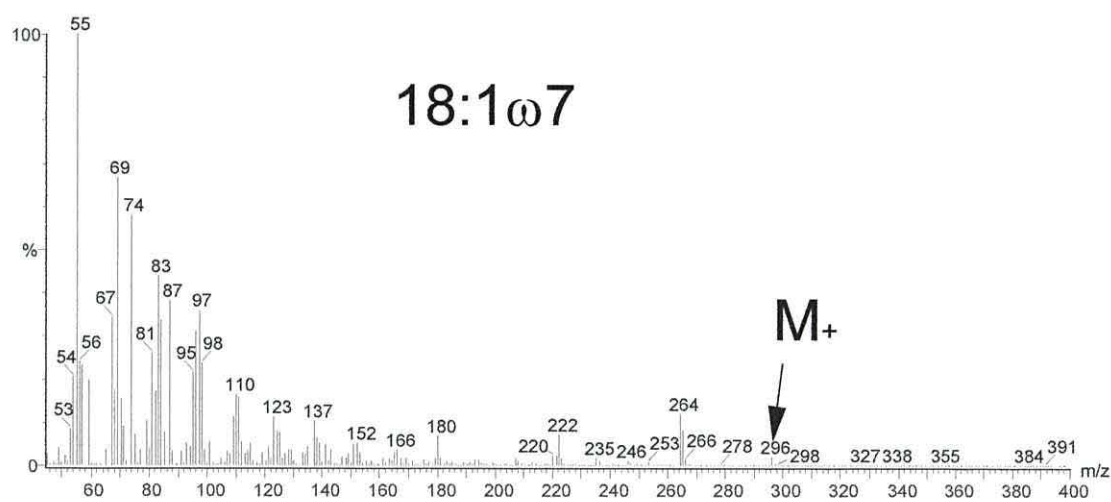
Figure 2.4: Mass Fragmentation patterns for example compounds quantified during these analyses.

whole extraction process was confirmed by repeat reflux of some sediment samples; no further fatty alcohols, sterols and fatty acids could be detected in these later extractions. Blanks and calibration standards were used throughout the GC injections. A blank was injected first and followed by the calibration standard. Five samples were injected afterwards, and followed by the blank and calibration standard again. Since completing this work, a new accredited reference material has become available in November 1999 (IAEA-408) and was not at the time of analyses. IAEA-408 contains only 6 sterol compounds and more than 20 compounds were found in this study.

Random samples were extracted three times to test the reproducibility of the extraction. The mean and standard deviation of fatty acid 18:1ω7, saturated alcohol 18:0 and cholesterol from sample 1 in the Mawddach Estuary are $1426 \pm 45$ ng g$^{-1}$, $24.84 \pm 0.83$ ng g$^{-1}$ and $1342 \pm 52$ ng g$^{-1}$ respectively. Therefore, the reproducibility of the extraction has been found to be better than 90% for all three classes of compound. Procedural blanks were also analysed and no compounds of interest were measured in any sample. All glassware and Teflon-lined caps used in these analyses were rinsed with organic solvents prior to work.

### 2.2.4 Multivariate statistical techniques

The whole data sets were investigated using multivariate statistical methods to determine sources of organic material. Analyses such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) path modelling are very useful in extracting information on mixtures of compounds (Yunker *et al.*, 1995; Mudge *et al.*, 1999). Factor analysis and cluster analysis are also carried out in this study.

Most quantitative statistical methods required the normally distributed data in order to run the statistical analysis. In most environmental data, there is a spread of values from less than the limit of detection to relatively high values in source or near source sample (Mudge, 2002). An example to the distribution of brassicasterol from the Clyde Sea surface sample can be seen in Figure 2.5. This distribution is typical of most environmental data, where the distribution skewed more to the lower values. Therefore, by transforming the data with log transformation, this problem can be overcome even though the zero
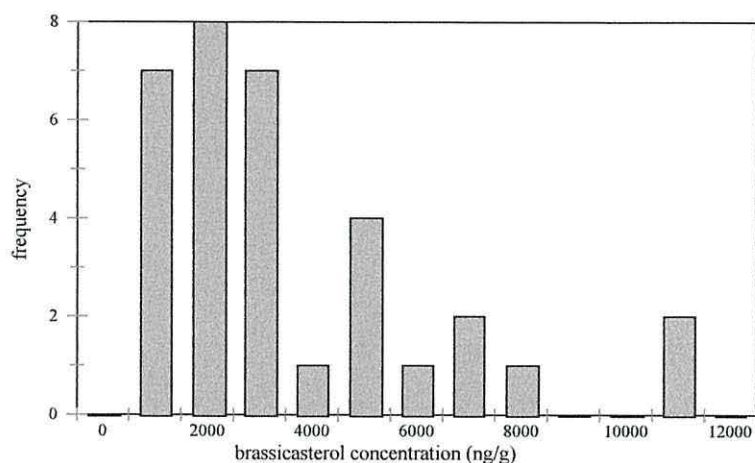
Figure 2.5: Frequency histogram of the brassicasterol in the Clyde Sea sediment sample

values will be lost. To overcome the zeros when taking logarithms, a small value less than the limit of detection can be added (Mudge, 2002), for example in this study value 0.001 ng g$^{-1}$ was used. Figure 2.6 shows the distribution of brassicasterol plotted as a logarithmic frequency plot.
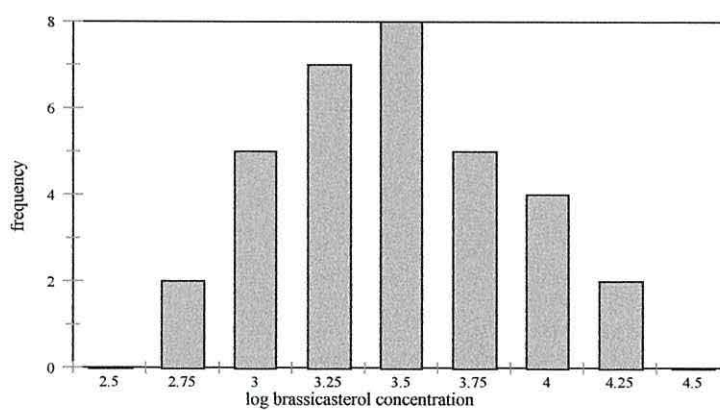


Figure 2.6: Logarithmic frequency histogram of the data from Figure 2.5 with associated normal distribution

**2.2.4.1 Principal component analysis**

A SIMCA-P program from Umetri and a Minitab statistical program (Release 12) were used to perform the PCA in order to determine the biomarker distributions throughout the sampling sites. PCA was carried out on individual chemical groups as well as on combination of fatty acids, fatty alcohols and sterols, and all compounds were used for this analysis.

1. Mawddach Estuary

Raw and proportion data were selected to perform PCA for fatty acids in the Mawddach Estuary.

Raw data

a) Raw data without transformation

b) Raw data with log transformation

c) Raw data, added 0.001 with log transformation

The value 0.001 was added to the 'zero' data as the mean limit of detection.

Proportion data

Proportion data was used in most cases as it removes any variable recovery differences.

a) Proportion data without transformation

b) Proportion data with log transformation

c) Proportion data, added 0.001 with log transformation

Proportion data, added 0.001 with log transformation were used for fatty alcohols, sterols and mixed compounds.

2. Conwy Estuary

For individual PCA, proportion data without transformation was used throughout the analysis. Meanwhile, raw and proportion data (with and without log transformation) were used to perform PCA with mixed compounds.

3. Loch Riddon and Clyde Sea

PCA performed for these sets of data was using proportion data, added 0.001 with log transformation.

**2.2.4.2 Partial least squares path modelling**

SIMCA-P program from Umetri (Sweden) was used to run the PLS. With this method a large number of variables are replaced by a small number of latent variables, which are related to one another by a logical path. In this study, the logical path is the geochemical relationship between samples. The same mixed variables used for the PCA were used to perform the PLS.

Two blocks of data were required:

a) *X*-block data - "signatures" data representing the 100% condition.

b) *Y*-block data - the data to be characterised.

The *X*-blocks chosen to perform PLS in the Mawddach Estuary are:

a) S23, S24 and S25 - to characterise terrestrial signatures

b) S1, S2 and S4 - to characterise marine signatures

c) S6, S9 and S14 - to characterise sewage signatures

In performing PLS for the Loch Riddon core, the top samples of the core were used as the *X*-block to characterise the biomarker signatures. PLS then was carried out with 3 different sets of signatures, which were chosen, from the Clyde Sea samples.

a) S31, S32 and S33 - to characterise terrestrial signatures

b) S1, S2 and S10 - to characterise marine signatures

c) S12, S13 and S21 - to characterise sewage signatures

The same sets of samples (*X*-block) were used to perform PLS for the Clyde Sea samples.

**2.2.4.3 Cluster analysis**

A Minitab statistical program (Release 12) was used to perform the cluster analysis. Cluster analysis was only performed for sediment samples collected from the Clyde Sea and the core from Loch Riddon. Cluster analysis was carried out separately on fatty acids,

fatty alcohols, sterols and mixed compounds. The Ward's method with correlation coefficient distance was used throughout the analysis.

### 2.2.4.4 Factor analysis

An SPSS statistical program (Release 11.0) was used to perform the factor analysis in this study. Factor analysis was carried out to study the distribution of lipid compounds in the sediment samples. Factor analysis was only performed for sediment samples collected from the Mawddach Estuary and the core from Loch Riddon. The data matrix contained the autoscored data that can be obtained by subtracting each observation from the mean and dividing by the standard deviation. Compounds with high percentage of coefficient of variation will be rejected when the factors can not be extracted. The measure of similarity used in the study was correlation. Factor analysis was carried out using the 'Maximum Likelihood' method with 'Varimax' rotation.

The steps used in factor analysis:

1. "Initial extraction" was carried out to select the final number of factors to extract. Two methods were used to determine the factor number.

   - The Kaiser-Guttman rule

     This rule states that number of factors to be extracted should be equal to the number of factors having an eigenvalue greater than 1.0.

   - Scree Test

     Maximum number of factors to extract can be determined by plotting the eigenvalues against the factor number. The "elbow", or the point, at which the curve bends, is considered to indicate the maximum number of factors to extract.

2. Extraction with fixed number of factors was then performed.

3. Factors then need to be interpreted.

   A matrix of factor loadings is one part of the output from factor analysis. Therefore significant loading then have to be determined. A rule frequently used is factor loadings greater than 0.3 in absolute value are considered to be significant. Once all significant loadings are identified, plots and graphs can be used to examine the loadings.

The contribution of each variable to end member's total composition can be determined by dividing the absolute value of the variable's loading for that factor by the sum of the absolute values of all loadings for that factor. Thus, by doing this calculation, the composition of that factor's end member is obtained. A bar graph of percentage composition *vs.* compounds was then produced.

In this study, factor scores for subjects (samples) were also used to determine the changes of each factor among the samples. Factor scores were squared and used to plot factor *vs.* station graphs.

## CHAPTER 3: SURFACE SAMPLES FROM MAWDDACH ESTUARY

### 3.1 Introduction

Estuaries are complex environments in which freshwaters mix with marine waters. Therefore organic matter in estuaries is a complex mixture of compounds from different origins such as terrestrial plants, plankton as well as from bacteria. Wastewater discharges can also contribute to this matter. The lipid biomarker approach can give valuable insights concerning the origin of these diverse sources of organic matter. They also provide information on distribution of organic matter in general (Saliot *et al.*, 1991; Laureillard and Saliot, 1993). The lipid biomarkers used in the present study are fatty acids, fatty alcohols and sterols.

The Mawddach Estuary is situated approximately 52° 43'N and 4° 00'W on the west coast of Wales and lies within the Snowdonia National Park (Figure 3.1). It has an area of 1000 ha and a coastline of 33 km. The catchment is drained by the Afon Wnion and Afon Mawddach, which converge just below their tidal limits. It is a wide estuary with extensive sandflats throughout its length and 220 ha of salt marsh (Burd, 1989). Mawddach Estuary opens into Cardigan Bay at Barmouth, which is a popular seaside town with a sheltered harbour for small fishing boats. The estuary is a popular recreational area for water sports and angling. The estuary is an area of immense beauty and offers visitors a range of walks to suit the determined and the casual stroller. It is a haven for bird spotters and offers great picture taking opportunities, especially at sundown. A spit of sand and shingle (Fairbourne sands) protects the mouth of the estuary.

Surface sediment samples (the top 4 cm) were collected from 25 sites around the Mawddach Estuary (Figure 3.2). The samples were stored at 4°C until further analysis. Table 3.1 shows the distance of each sampling site from S1, which is the marine site. Limited numbers of published works on Mawddach Estuary were found and unfortunately the salinity profile in the estuary was not available.

Table 3.1: Distance of other sampling sites from S1

| Site | Distance inland (km) |
| --- | --- |
| S2 | 0.38 |
| S3 | 0.5 |
| S4 | 0.55 |
| S5 | 1.32 |
| S6 | 2.02 |
| S7 | 2.28 |
| S8 | 3.9 |
| S9 | 4.12 |
| S10 | 4.4 |
| S11 | 4.88 |
| S12 | 6.05 |
| S13 | 6.75 |
| S14 | 8.32 |
| S15 | 8.42 |
| S16 | 8.88 |
| S17 | 9.98 |
| S18 | 10.1 |
| S19 | 11.08 |
| S20 | 14.68 |
| S21 | 15.65 |
| S22 | 15.72 |
| S23 | 17.45 |
| S24 | 17.95 |
| S25 | 19.85 |

## 3.2 Multivariate statistical analysis

In this study, the results were investigated by multivariate statistical analysis such as Principal Component Analysis (PCA), Partial Least Squares (PLS) path modelling and Factor Analysis (FA) to extract and quantify individual compound sources from complex mixtures of compounds collected from the estuary. PCA and Factor Analysis were carried out separately on each chemical group as well as on a mixture of fatty acids, fatty alcohols and sterols.

## 3.3 Results

The results were expressed in ng $g^{-1}$ WW due to lack of sample for dry weight analysis. Arrows were applied to the figures to show the sewage outfalls and ST for the septic tanks.
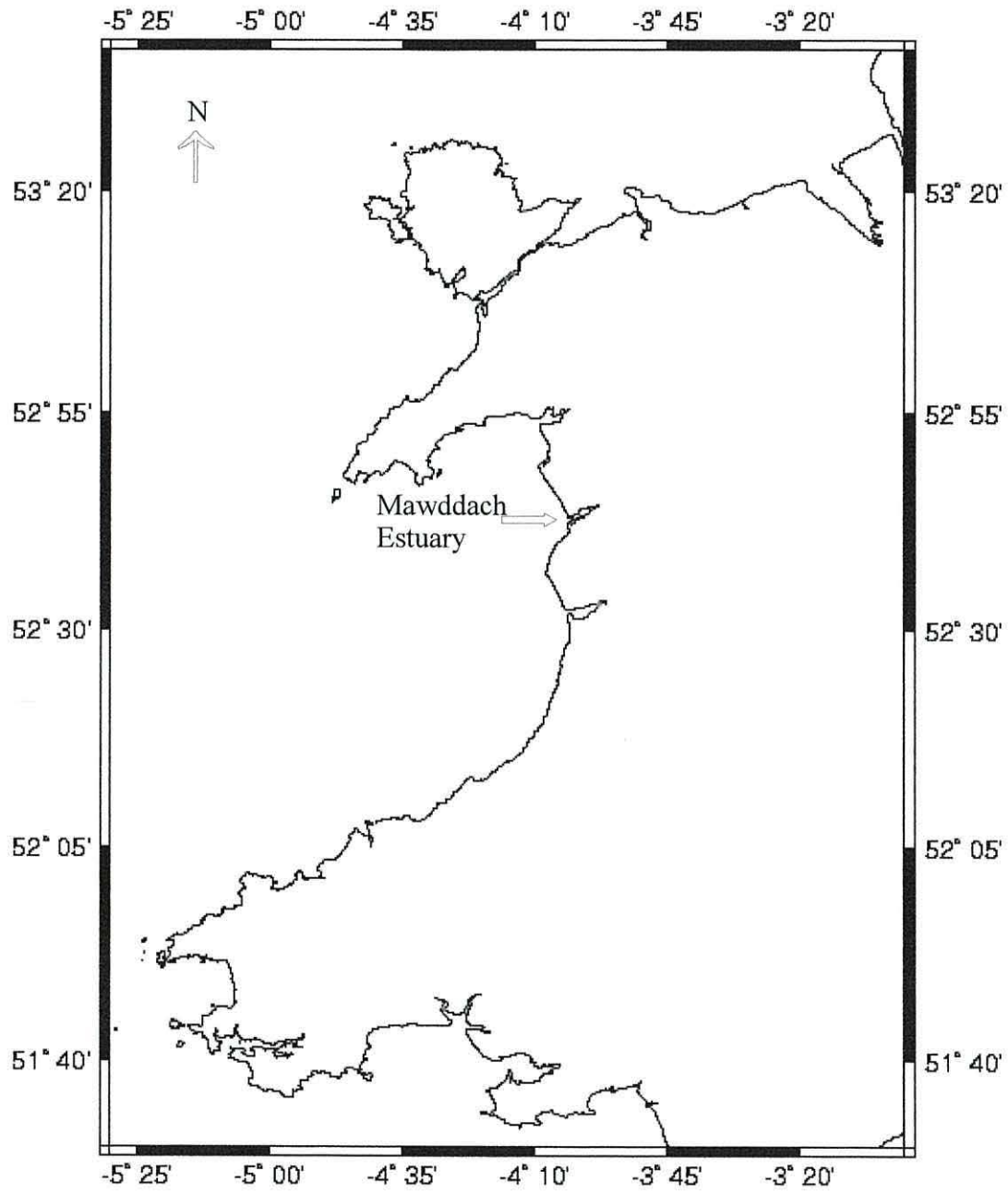
Figure 3.1: Location of Mawddach Estuary
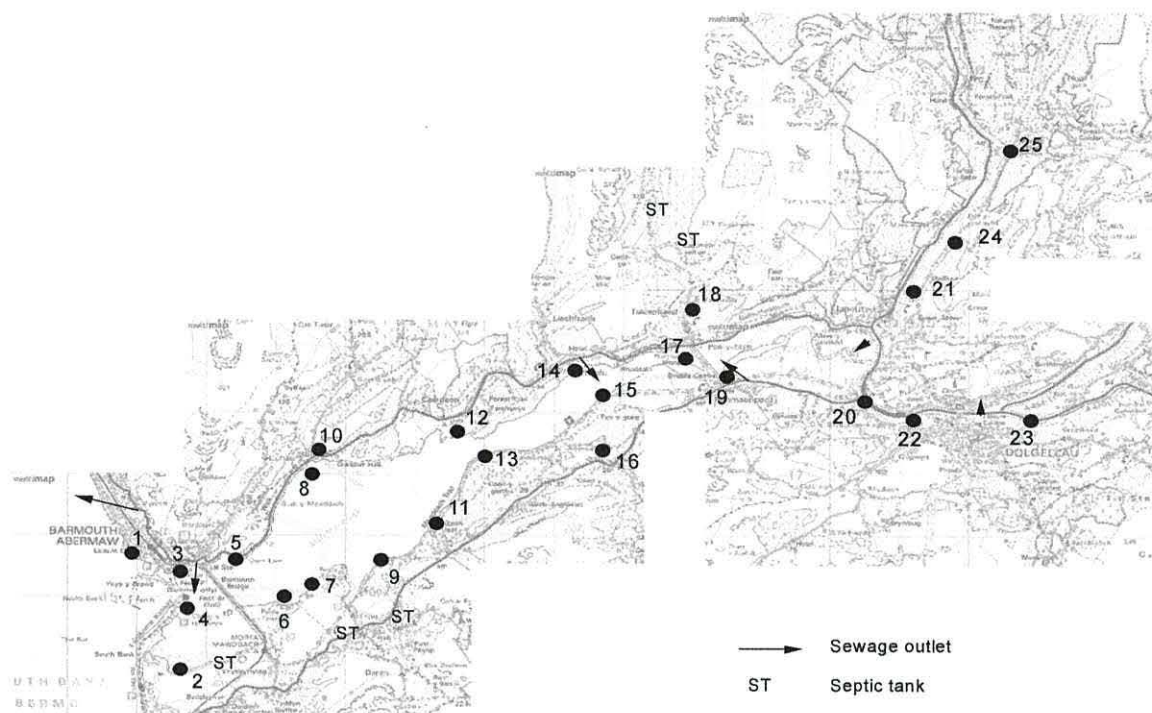
Figure 3.2: Location of sampling sites and sewage outfalls throughout the Mawddach Estuary

### 3.3.1 Fatty acids

Fatty acids are essential components of every living cell including, those from marine and terrestrial environments (Reitan *et al*., 1994; Albers *et al*., 1996) and have been used as sediment biomarkers by many researchers (Harvey, 1994; Colombo *et al*., 1997; Carrie *et al*., 1998). They have great structural diversity with high biological specificity (Parkes, 1987) and therefore useful in determining the origin of the organic materials in sediments

A total of 32 fatty acids were identified from the 25 sampling sites with 16:0 and 18:0 the two most abundant (Appendix 1). Figure 3.3a and 3.3b illustrate the distribution of the monounsaturated and polyunsaturated fatty acids (expressed as a percentage of the total fatty acids concentration), which are similar to each other (correlation coefficient *r*=0.58, p<0.01). High values of monounsaturated fatty acids (>30% of total fatty acids) can be seen at S1. The unsaturated fatty acids together with the short chain saturated fatty acids

Have high concentrations at stations towards the sea (S1 and S2) and are low at stations where sewage is likely to have a major influence on sedimentary organic matter (S3, S4, S6, S7, S9, S14 and S22) and at freshwater end of the estuary (S24 and S25).
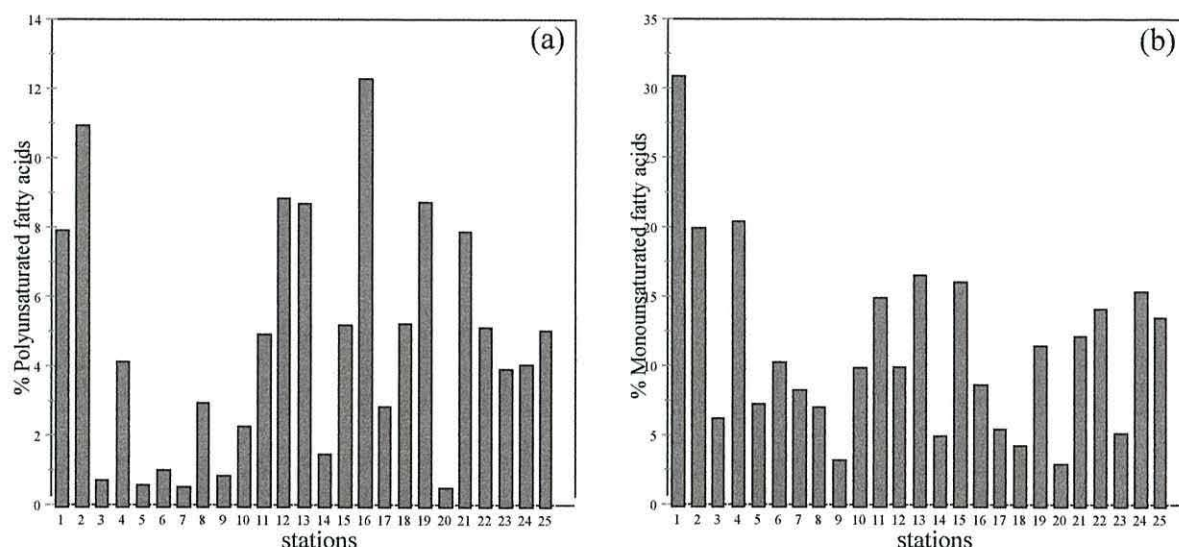


Figure 3.3: Percentage of (a) polyunsaturated and (b) monounsaturated fatty acids in Mawddach estuary

$C_{14}$-$C_{22}$ fatty acids are typical of plankton and have been reported in marine environments (Wakeham and Lee, 1989; Reemtsma *et al.*, 1990; Nadjek, 1993; Colombo *et al.*, 1997; Mudge *et al.*, 1998). Fatty acids 14:0, 16:0, 16:1ω7 and 20:3ω3 are abundant in phytoplankton, while 16:0, 18:1ω9 and 18:0 are characteristics of zooplankton. Diatom fatty acids are characterised by low concentrations of 18:0 fatty acids and high of 14:0, 16:0 and 16:1 (Smith *et al.*, 1983; Wakeham and Lee, 1989; Hama, 1991; Saliot *et al.*, 1991; Colombo *et al.*, 1997; Carrie *et al.*, 1998). Three monounsaturated 16-carbon fatty acids (16:1ω7, 16:1ω9 and 16:1ω11) were identified in this study. 16:1ω7, which is the major monounsaturated 16-carbon fatty acid, is a diatom derived biomarker (Carrie *et al.*, 1998, Mudge *et al.*, 1999). This compound contributes more than 10% of the total fatty acids in S1 and S2, which are marine in nature.

In general, correlation within the fatty acids was low with 21% of compound pairs showing an *r* value greater than 0.5 (Table 3.2). Short chain and polyunsaturated fatty acids are

Table 3.2: Coefficients of correlation between the fatty acids in the Mawddach Estuary

| | 12:0 | 13:0 | br14:0 | 14:0 | iso15:0 | ante15:0 | 15:0 | br16:0 | 16:0 | 16:1ω11 | 16:1ω9 | 16:1ω7 | iso17:0 | ante17:0 | 17:0 | 16:2 | 17:1 | 16:3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13:0 | 0.95*** | | | | | | | | | | | | | | | | | |
| br14:0 | 0.22 | 0.27 | | | | | | | | | | | | | | | | |
| 14:0 | 0.92*** | 0.84*** | 0.29 | | | | | | | | | | | | | | | |
| iso15:0 | 0.18 | 0.11 | 0.50* | 0.36 | | | | | | | | | | | | | | |
| ante15:0 | 0.07 | 0.09 | 0.50* | 0.29 | 0.97*** | | | | | | | | | | | | | |
| 15:0 | 0.28 | 0.26 | 0.42 | 0.41 | 0.68*** | 0.66*** | | | | | | | | | | | | |
| br16:0 | -0.06 | 0.02 | 0.45 | 0.04 | 0.47 | 0.56* | 0.35 | | | | | | | | | | | |
| 16:0 | 0.41 | 0.50* | 0.34 | 0.54** | 0.56** | 0.51** | 0.38 | 0.18 | | | | | | | | | | |
| 16:1ω11 | 0.56** | 0.58** | 0.05 | 0.48 | -0.16 | -0.05 | 0.10 | 0.04 | 0.04 | | | | | | | | | |
| 16:1ω9 | 0.63** | 0.61** | 0.03 | 0.56** | 0.05 | 0.14 | 0.22 | -0.03 | 0.28 | 0.66*** | | | | | | | | |
| 16:1ω7 | 0.84*** | 0.84*** | 0.06 | 0.77*** | 0.08 | 0.07 | 0.27 | -0.14 | 0.46 | 0.55* | 0.79*** | | | | | | | |
| iso17:0 | 0.005 | 0.03 | 0.50* | 0.19 | 0.89*** | 0.92*** | 0.5* | 0.57** | 0.39 | 0.01 | 0.04 | -0.001 | | | | | | |
| ante17:0 | 0.31 | 0.30 | 0.50* | 0.43 | 0.77*** | 0.83*** | 0.60** | 0.52* | 0.38 | 0.23 | 0.24 | 0.20 | 0.82*** | | | | | |
| 17:0 | 0.48 | 0.42 | 0.23 | 0.60** | 0.43 | 0.46 | 0.66*** | 0.16 | 0.33 | 0.53* | 0.74*** | 0.57* | 0.33 | 0.46 | | | | |
| 16:2 | 0.25 | 0.22 | -0.07 | 0.18 | -0.17 | -0.13 | -0.12 | 0.06 | -0.08 | 0.10 | 0.22 | 0.26 | -0.21 | -0.05 | -0.01 | | | |
| 17:1 | 0.53** | 0.54** | -0.03 | 0.45 | -0.11 | 0.01 | 0.02 | 0.03 | 0.18 | 0.65*** | 0.74*** | 0.61** | -0.10 | 0.18 | 0.40 | 0.64** | | |
| 16:3 | 0.38 | 0.40 | -0.10 | 0.29 | -0.09 | -0.03 | 0.01 | -0.03 | 0.12 | 0.23 | 0.75*** | 0.60** | -0.17 | -0.10 | 0.39 | 0.28 | 0.48 | |
| 18:0 | -0.06 | -0.06 | -0.29 | -0.02 | 0.07 | 0.07 | 0.03 | -0.21 | 0.39 | -0.07 | 0.38 | 0.31 | -0.02 | -0.18 | 0.33 | 0.22 | 0.27 | 0.36 |
| 18:1ω11 | -0.14 | -0.11 | -0.05 | -0.135 | 0.02 | 0.18 | 0.18 | 0.06 | 0.05 | 0.27 | 0.63** | 0.11 | 0.05 | 0.08 | 0.49 | -0.03 | 0.41 | 0.49 |
| 18:1ω9 | 0.27 | 0.21 | -0.39 | 0.19 | -0.32 | -0.32 | -0.22 | 0.50* | -0.33 | 0.14 | 0.28 | 0.50* | 0.49 | -0.15 | 0.24 | 0.29 | 0.46 | 0.36 |
| 18:1ω7 | 0.38 | 0.42 | 0.50* | 0.45 | 0.76*** | 0.76*** | 0.78*** | 0.44 | 0.50* | 0.08 | 0.18 | 0.27 | 0.68*** | 0.80*** | 0.47 | -0.18 | 0.07 | -0.02 |
| 18:2ω6 | 0.10 | 0.12 | -0.31 | 0.10 | -0.22 | -0.14 | -0.06 | -0.07 | 0.14 | 0.41 | 0.54** | 0.41 | -0.10 | -0.14 | 0.36 | 0.10 | 0.36 | 0.48 |
| 18:2 | 0.75*** | 0.56** | -0.08 | 0.60** | -0.15 | -0.18 | -0.001 | 0.24 | -0.09 | 0.44 | 0.56* | 0.62** | -0.18 | 0.15 | 0.38 | 0.37 | 0.48 | 0.37 |
| 18:3ω3 | 0.20 | 0.17 | -0.30 | 0.10 | -0.38 | -0.40 | -0.34 | -0.18 | -0.02 | 0.09 | 0.18 | 0.33 | -0.42 | -0.26 | -0.06 | 0.66*** | 0.46 | 0.18 |
| 20:0 | -0.18 | -0.20 | -0.30 | -0.17 | -0.04 | -0.08 | -0.17 | -0.31 | 0.36 | -0.29 | 0.08 | 0.10 | -0.15 | -0.22 | -0.03 | 0.14 | 0.12 | 0.08 |
| 20:1 | 0.61** | 0.52* | -0.13 | 0.51* | -0.14 | -0.08 | -0.04 | -0.17 | -0.04 | 0.67*** | 0.78*** | 0.74*** | -0.05 | 0.08 | 0.50* | 0.30 | 0.63** | 0.63** |
| 21:0 | -0.23 | -0.17 | 0.21 | -0.24 | 0.17 | 0.30 | 0.32 | 0.34 | -0.04 | 0.18 | 0.36 | -0.04 | 0.25 | 0.20 | 0.54* | -0.18 | 0.05 | 0.25 |
| 20:4ω6 | 0.73*** | 0.74*** | -0.06 | 0.59* | -0.12 | -0.06 | 0.11 | -0.10 | 0.26 | 0.61** | 0.91*** | 0.83*** | -0.17 | 0.09 | 0.56* | 0.37 | 0.79*** | 0.76*** |
| 22:0 | -0.25 | -0.25 | -0.09 | -0.20 | 0.10 | 0.14 | 0.08 | 0.05 | 0.38 | -0.16 | 0.18 | -0.02 | -0.02 | -0.04 | 0.18 | 0.06 | 0.17 | 0.11 |
| 20:5ω3 | 0.53* | 0.73*** | 0.06 | 0.44 | -0.17 | -0.14 | -0.003 | 0.00 | 0.39 | 0.50* | 0.40 | 0.55* | -0.16 | -0.08 | 0.15 | 0.11 | 0.43 | 0.37 |
| 24:0 | -0.33 | -0.35 | -0.36 | -0.29 | 0.01 | -0.05 | -0.12 | -0.20 | 0.19 | -0.38 | -0.12 | -0.07 | -0.07 | -0.21 | -0.09 | 0.13 | -0.02 | -0.08 |
| 25:0 | -0.15 | -0.16 | -0.38 | -0.11 | 0.05 | 0.09 | -0.16 | -0.08 | 0.23 | 0.06 | 0.20 | 0.09 | 0.14 | 0.07 | 0.15 | 0.11 | 0.23 | 0.06 |

|          | 18:0 | 18:1ω11 | 18:1ω9 | 18:1ω7 | 18:2ω6 | 18:2 | 18:3ω3 | 20:0 | 20:1 | 21:0 | 20:4ω6 | 22:0 | 20:5ω3 | 24:0 |
|----------|------|---------|--------|--------|--------|------|--------|------|------|------|--------|------|--------|------|
| 18:1ω11 | 0.43 | | | | | | | | | | | | | |
| 18:1ω9 | 0.69*** | 0.23 | | | | | | | | | | | | |
| 18:1ω7 | -0.03 | -0.002 | -0.19 | | | | | | | | | | | |
| 18:2ω6 | 0.56** | 0.42 | 0.66*** | -0.20 | | | | | | | | | | |
| 18:2 | 0.04 | -0.06 | 0.44 | 0.06 | 0.09 | | | | | | | | | |
| 18:3ω3 | 0.47 | -0.08 | 0.58** | -0.33 | 0.19 | 0.34 | | | | | | | | |
| 20:0 | 0.85*** | 0.22 | 0.68*** | -0.17 | 0.31 | -0.06 | 0.44 | | | | | | | |
| 20:1 | 0.18 | 0.22 | 0.44 | -0.09 | 0.50* | 0.74*** | 0.25 | -0.08 | | | | | | |
| 21:0 | 0.30 | 0.66*** | 0.09 | 0.16 | 0.30 | -0.13 | -0.23 | 0.06 | 0.08 | | | | | |
| 20:4ω6 | 0.33 | 0.41 | 0.54* | 0.17 | 0.18 | 0.65*** | 0.30 | 0.08 | 0.75*** | 0.16 | | | | |
| 22:0 | 0.74*** | 0.49 | 0.52* | -0.06 | 0.36 | -0.21 | 0.18 | 0.81*** | -0.15 | 0.45 | 0.07 | | | |
| 20:5ω3 | -0.03 | -0.01 | 0.14 | 0.16 | 0.29 | 0.04 | 0.14 | -0.16 | 0.24 | -0.12 | 0.58* | -0.21 | | |
| 24:0 | 0.74*** | 0.08 | 0.56* | -0.12 | 0.20 | -0.15 | 0.42 | 0.90*** | -0.22 | 0.07 | -0.10 | 0.68*** | -0.21 | |
| 25:0 | 0.78*** | 0.26 | 0.71*** | -0.04 | 0.55* | -0.03 | 0.40 | 0.72*** | 0.09 | 0.20 | 0.15 | 0.61** | -0.03 | 0.76*** |

| * | $p < 0.05$ |
|------|------------|
| ** | $p < 0.01$ |
| *** | $p < 0.001$ |

55

characteristic of marine derived fatty acids. Saturated 14:0 acid, which appeared to be prominent in the marine samples correlated most strongly with 12:0 and 13:0 saturated acids with $r$ values of 0.92 and 0.84 respectively. Polyunsaturated fatty acids such as 20:5ω3 showed correlation coefficients of 0.73, 0.55 and 0.58 respectively with 13:0, 16:1ω7 and 20:4ω6 acids. The monounsaturated acid, 16:1ω7, which is abundant in diatoms, was strongly correlated with short chain saturated acids such as 12:0, 13:0, 14:0 with $r$ values of 0.84, 0.84 and 0.77 respectively; and also with polyunsaturated fatty acids such as 16:3, 18:2, 20:4ω6 and 20:5ω3 with $r$ values of 0.60, 0.62, 0.83 and 0.55 respectively. Branched fatty acids and 18:1ω7 acid, which are bacterial derived, showed good correlation with each other. For example, *iso* 15:0 acid correlated most strongly with *anteiso* 15:0, *iso* 17:0, *anteiso* 17:0 and 18:1ω7 fatty acids with $r$ values of 0.97, 0.89, 0.77 and 0.76 respectively.

Branched chain fatty acids; principally *iso* and *anteiso* odd chain length compounds are commonly used as bacterial biomarkers because of their strong predominance in micro organisms (Saliot *et al.*, 1991). The distribution of percentage branched fatty acids (Figure 3.4) is the inverse of the distribution of monounsaturated and polyunsaturated fatty acids ($r$=-0.32, p<0.01). High percentages (>15% of total fatty acids) were generally seen at S3, S4, S5, S6, S7, S9, S12, S14, S21 and S22 that are near the sewage discharge points, indicating those areas had increased bacterial biomass. This observation is supported by the low concentration of polyunsaturated fatty acids in those stations.

The odd/even ratio (Figure 3.5) shows a similar distribution as the percentage branched fatty acids reinforcing their link with bacterial biomass. Mudge *et al.* (1998) found a similar distribution of branched fatty acids and odd/even ratio of fatty acids in the Ria Formosa lagoon in Portugal. Fatty acid 18:1ω7 has also been used as bacterial biomarker (Claustre *et al.*, 1989; Saliot *et al.*, 1991). The concentration of this fatty acid was strongly correlated to branched fatty acids and total concentrations of odd chain fatty acids with correlation factors of 0.81 and 0.66 respectively (p<0.001).
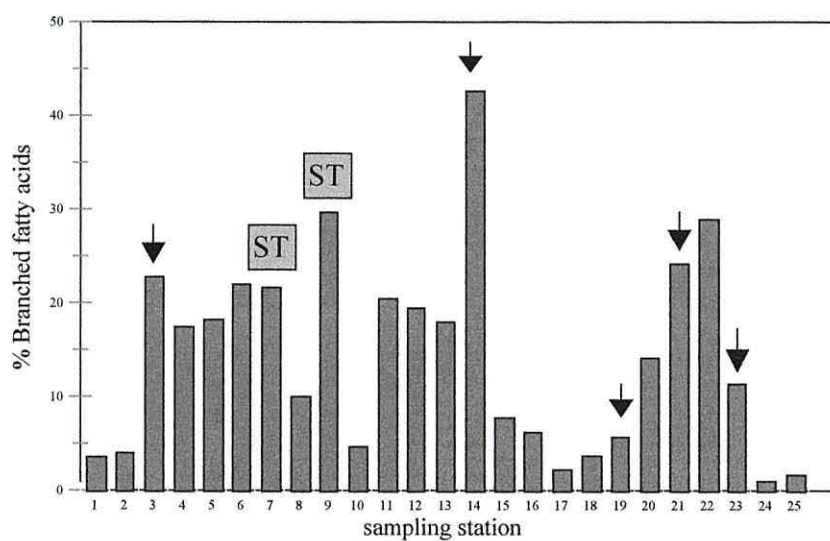
Figure 3.4: Percentage of branched fatty acids in Mawddach estuary
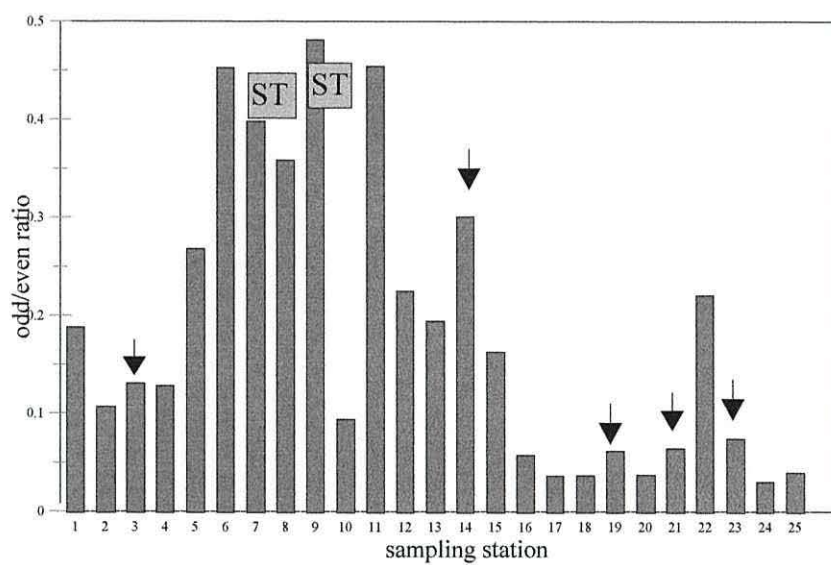


Figure 3.5: Distribution of odd/even ratio of fatty acids throughout the Mawddach sampling stations
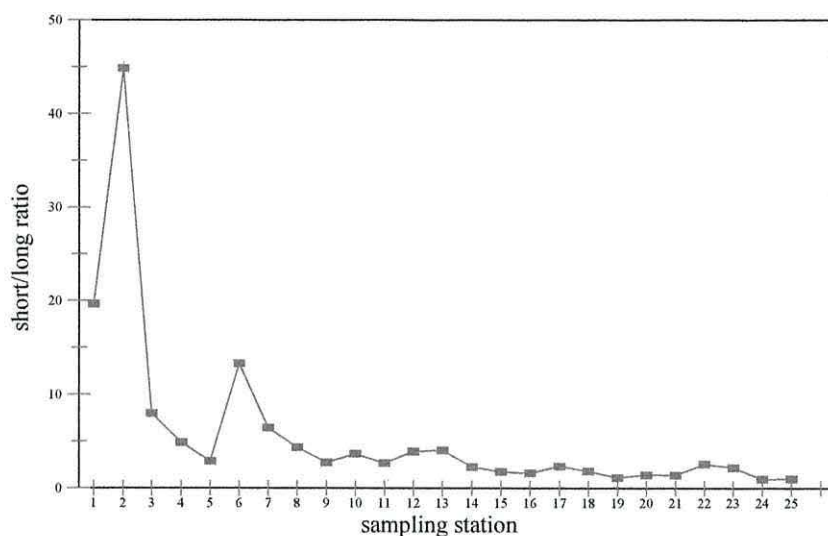
Figure 3.6: Change in short/long ratio of fatty acids throughout the Mawddach sampling stations

Long chain fatty acids ($\geq C_{24}$) are generally associated with the waxy leaf coatings of higher plants and are considered as indicator of terrigenous inputs (Saliot *et al.*, 1991; Colombo *et al.*, 1997; Carrie *et al.*, 1998). However longer chain fatty acids may also be present in some diatoms (Volkman, 1986; Volkman *et al.*, 1998). Only two long chain components were identified in this study, 24:0 and 25:0. These two saturated fatty acids were correlated strongly with each other with correlation coefficient of 0.76 (p<0.001). The distribution of these compounds is consistent with freshwater sites (S16, S18, S24 and S25). The short/long ratios ($\sum$ 12:0 - 20:0)/($\sum$ 21:0 - 25:0) in Figure 3.6 indicate increasing values from the freshwater samples to the marine samples.

### 3.3.2 Fatty alcohols

Fatty alcohols are derived from wax esters, which can be found in marine plankton and terrestrial higher plants (Fukushima and Ishiwatari, 1984; Parameswaran *et al.*, 1994). Short chain fatty alcohols are often used as marine indicators while longer chain fatty alcohols are indicative of terrestrial inputs (Grimalt and Albaiges, 1990; Mudge and Norris, 1997). A total of 19 straight chain alcohols from $C_9$ to $C_{30}$ with 8 branched chain alcohols and 1 monounsaturated compounds were identified in the samples (Appendix 2).
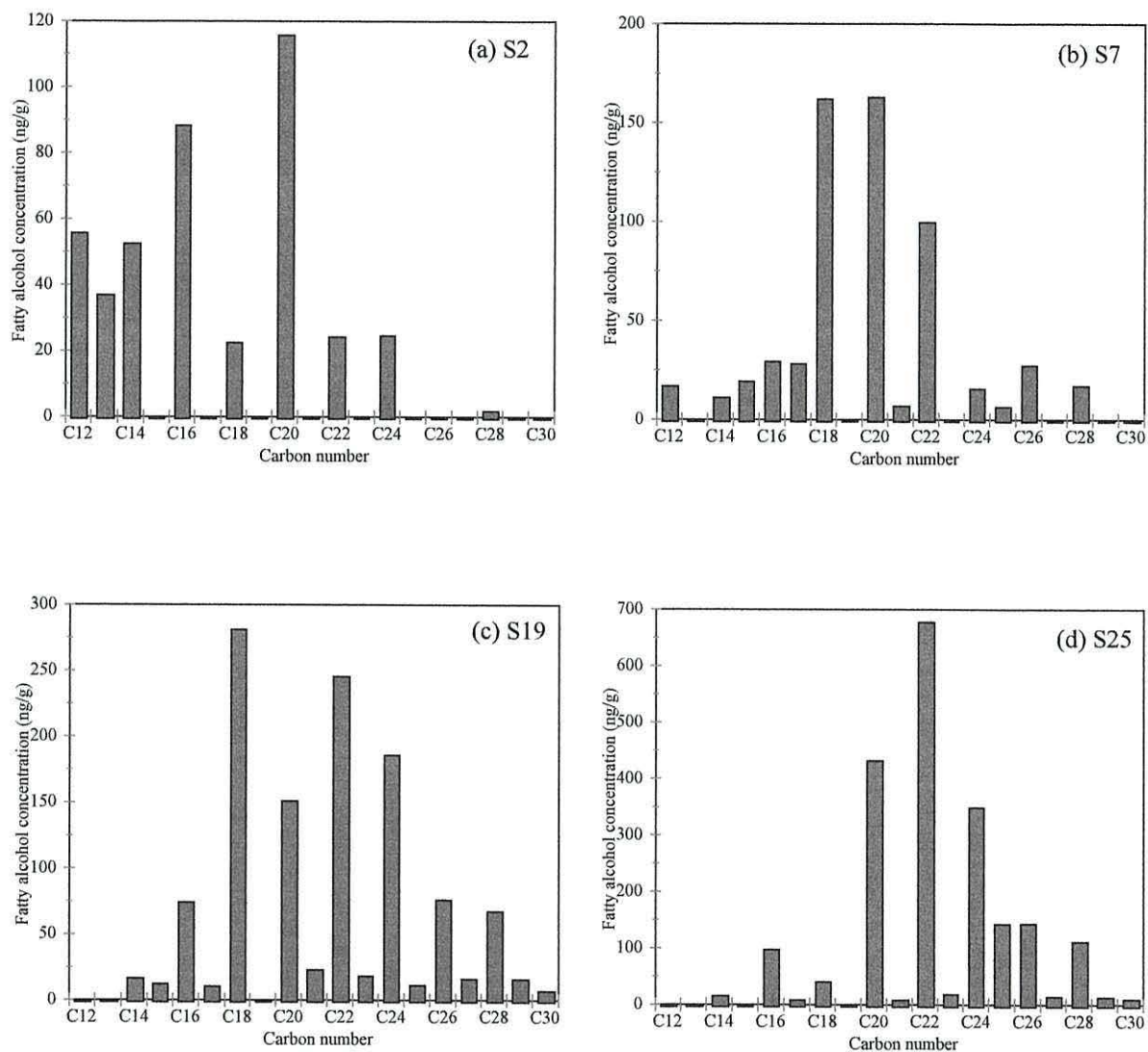
Figure 3.7: Distribution of *n*-alkanols at selected stations in the Mawddach estuary

Figure 3.7 shows the profiles of the straight chain fatty alcohols throughout the sampling stations. The distribution between long and short chain fatty alcohols changes between stations. $C_{14}$ and $C_{20}$ are dominant at the marine end (S2), and at the terrestrial station (S25) $C_{22}$ to $C_{30}$ dominated. Mean chain length of saturated fatty alcohols in each sampling station is showed in Figure 3.8. The mean chain length clearly shows the changes from the short chain fatty alcohols in the marine samples and longer chain fatty alcohols in the freshwater samples.
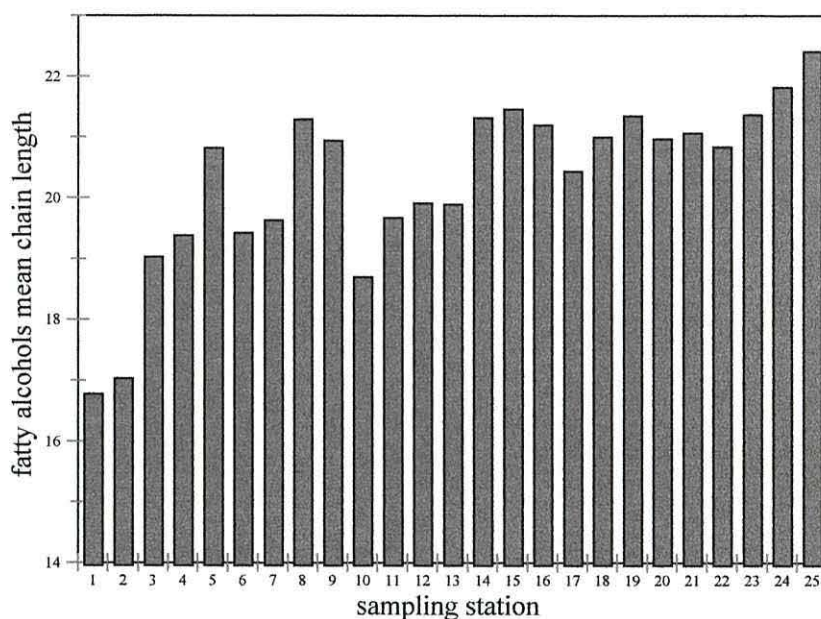


Figure 3.8: Distribution of mean chain length of fatty alcohols throughout the sampling stations in Mawddach Estuary

Within the fatty alcohols, correlation was generally low, with only 13% of compound pairs showing an *r* value greater than 0.5 (Table 3.3). Saturated $C_{16}$, which appeared to be most prominent an all samples did not correlate with any compounds except with monounsaturated 20:1 and $C_{20}$ with *r* values of 0.57 and 0.54 respectively. Short chain fatty alcohols such as $C_{12}$ also did not correlate strongly with other short chain compounds. Meanwhile branched fatty alcohols were clearly correlated with each other. For example, *iso* $C_{15}$ correlated most strongly with *iso* $C_{17}$ and *anteiso* $C_{17}$, with *r* values of 0.67 and

Table 3.3: Coefficients of correlation between the fatty alcohols in the Mawddach Estuary

| | $C_9$ | $C_{12}$ | $C_{13}$ | $brC_{14}$ | $C_{14}$ | $isoC_{15}$ | $anteC_{15}$ | $C_{15}$ | $C_{16}$ | $isoC_{17}$ | $anteC_{17}$ | $C_{17}$ | $C_{18}$ | $isoC_{19}$ | $anteC_{19}$ | $C_{19}$ | $C_{20}$ | $C_{21}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_{12}$ | 0.01 | | | | | | | | | | | | | | | | | |
| $C_{13}$ | 0.30 | 0.70*** | | | | | | | | | | | | | | | | |
| $brC_{14}$ | 0.12 | -0.02 | 0.05 | | | | | | | | | | | | | | | |
| $C_{14}$ | -0.13 | 0.48 | 0.44 | -0.06 | | | | | | | | | | | | | | |
| $isoC_{15}$ | -0.02 | -0.11 | -0.07 | 0.67*** | 0.01 | | | | | | | | | | | | | |
| $anteC_{15}$ | -0.04 | 0.07 | -0.19 | 0.66*** | -0.01 | 0.62** | | | | | | | | | | | | |
| $C_{15}$ | 0.26 | -0.25 | -0.19 | 0.03 | 0.02 | 0.47 | 0.16 | | | | | | | | | | | |
| $C_{16}$ | 0.28 | -0.02 | 0.38 | -0.20 | 0.12 | 0.01 | -0.29 | 0.17 | | | | | | | | | | |
| $isoC_{17}$ | 0.43 | -0.06 | 0.03 | 0.62** | -0.13 | 0.67*** | 0.54* | 0.30 | -0.003 | | | | | | | | | |
| $anteC_{17}$ | 0.12 | 0.02 | -0.10 | 0.66*** | -0.19 | 0.73*** | 0.65*** | 0.27 | -0.20 | 0.87*** | | | | | | | | |
| $C_{17}$ | 0.44 | -0.21 | -0.17 | 0.28 | -0.26 | 0.46 | 0.31 | 0.53* | 0.32 | 0.66*** | 0.59* | | | | | | | |
| $C_{18}$ | 0.21 | -0.46 | -0.26 | 0.01 | -0.19 | 0.40 | 0.05 | 0.48 | 0.08 | 0.25 | 0.26 | 0.35 | | | | | | |
| $isoC_{19}$ | 0.10 | -0.17 | 0.26 | 0.49 | 0.14 | 0.48 | 0.11 | 0.11 | 0.46 | 0.44 | 0.34 | 0.16 | 0.19 | | | | | |
| $anteC_{19}$ | 0.53* | -0.06 | 0.33 | 0.36 | 0.18 | 0.53* | 0.18 | 0.45 | 0.37 | 0.57* | 0.41 | 0.31 | 0.33 | 0.72*** | | | | |
| $C_{19}$ | 0.07 | -0.28 | 0.01 | 0.29 | 0.06 | 0.51* | 0.12 | 0.44 | 0.48 | 0.23 | 0.27 | 0.34 | 0.62** | 0.68*** | 0.52* | | | |
| $C_{20}$ | 0.06 | -0.33 | -0.07 | 0.07 | -0.24 | -0.01 | -0.19 | 0.01 | 0.54* | 0.12 | 0.05 | 0.12 | -0.14 | 0.53* | 0.25 | 0.33 | | |
| $C_{21}$ | -0.09 | -0.51* | -0.21 | 0.20 | -0.06 | 0.32 | 0.03 | 0.41 | 0.36 | 0.24 | 0.16 | 0.27 | 0.26 | 0.50* | 0.28 | 0.49 | 0.58* | |
| $C_{22}$ | -0.21 | -0.54* | -0.34 | -0.17 | -0.21 | -0.07 | -0.28 | 0.17 | 0.41 | -0.14 | -0.18 | 0.03 | -0.09 | 0.26 | -0.03 | 0.17 | 0.81*** | 0.64** |
| $C_{23}$ | -0.07 | -0.46 | -0.31 | 0.01 | -0.07 | 0.10 | 0.01 | 0.32 | 0.32 | 0.06 | -0.07 | 0.26 | 0.18 | 0.24 | 0.05 | 0.30 | 0.34 | 0.78*** |
| $C_{24}$ | 0.16 | -0.48 | -0.30 | -0.14 | -0.14 | -0.04 | -0.11 | 0.50* | 0.41 | 0.04 | -0.11 | 0.33 | 0.19 | 0.12 | 0.07 | 0.27 | 0.43 | 0.48 |
| $C_{25}$ | -0.02 | -0.23 | -0.19 | -0.12 | -0.21 | -0.03 | -0.11 | -0.07 | 0.32 | -0.04 | -0.10 | 0.04 | -0.13 | 0.05 | 0.003 | 0.12 | 0.34 | 0.09 |
| $C_{26}$ | -0.23 | -0.57* | -0.47 | 0.05 | -0.05 | 0.43 | 0.15 | 0.50* | 0.18 | 0.13 | 0.13 | 0.24 | 0.53* | 0.21 | 0.10 | 0.60** | 0.21 | 0.46 |
| $brC_{27}$ | -0.12 | -0.03 | -0.25 | 0.53* | 0.16 | 0.57* | 0.84*** | 0.21 | -0.26 | 0.36 | 0.37 | 0.10 | 0.06 | 0.07 | 0.13 | 0.14 | -0.21 | 0.14 |
| $C_{27}$ | 0.12 | -0.34 | -0.30 | -0.26 | -0.24 | -0.12 | -0.16 | 0.04 | 0.01 | -0.02 | 0.001 | 0.09 | 0.08 | -0.12 | -0.04 | -0.10 | 0.07 | -0.01 |
| $C_{28}$ | -0.19 | -0.40 | -0.31 | -0.19 | -0.37 | -0.30 | -0.33 | -0.16 | 0.18 | -0.23 | -0.26 | -0.20 | -0.35 | -0.11 | -0.12 | -0.17 | 0.67*** | 0.31 |
| $C_{29}$ | -0.16 | -0.34 | -0.25 | -0.12 | -0.17 | 0.004 | -0.15 | 0.05 | 0.06 | -0.09 | -0.20 | -0.21 | -0.00 | -0.17 | 0.09 | -0.07 | 0.22 | 0.35 |
| $C_{30}$ | -0.18 | -0.32 | -0.37 | -0.34 | 0.04 | -0.12 | -0.24 | 0.25 | 0.35 | -0.30 | -0.33 | 0.24 | -0.10 | -0.14 | -0.21 | -0.05 | 0.25 | 0.21 |
| Phytol | 0.29 | 0.65*** | 0.63** | -0.10 | 0.34 | -0.04 | 0.02 | 0.09 | 0.22 | 0.11 | 0.04 | -0.01 | -0.14 | -0.04 | 0.21 | -0.07 | -0.08 | -0.21 |
| 20:1 | 0.25 | 0.20 | 0.31 | -0.17 | 0.22 | 0.22 | -0.03 | 0.55* | 0.57* | 0.17 | 0.14 | 0.44 | 0.21 | 0.15 | 0.40 | 0.37 | 0.20 | 0.35 |

|         | $C_{22}$ | $C_{23}$ | $C_{24}$ | $C_{25}$ | $C_{26}$ | $brC_{27}$ | $C_{27}$ | $C_{28}$ | $C_{29}$ | $C_{30}$ | Phytol |
|---------|----------|----------|----------|----------|----------|------------|----------|----------|----------|----------|--------|
| $C_{23}$ | 0.54* | | | | | | | | | | |
| $C_{24}$ | 0.59* | 0.58* | | | | | | | | | |
| $C_{25}$ | 0.46 | 0.37 | 0.50* | | | | | | | | |
| $C_{26}$ | 0.34 | 0.38 | 0.54* | 0.26 | | | | | | | |
| $brC_{27}$ | -0.20 | 0.18 | -0.002 | -0.03 | 0.38 | | | | | | |
| $C_{27}$ | 0.16 | -0.01 | 0.29 | 0.37 | 0.37 | -0.17 | | | | | |
| $C_{28}$ | 0.77*** | 0.34 | 0.40 | 0.60** | 0.07 | -0.25 | 0.45 | | | | |
| $C_{29}$ | 0.42 | 0.45 | 0.39 | 0.52* | 0.24 | 0.08 | 0.45 | 0.70*** | | | |
| $C_{30}$ | 0.60** | 0.39 | 0.50* | 0.35 | 0.24 | -0.14 | 0.31 | 0.47 | 0.30 | | |
| Phytol | -0.31 | -0.23 | -0.16 | -0.15 | -0.27 | 0.03 | -0.21 | -0.27 | -0.14 | -0.23 | |
| 20:1 | 0.12 | 0.21 | 0.12 | -0.15 | 0.10 | -0.04 | -0.08 | -0.17 | -0.16 | 0.19 | 0.34 |

\*      $p < 0.05$

\*\*     $p < 0.01$

\*\*\*    $p < 0.001$

62

0.73 respectively. Phytol, which is a potential marker for chlorophyll showed good correlation with $C_{12}$ and $C_{13}$ with $r$ values of 0.65 and 0.63 respectively. Long chain saturated fatty alcohols such as $C_{22}$, showed $r$ values of 0.64, 0.77 and 0.60 with $C_{21}$, $C_{28}$ and $C_{30}$ respectively.

Branched fatty alcohols can be used as bacterial markers as they result from bacterial metabolism of even chain length precursors (Parkes, 1987). *Iso* $C_{13}$ is formed from a straight chain $C_{12}$ with the addition of one methyl group at carbon number 11. Therefore it may be possible to use the ratio between the even chain length precursor and the odd carbon numbered methyl derivatives of fatty alcohols to indicate the degree of bacterial metabolism in the samples (Mudge and Norris, 1997). Figure 3.9 shows the results of those ratios. High ratios can be seen at S3, S5, S6, S7, S9, S14 and S22. These values coincided with the percentage of branched fatty acids (Figure 3.4) and concentration of 18:1ω7 (as discussed in the fatty acids section) and this indicates bacteria present in the sewage discharges. The increase in branched fatty alcohol concentrations at those stations may be because of the sewage discharges itself or from the post-depositional changes mediated by the bacteria.
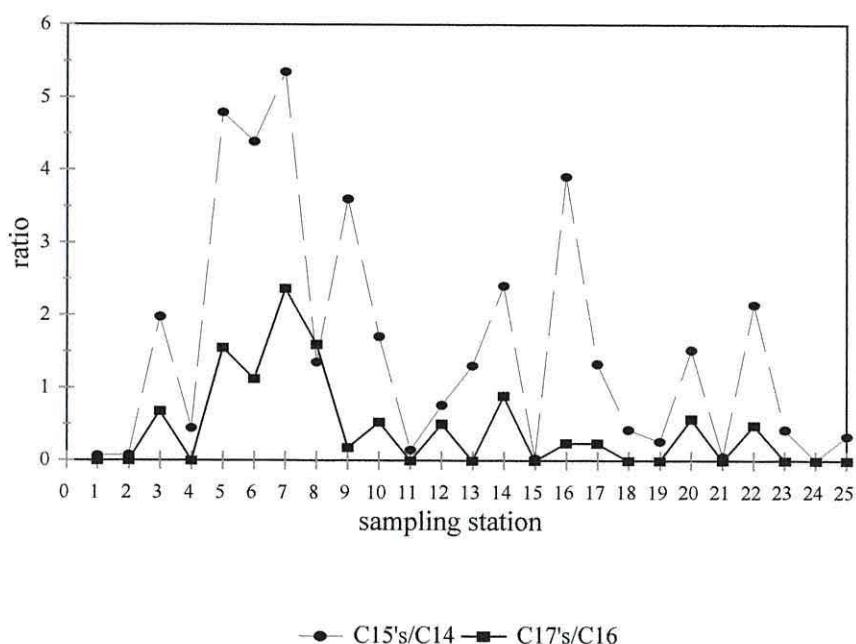


Figure 3.9: Change in the ratio of combined *iso* and *anteiso* isomers of odd chain length fatty alcohols to the even chain length precursor through the Mawddach sampling stations

Chain length distribution may reflect input of fatty alcohols from particular source organisms by using the L/H ratio (Fukushima and Ishiwatari, 1984). Therefore the highest value of the short/long ratio $(\sum C_9 - C_{20})/(\sum C_{21}-C_{30})$ (similar to L/H ratio) would be observed in the marine samples and the lowest in the freshwater samples. The results from this study agree with the hypothesis as seen in Figure 3.10. In the estuarine samples, the short/long ratio was generally >1.0, showing that shorter chain fatty alcohols were more predominant than the longer chain compounds. This may be due to high concentration of odd chain compounds (e.g. $C_{15}$ and $C_{17}$) that associated with bacteria present in the sewage discharges near those areas.
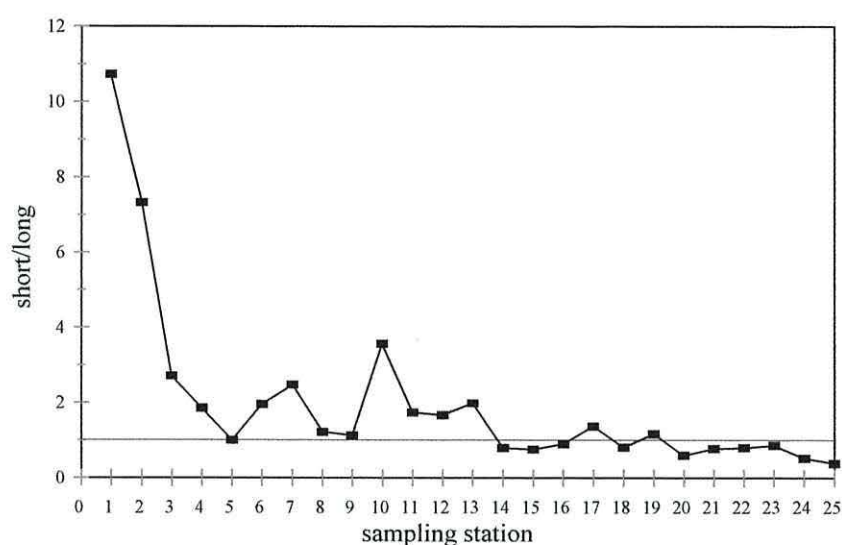


Figure 3.10: Change in short/long ratio of fatty alcohols throughout the Mawddach estuary

The degree of influence of terrestrial organic matter within marine sediments can be described using an index such as Alcohol Source Index (ASI). ASI is calculated by dividing the concentration of terrestrial fatty alcohol with concentration of marine fatty alcohols (Mudge and Norris, 1997). $C_{22}$ and $C_{24}$ were used as terrestrial markers while $C_{14}$ and $C_{16}$ as marine fatty alcohols. Figure 3.11 shows the distribution of ASIs throughout the stations. The values increase from marine to freshwater samples. $C_{14}$ appears to be the strongest marine marker. For example, $C_{24}/C_{14}$ ratio is increased by a factor of 60, whilst $C_{24}/C_{16}$ increased by a factor of 18. Similarly, $C_{22}$ appears to be the strongest terrestrial

marker. $C_{22}/C_{14}$ ratio increased by a factor of 127, whilst $C_{24}/C_{14}$ increased by a factor of 60.
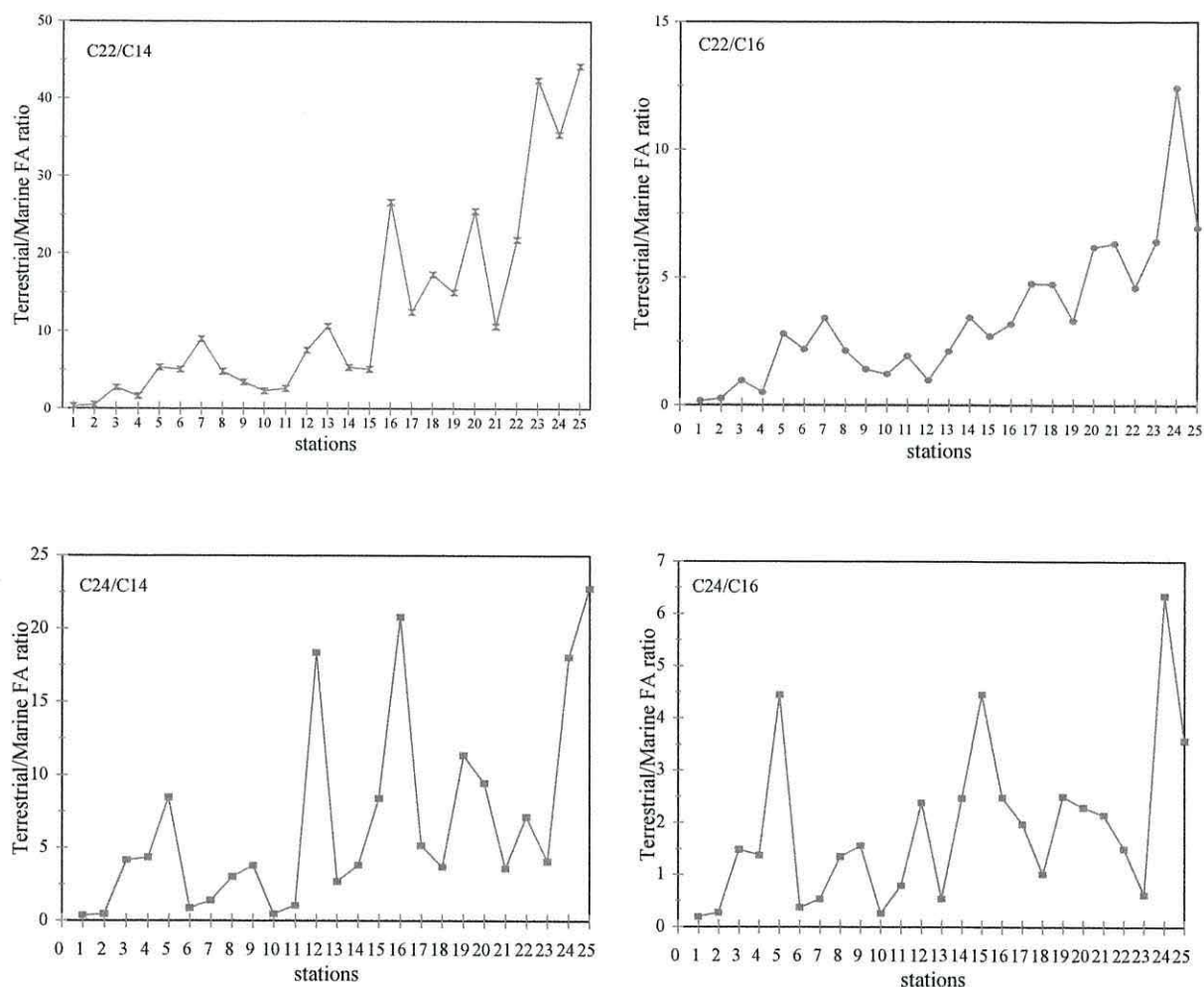


Figure 3.11: Change of ASIs through the Mawddach estuary

The distribution of the branched monounsaturated fatty alcohol, phytol, is shown in Figure 3.12. Phytol is a potential marker for chlorophyll (Killops and Killops, 1993). The phytol distribution is similar to the short/long ratio distribution ($r$=0.74, p<0.001) and cholesta-5,22-dien-3β-ol ($r$=0.51, p<0.01) suggesting that phytol may have a marine source such as

algae and other microorganisms. Phytol may be terrestrial or marine in origin but other work in estuaries using stable isotopes (Tolosa, pers. comm.) show it is predominantly marine.
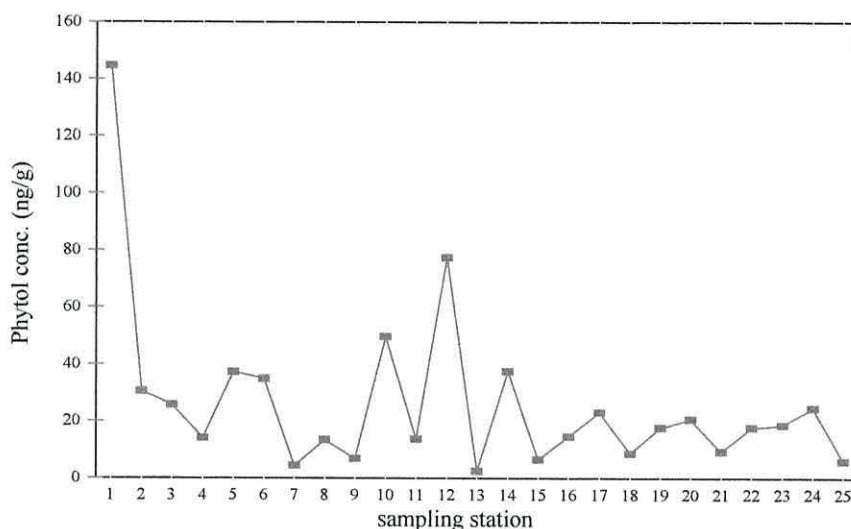


Figure 3.12: Phytol concentration in Mawddach estuary

### 3.3.3 Sterols

Sterols are a very popular series of biogeochemical markers and can be used to characterise the origins of the organic matter from rivers to coastal zones as they are better preserved in sedimentary environments than most other biological products (Saliot *et al.*, 1991). Cholesterol is present in many marine organisms as well as in sewage discharges (Volkman, 1986; Mudge *et al.*, 1999). Twenty-three sterols were identified and quantified in the sampling area (Appendix 3, Table 3.4). Cholesterol was present in all samples but there is no clear trend of cholesterol distribution throughout the estuary.

Within the sterols found in the Mawddach, correlation was low with 39 out of 276 compound pairs showing an *r* value greater than 0.5 (Table 3.5). Positive correlations were observed between some sterols from marine phytoplankton. Brassicasterol which has been found in several algal groups (Colombo *et al.*, 1997; Volkman *et al.*, 1998) has similar distribution to 24nor-cholesta-5,22(E)-dien-3β-ol ($r$=0.53, p<0.01) and cholesta-5,22-dien-3β-ol ($r$=0.56, p<0.01). They appear to have high concentrations in marine samples and low in freshwater samples. 24 nor-cholesta-5,22(E)-dien-3β-ol is often found in the marine

environment (Yunker *et al.*, 1995) such as in sponges and marine worms (Bohlin *et al.*, 1982; Ponomarenko *et al.*, 1995). Nichols *et al.* (1993) and Skerratt *et al.* (1995) have reported that cholesta-5,22-dien-3β-ol is a good biomarker for diatoms. Another type of sterol, dinosterol, is usually considered a reliable biomarker of dinoflagellates. Dinosterol also has a similar distribution with cholesta-5,22-dien-3β-ol ($r$=0.81, p<0.001).

Table 3.4: Trivial and systematic names of the sterols identified

| Abbreviation | Systematic names |
| --- | --- |
| st1 | 24 nor-cholesta-5,22(E)-dien-3β-ol |
| cop | 5β-cholestan-3β-ol (coprostanol) |
| epi | 5β-cholestan-3α-ol (epicoprostanol) |
| st2 | cholesta-5,22(E)-dien-3β-ol |
| st3 | 5α-cholest-22(E)-en-3β-ol |
| chol | cholest-5-en-3β-ol (cholesterol) |
| cholest | 5α-cholestan-3β-ol (cholestanol) |
| brass | 24-methylcholesta-5,22(E)-dien-3β-ol (brassicasterol) |
| st4 | 24-methyl-5α(H)-cholest-22(E)-en-3β-ol |
| st5 | 5α(H)-cholest-7-en-3β-ol |
| ergo | ergosta-5,7,22(E)-trien-3β-ol (ergosterol) |
| st6 | 24-methylenecholest-5-en-3β-ol |
| camp | 24-methylcholest-5-en-3β-ol (campesterol) |
| st7 | 24-methyl-5α(H)- cholestan-3β-ol |
| stig | 24-ethylcholesta-5,22(E)-dien-3β-ol (stigmasterol) |
| st8 | 24-methyl-5α(H)- cholest-7-en-3β-ol |
| st9 | 24-ethyl-5α(H)- cholest-22(E)-en-3β-ol |
| st10 | 4α,24-dimethyl-5α(H)- cholest-22(E)-en-3β-ol |
| st11 | 23,24- dimethylcholest-5-en-3β-ol |
| sito | 24-ethylcholest-5-en-3β-ol (sitosterol) |
| st12 | 24-ethyl-5α(H)- cholestan-3β-ol |
| st13 | 4α,24-dimethyl-5α(H)- cholestan-3β-ol |
| dino | 4α,23,24-trimethyl-5α(H)- cholest-22(E)-en-3β-ol (dinosterol) |
| st14 | 4α-methyl,24-ethyl-5α(H)- cholest-22(E)-en-3β-ol |

Three sterols are often found in terrestrial higher plants: β-sitosterol, stigmasterol and campesterol (Volkman, 1986; Laureillard and Saliot, 1993). These compounds therefore are commonly used as biomarkers of terrestrial derived organic matter inputs into marine systems. Ergosterol can also be used to indicate terrestrial input due to the presence of fungal organic matter in decaying leaves and wood products (Mudge and Norris, 1997).

Table 3.5: Coefficients of correlation between sterols in the Mawddach Estuary

| | st1 | cop | epi | st2 | st3 | chol | cholest | brass | st4 | st5 | ergo | st6 | camp | st7 | stig | st8 | st9 | st10 | st11 | sito | st12 | st13 | dino |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cop | 0.21 | | | | | | | | | | | | | | | | | | | | | | |
| epi | 0.44 | 0.65** | | | | | | | | | | | | | | | | | | | | | |
| st2 | 0.52** | 0.26 | 0.10 | | | | | | | | | | | | | | | | | | | | |
| st3 | 0.38 | 0.33 | 0.34 | 0.32 | | | | | | | | | | | | | | | | | | | |
| chol | 0.19 | 0.21 | 0.35 | 0.24 | 0.19 | | | | | | | | | | | | | | | | | | |
| cholest | 0.03 | 0.79*** | 0.62** | 0.32 | 0.07 | 0.34 | | | | | | | | | | | | | | | | | |
| brass | 0.52** | 0.64** | 0.59** | 0.56** | 0.36 | 0.26 | 0.55** | | | | | | | | | | | | | | | | |
| st4 | 0.36 | -0.10 | -0.15 | 0.69*** | 0.02 | 0.30 | 0.02 | 0.27 | | | | | | | | | | | | | | | |
| st5 | 0.29 | 0.49 | 0.60** | 0.003 | 0.33 | 0.22 | 0.38 | 0.25 | -0.38 | | | | | | | | | | | | | | |
| ergo | -0.11 | 0.08 | -0.10 | -0.23 | 0.14 | 0.28 | -0.004 | -0.02 | -0.33 | 0.004 | | | | | | | | | | | | | |
| st6 | 0.24 | 0.66*** | 0.59** | 0.24 | 0.23 | 0.33 | 0.72*** | 0.52** | -0.19 | 0.50** | 0.27 | | | | | | | | | | | | |
| camp | -0.37 | -0.14 | -0.22 | -0.23 | -0.07 | 0.29 | -0.04 | -0.19 | -0.23 | -0.15 | 0.75*** | 0.14 | | | | | | | | | | | |
| st7 | -0.12 | -0.08 | 0.07 | -0.32 | 0.16 | 0.20 | -0.03 | -0.13 | -0.19 | 0.25 | 0.34 | -0.05 | 0.35 | | | | | | | | | | |
| stig | -0.33 | -0.008 | -0.18 | -0.26 | 0.14 | 0.37 | 0.02 | -0.28 | -0.36 | 0.05 | 0.81*** | 0.17 | 0.82*** | 0.51** | | | | | | | | | |
| st8 | 0.42 | 0.04 | 0.02 | 0.67*** | 0.02 | 0.25 | 0.16 | 0.36 | 0.88*** | -0.30 | -0.30 | -0.05 | -0.25 | -0.34 | -0.40 | | | | | | | | |
| st9 | -0.21 | -0.04 | 0.10 | -0.23 | 0.28 | 0.18 | -0.04 | -0.00 | -0.20 | 0.08 | 0.18 | -0.16 | 0.20 | 0.09 | 0.22 | -0.35 | | | | | | | |
| st10 | -0.25 | 0.16 | 0.47 | -0.06 | -0.03 | 0.23 | 0.46 | 0.06 | -0.03 | 0.25 | -0.26 | 0.09 | -0.17 | 0.03 | -0.18 | 0.05 | 0.32 | | | | | | |
| st11 | 0.11 | 0.05 | 0.40 | -0.25 | 0.14 | 0.10 | 0.02 | -0.04 | -0.22 | 0.34 | -0.10 | -0.13 | -0.33 | 0.24 | -0.16 | -0.30 | 0.50** | 0.54** | | | | | |
| sito | -0.09 | -0.21 | -0.19 | -0.17 | 0.18 | 0.26 | -0.22 | -0.31 | -0.16 | -0.21 | 0.57** | 0.15 | 0.74*** | 0.26 | 0.68*** | -0.20 | -0.03 | -0.34 | -0.22 | | | | |
| st12 | -0.24 | 0.004 | -0.09 | -0.07 | -0.06 | 0.15 | 0.12 | 0.18 | -0.16 | 0.08 | 0.46 | 0.17 | 0.61** | 0.22 | 0.40 | -0.03 | 0.12 | -0.04 | -0.22 | 0.18 | | | |
| st13 | 0.38 | 0.43 | 0.51** | 0.26 | 0.32 | 0.22 | 0.42 | 0.68*** | -0.11 | 0.51** | 0.16 | 0.67*** | 0.03 | 0.13 | 0.02 | 0.001 | -0.08 | -0.07 | -0.07 | -0.10 | 0.52** | | |
| dino | 0.36 | 0.42 | 0.20 | 0.81*** | 0.13 | 0.26 | 0.54** | 0.51** | 0.69*** | 0.01 | -0.38 | 0.28 | -0.40 | -0.33 | -0.38 | 0.74*** | -0.33 | 0.20 | -0.22 | -0.31 | -0.29 | 0.05 | |
| st14 | 0.31 | 0.42 | 0.006 | 0.30 | 0.15 | 0.07 | 0.09 | 0.42 | 0.46 | -0.17 | -0.06 | -0.17 | -0.31 | -0.27 | -0.28 | 0.55** | -0.18 | -0.22 | -0.12 | -0.33 | -0.09 | -0.06 | 0.42 |

| * | $p < 0.05$ |
|---|---|
| ** | $p < 0.01$ |
| *** | $p < 0.001$ |

All four sterols had a similar distribution with increased concentrations from marine samples to freshwater samples (Figure 3.13). For example, stigmasterol correlated most strongly with ergosterol, campesterol and β-sitosterol with *r* values of 0.81, 0.82 and 0.68 respectively.
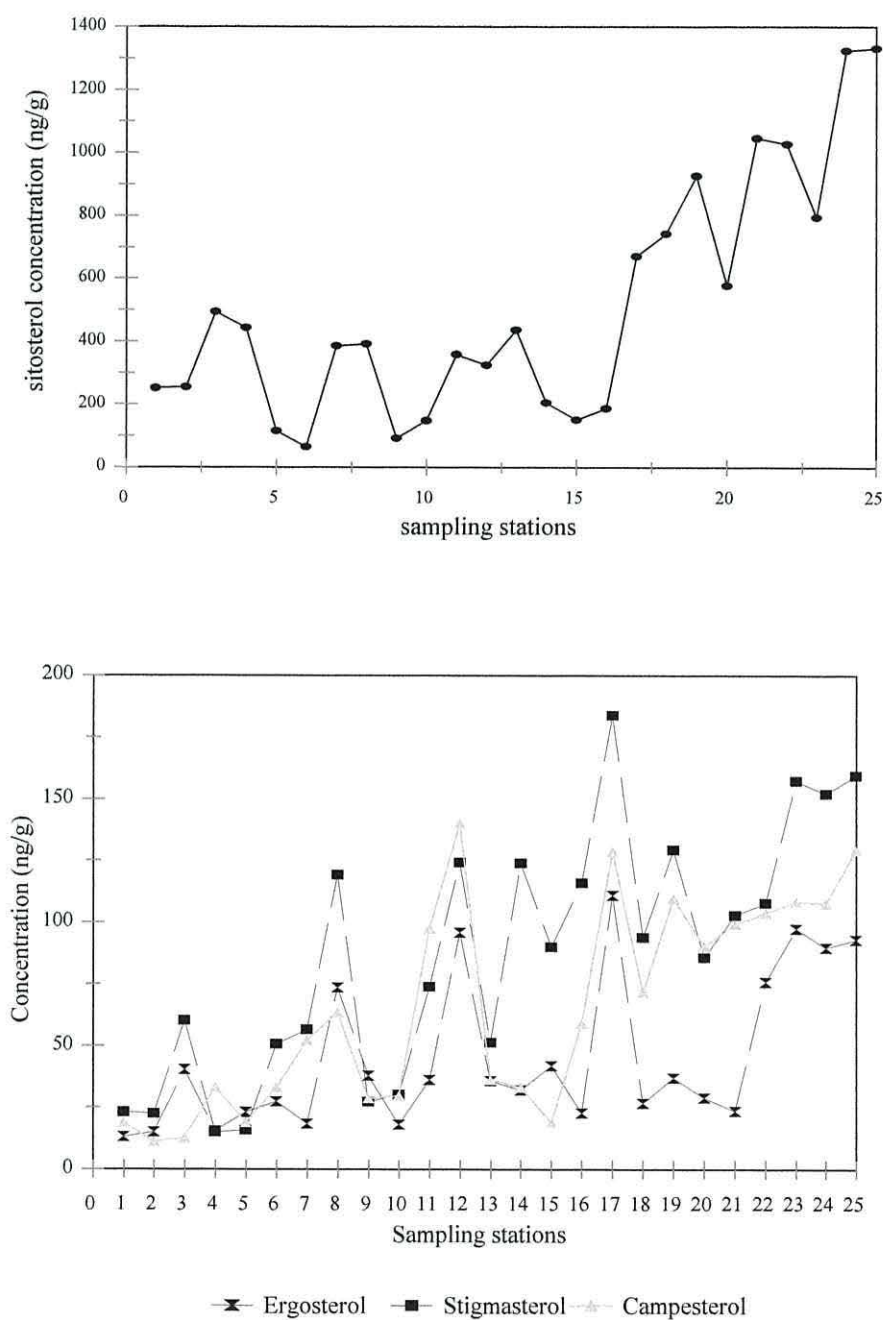




Figure 3.13: Change in concentration of β-sitosterol, stigmasterol, campesterol and ergosterol in Mawddach estuary
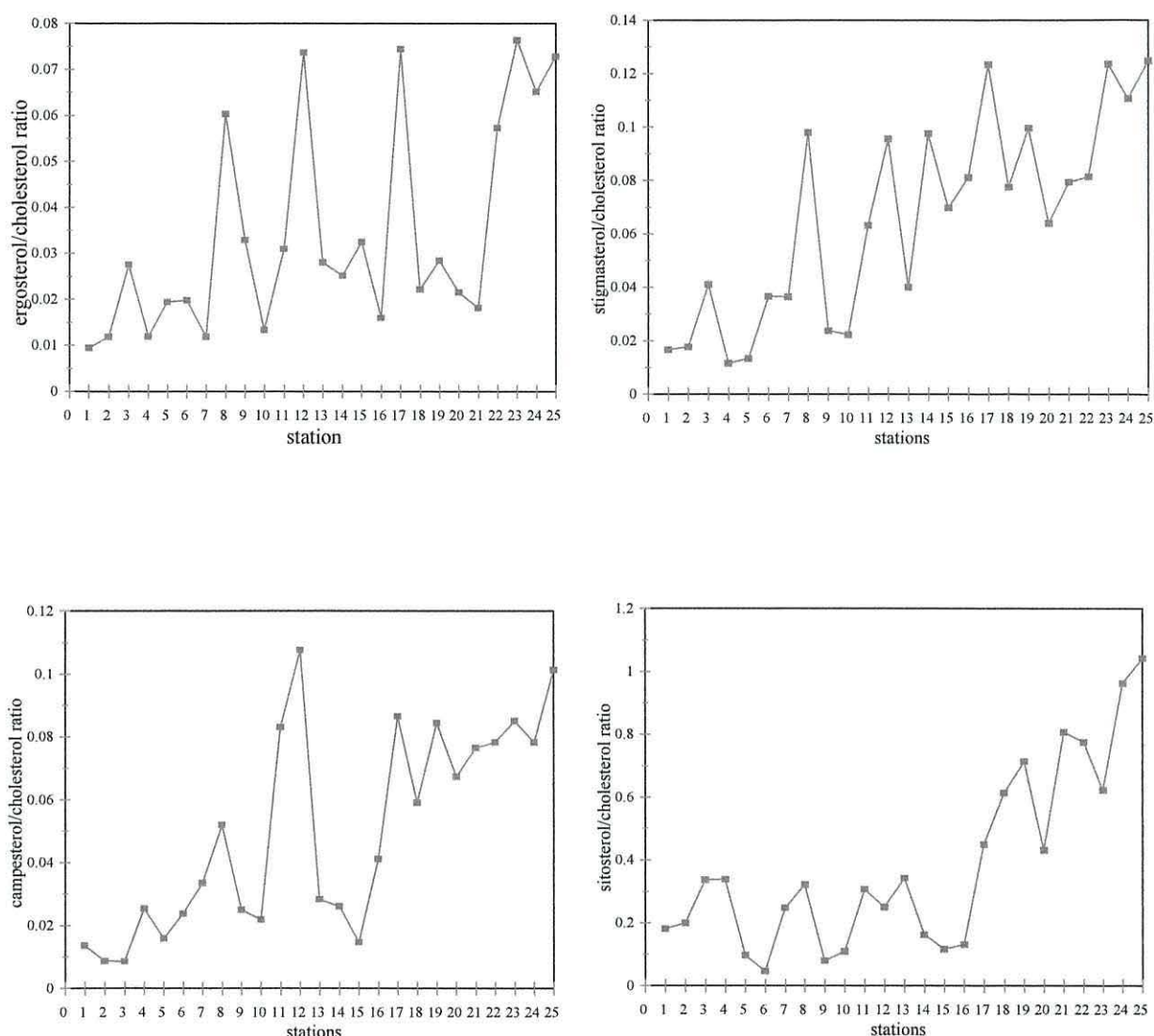
Figure 3.14: Sterol ratios for SSI throughout the Mawddach Estuary

Sterol source indices (SSI) were calculated with cholesterol as the assumed "marine" sterol (Grimalt and Albaiges, 1990) by dividing the terrestrially derived sterols (β-sitosterol, stigmasterol, campesterol and ergosterol) with cholesterol. For example, Figure 3.14 shows the β-sitosterol/cholesterol ratios. Ergosterol/cholesterol, campesterol/cholesterol and stigmasterol/cholesterol increased by a factor of 8 between the marine samples and

freshwater samples while sitosterol/cholesterol increased by a factor of 6. These figures do not work as well as the fatty alcohols even though these are traditional "marine"/terrestrial markers. Correlation matrix between SSI and ASI was measured to find the relationship between them, where they were correlated positively ($r$=0.72, $p$<0.001). Figure 3.15 shows an example of the relationship between β-sitosterol/cholesterol and $C_{22}/C_{14}$. These results suggest that SSI can be used as indicator of terrestrially derived organic matter.
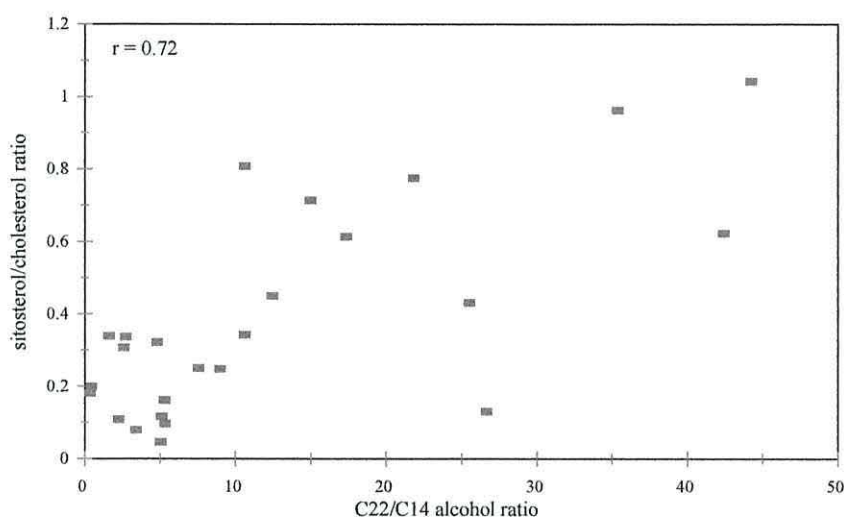


Figure 3.15: The correlation between β-sitosterol/cholesterol ratio and $C_{22}/C_{14}$ ratio in the Mawddach Estuary

River Mawddach receives domestic sewage from sewage treatment plants at Barmouth, Bontddu, and Dolgellau and from small septic tanks near the footbridge (Figure 3.2). Coprostanol (5β-cholestan-3β-ol) has been used as an indicator of human sewage contamination (Nichols *et al.*, 1993; Mudge *et al.*, 1999). The coprostanol/cholesterol ratio is often used to identify sewage contamination (Nichols and Espey, 1991; Mudge and Bebianno, 1997; Mudge *et al.*, 1999). The ratio has greater values at stations near the discharge points (Figure 3.16). Epicoprostanol (5β-cholestan-3α-ol) is formed principally during the treatment of sewage and is only a trace constituent in human faeces (McCalley *et al.*, 1981). The ratio between epicoprostanol and coprostanol can be used to indicate the degree of treatment that sewage has received (Mudge and Lintern, 1999; Mudge *et al.*,

1999). A scatter plot of coprostanol/cholesterol ratios and epicoprostanol/coprostanol ratios (Figure 3.17) showing that sites with high sewage content have a low
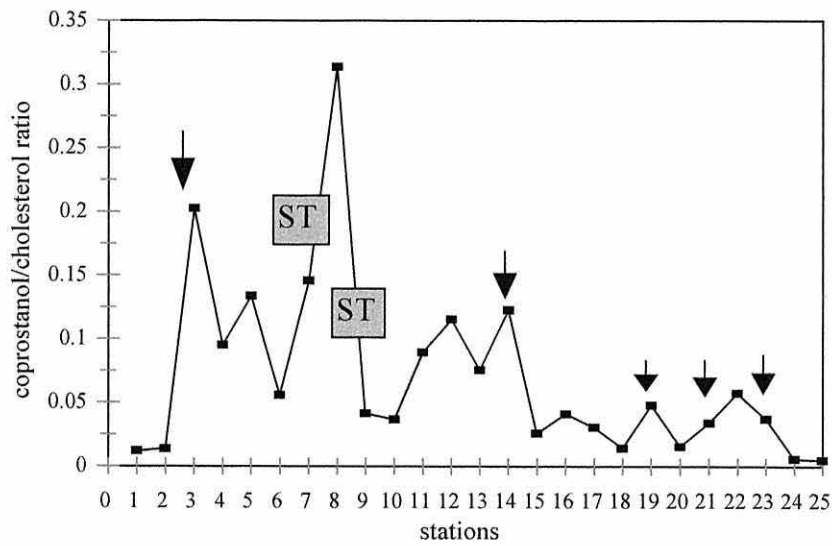


Figure 3.16: Sewage biomarker ratio using coprostanol/cholesterol in the Mawddach Estuary
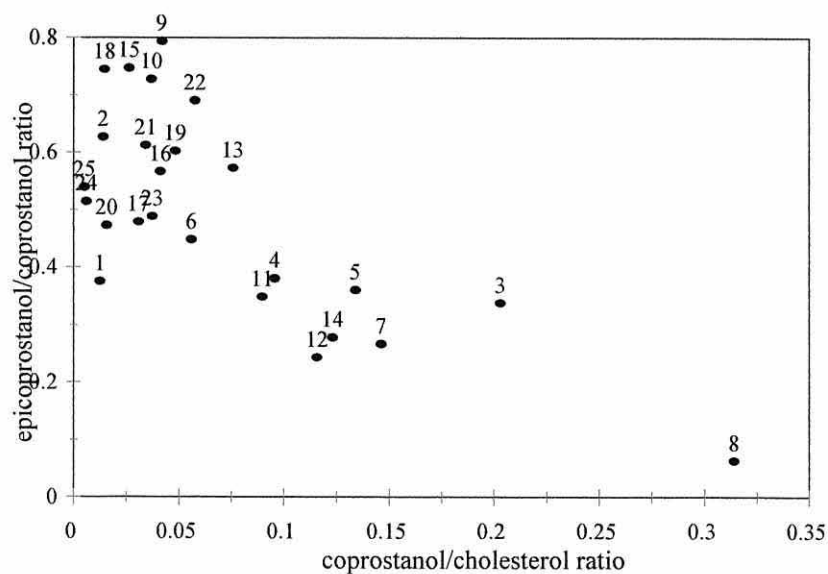


Figure 3.17: Sewage biomarker ratio using epicoprostanol/coprostanol and coprostanol/cholesterol ratios in the Mawddach Estuary

epicoprostanol/coprostanol ratio. This indicates little treatment at those sites (S8, S12, S14, S7 and S3). On the other hand, high epicoprostanol/coprostanol ratios indicate a low sewage component. Samples with high epicoprostanol/coprostanol ratios are S9, S15, S18 and S10. The regions of elevated sewage markers show the transportation of the sewage throughout the Mawddach Estuary by freshwater or by the tides.

## 3.4 Multivariate statistical analysis

### 3.4.1 Principal component analysis

#### 3.4.1.1 Fatty acids

All compounds were used to perform PCA. Raw and proportion data were selected in these analyses. All the data were mean centred to unit variance to remove the concentration effect. Table 3.6 shows the colour coding that are appropriate for all figures in Chapter 3 and 6.

Table 3.6: Colour coding for potential source and the associated compounds

| Potential source | Colour |
|---|---|
| Terrestrial | Green |
| Bacteria/Sewage | Orange |
| Marine | Blue |
| Algae | Red |

#### 3.4.1.1.1 Raw data

a) Raw data without transformation

Figure 3.18 shows the loadings on PC1 and PC2, which account for 28.4% and 21.5% of the total variance. Marine derived fatty acids such as short chain fatty acids are loaded positively on PC1. Meanwhile bacterial fatty acids (branched compounds) are positively loaded on PC2. Terrestrial and algal derived fatty acids are negatively loaded on PC2.
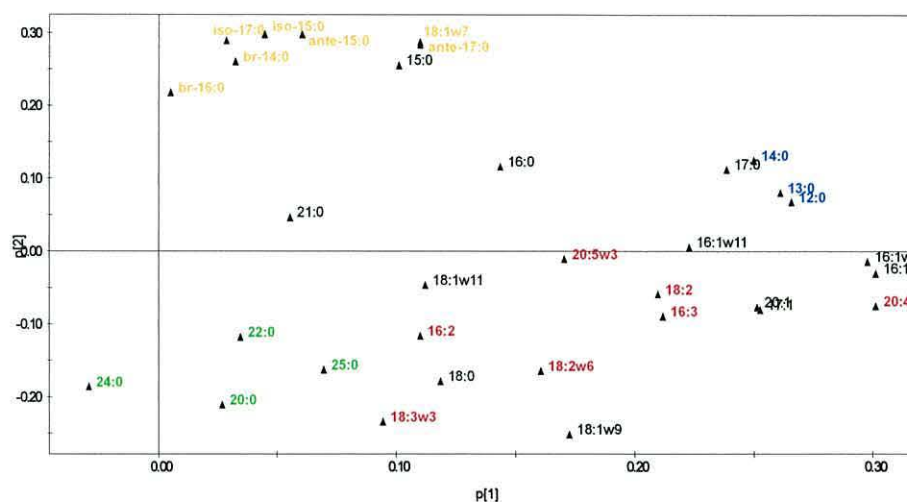
Figure 3.18: The loadings for each fatty acid on PC1 and PC2 in the PCA model with raw data

## b) Raw data with log transformation
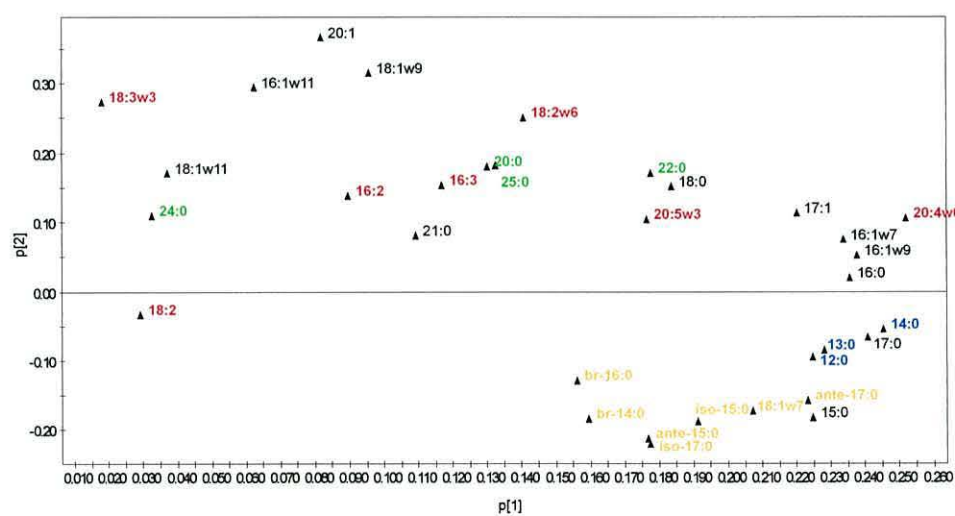


Figure 3.19: The loadings for each fatty acid on PC1 and PC2 in the PCA model with raw data, log transformed

PC1 and PC2 accounted for 42.0% and 20.0% of the variance in the data. The amount of variance in the data has decreased due to the loss of zeros. All the zeros were removed during log transformation. Therefore PC1 explains a high percentage of the variance. The

74

loadings of each compound on PC1 and PC2 are illustrated in Figure 3.19. It appears that all the compounds are positively loaded on PC1. Most marine derived fatty acids and bacterial markers are negatively loaded on PC2. PCA carried out with raw data (log transform) does not separate the compounds according to their geochemical source. Algal and terrestrial derived fatty acids were mixed together with positive loadings on PC2.

c) Raw data, added 0.001 with log transformation

The value 0.001 was added to the 'zero' data as the mean limit of detection. Figure 3.20 shows the compound loadings on PC1 and PC2. Again, PC1 and PC2, which account for 35.4% and 17.6% of the variance in the data does not related to source input. The algal and terrestrial markers still mixed together with positive loadings on PC2. Bacterial derived fatty acids are strongly loaded positively on PC1. Short chain fatty acids (marine markers) also have positive loadings on PC1.
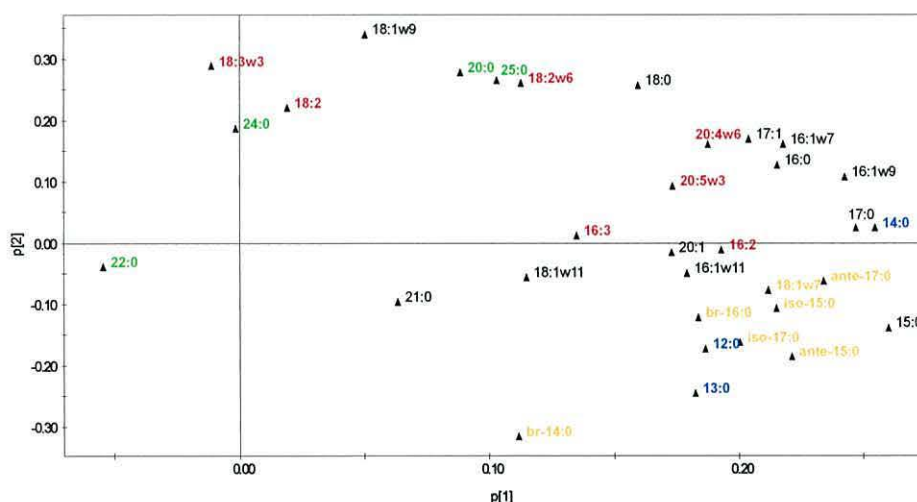


Figure 3.20: The loadings for each fatty acid on PC1 and PC2 in the PCA model with raw data, added 0.001 (log transformed)

### 3.4.1.1.2 Proportion data

a) Proportion data without transformation

Figure 3.21 illustrates the loadings of fatty acids on PC1 and PC2, which account for 24.8% and 19.6% respectively, of the variance in the data. Strong positive loadings on PC1 represent marine derived fatty acids and negative loadings represent terrestrial inputs. Branched fatty acids are negatively loaded on PC2. Unlike PCA that have been carried out

with the raw data (no transformation), the terrestrial fatty acids have strong negative loadings on PC1.
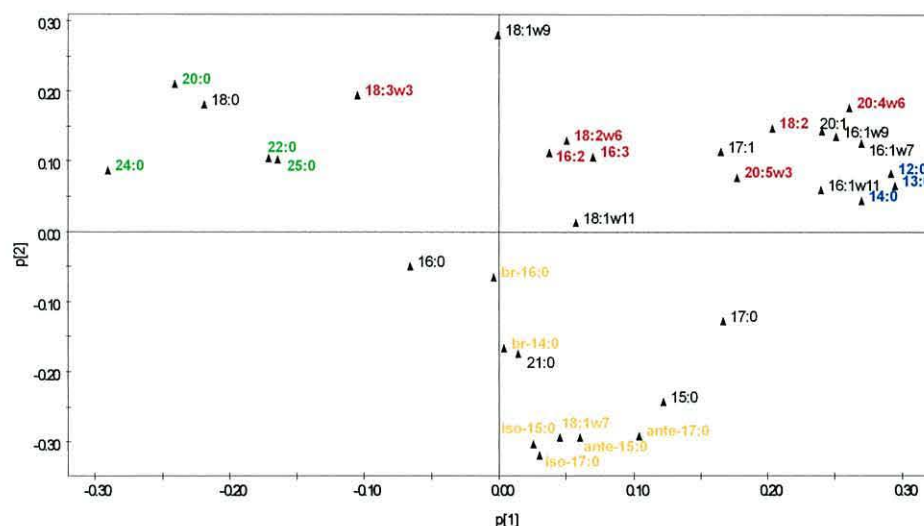


Figure 3.21: The loadings for each fatty acid on PC1 and PC2 in the PCA model with proportion data

## b) Proportion data with log transformation

The first two principal components account for 30.8% and 20.3% of the variance, respectively. From Figure 3.22, compounds that are marine markers are positively loaded on PC1 and terrestrial markers are loaded negatively. Algal derived fatty acids are negatively loaded on PC2. Branched fatty acids are clustered together and positively loaded on PC1 and PC2.
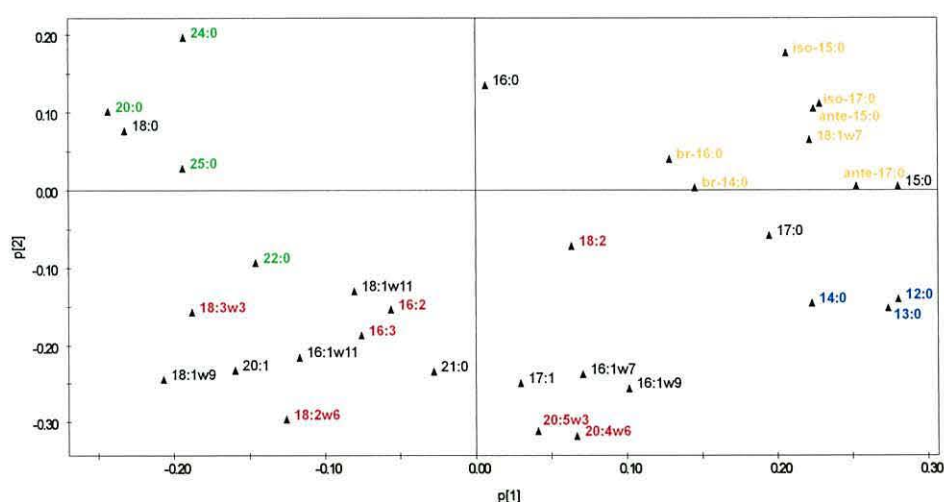


Figure 3.22: The loadings for each fatty acid on PC1 and PC2 in the PCA model with proportion data (log transformed)

c) Proportion data, added 0.001 with log transformation
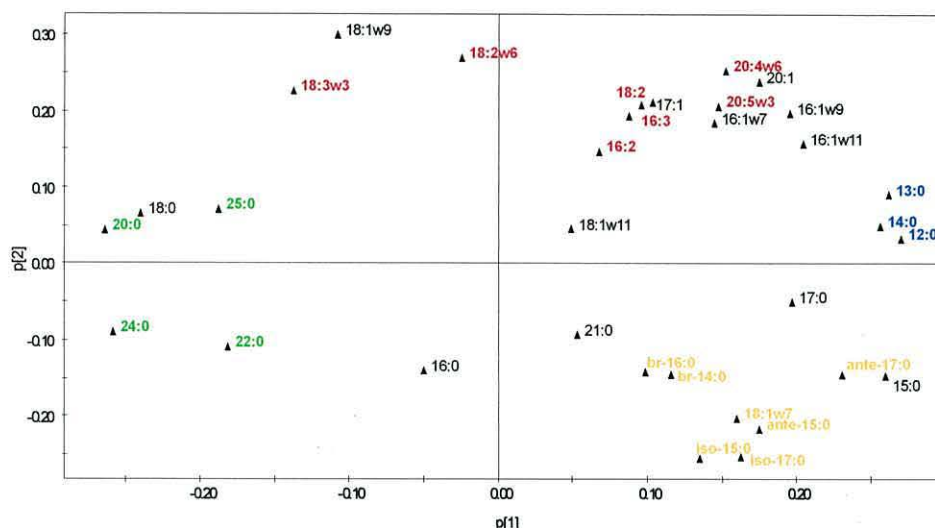


Figure 3.23a: The loadings for each fatty acid on PC1 and PC2 in the PCA model with proportion data, added 0.001 (log transformed)

PC1 and PC2 account for 27.5% and 20.6% of the variance in the data. The plot of the loadings on PC1 and PC2 (Figure 3.23a) indicate the geochemical sources. Marine markers such as 12:0, 13:0 and 14:0 fatty acids are positively loaded on PC1, whereas the terrestrial fatty acids are negatively loaded. The bacterial derived fatty acids are also positively loaded on PC1. All the polyunsaturated fatty acids representing the algal input are positively loaded on PC2. PCA carried out using proportion data with log transformation (+0.001) shows the clear separation compared to the other sets of data.

It can be seen that 17:0 acid behaves like "marine" derived compound in most cases, while the 15:0 acid behaves like branched fatty acids representing the "bacterial" marker. Monounsaturated fatty acids like 18:1ω9, 18:1ω11, 16:1ω7, 16:1ω9 and 16:1ω11 also behave like "algae" derived compounds but not as well defined as others.
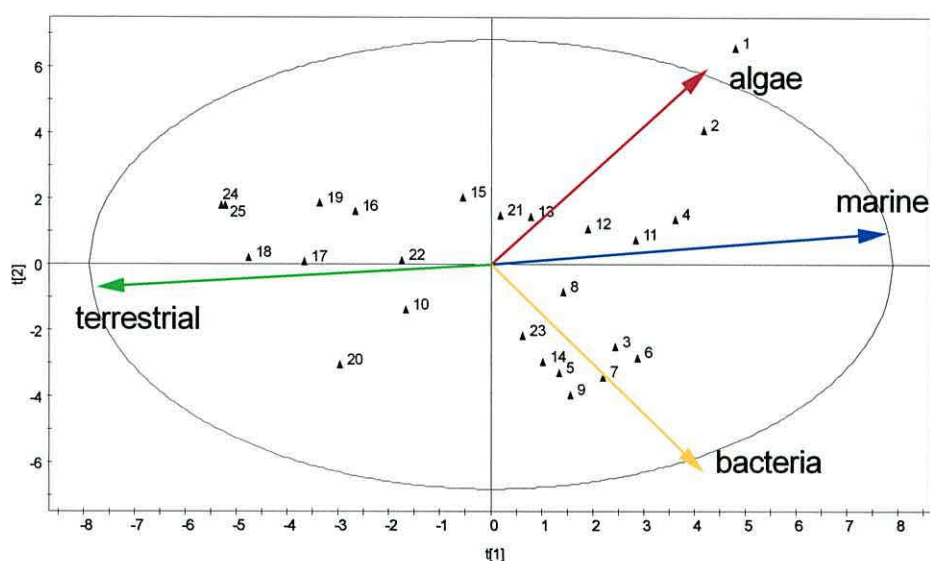
Figure 3.23b: The PCA scores for the first two components

Figure 3.23b shows the scores of the first two components based on the proportion data (+0.001) with log transformation as the PCA carried out with this set of data showed clearer compound separation. Terrestrial organic matter dominates samples projected on the left side of the diagram (terrestrial axis). Samples projected towards the red and blue arrows are influenced with algae and marine markers showing that sample 1 and 2 have the most marine derived compounds. Meanwhile samples that projected towards the orange arrow contain the greatest amount of bacterial derived fatty acids (bacterial axis).

### 3.4.1.2 Fatty alcohols

The proportion data (added 0.001) with log transformation was used to perform the PCA. The loadings of each individual compound are shown in Figure 3.24a and the scores are shown in Figure 3.24b. The PC1 and PC2 account for 24.3% and 23.0% respectively, of the variance in the data. The compounds are clearly separated according to their geochemical sources. Short chain marine derived fatty alcohols are loaded negatively on PC2 while the bacterial derived compounds; mainly branched fatty alcohols are loaded positively on PC1. Long chain fatty alcohols (terrestrial markers) are clustered together negatively loaded on PC1. In the diagram, $C_{18}$ seems to associate with bacterial fatty alcohols.
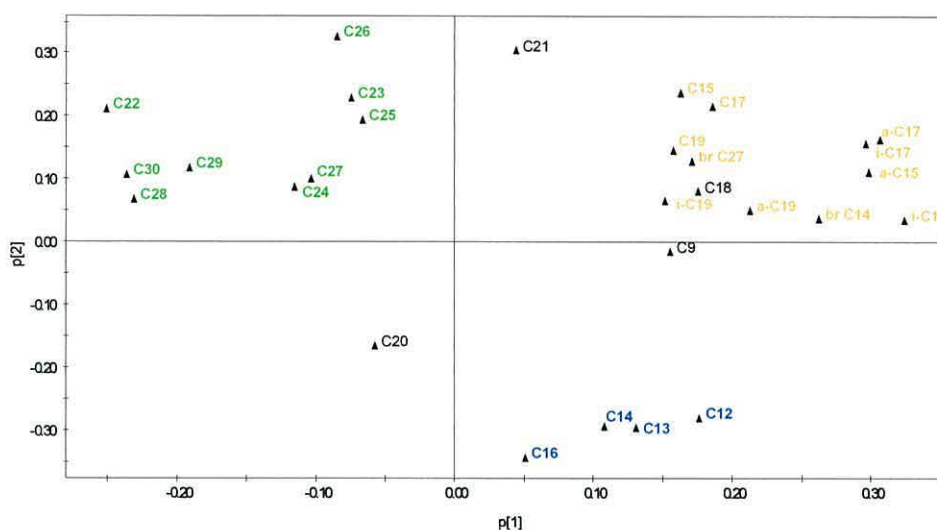
Figure 3.24a: The loadings for each fatty alcohol on PC1 and PC2 in the PCA model

From the score plot (Figure 3.24b) sample 1, 2 and 4 are clearly the true marine samples. Samples on the left associated with terrestrial fatty alcohols while bacterial derived organic matter are dominated samples along the bacterial axis as shown in the diagram. Sample 22 should be associated with terrestrial marker but it is clustered together with bacterial samples indicating that sample 22 may contain high bacterial derived fatty alcohols.
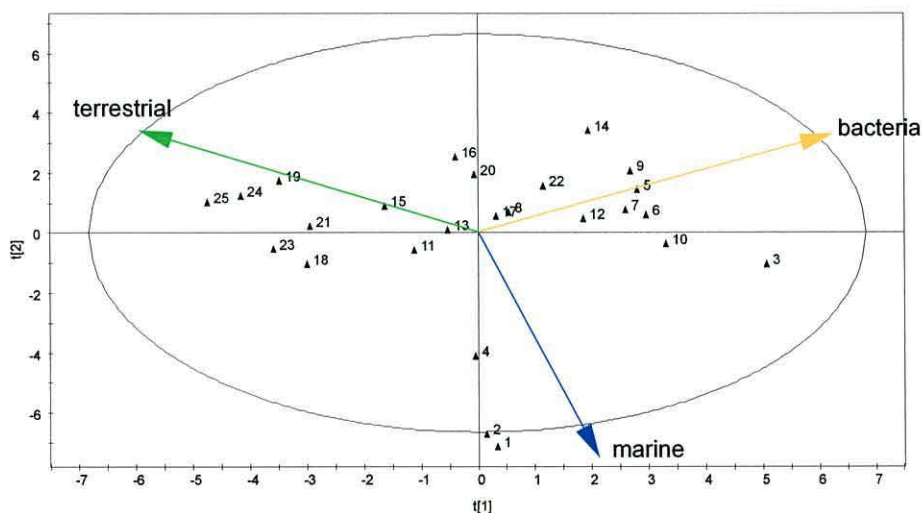


Figure 3.24b: The PCA scores for the first two components

### 3.4.1.3 Sterols

PCA carried out with the sterol compounds were using the proportion data (+0.001), log transformed. PC1 and PC2 account for 26.6% and 19.1% of the variance in the data. The plot of the loadings (Figure 3.25a) indicates the compounds geochemical sources. Terrestrial derived sterols (ergosterol, stigmasterol and β-sitosterol) are loaded negatively on PC1 whereas marine sterols (st1, st2, dinosterol and brassicasterol) are positively loaded with brassicasterol having the most positive loadings. Coprostanol and epicoprostanol (sewage markers), on the other hand, are loaded positively on PC2. Interestingly the reduction product compounds are also clustered together with their parent compounds. For example, st9 and st12 are formed from stigmasterol and β-sitosterol by *in situ* reduction. They are loaded negatively on PC1. Sterol st4 is loaded positively together with its parent compound, brassicasterol, on PC1. Meanwhile cholestanol has positive loadings on PC2 with cholesterol. Figure 3.25b shows the scores of the first two components in the analysis. The trend line shows the sequence in the Mawddach sampling sites. Sample 1 and 2 are dominated with marine sterols. Sample 24 and 25 (above tidal limit) have terrestrial influenced compounds while samples in the middle contain sewage derived sterols.
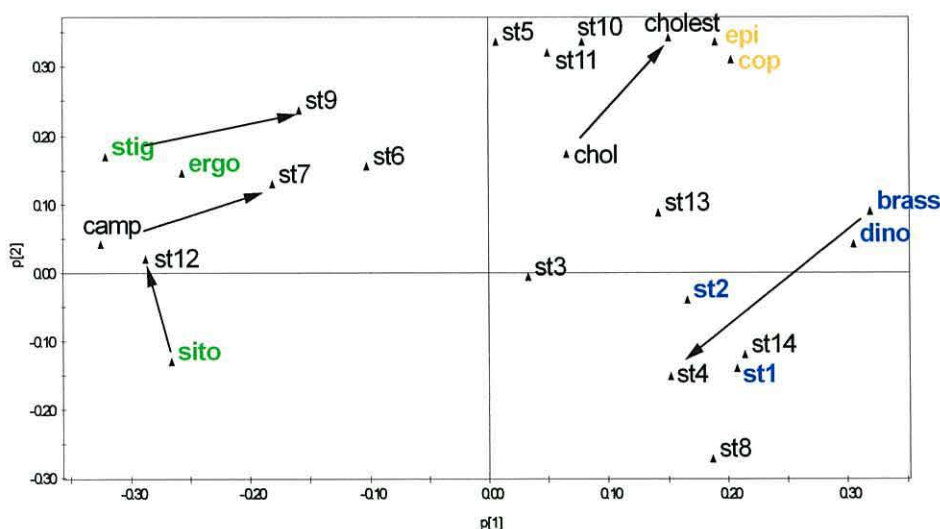


Figure 3.25a: The loadings for each sterol on PC1 and PC2 in the PCA model
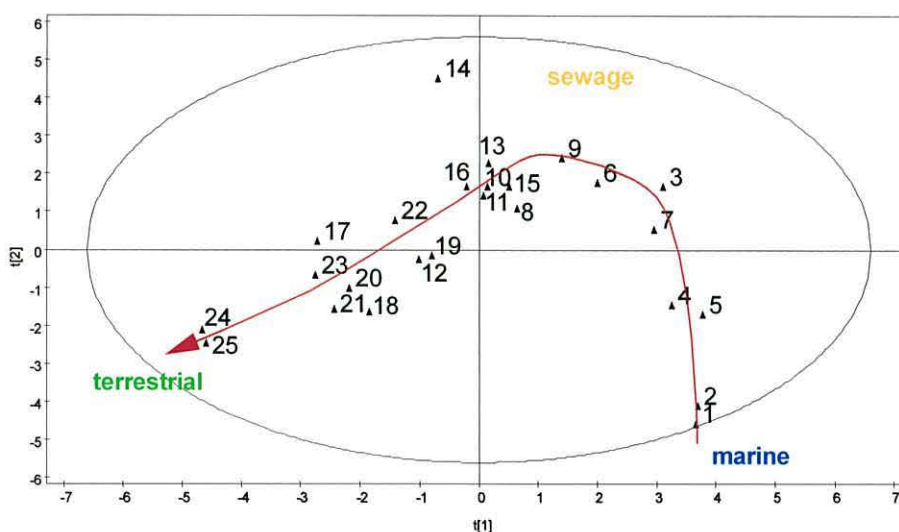
Figure 3.25b: The PCA scores for the first two components

## 3.4.1.4 Mixed PCA

PC1 and PC2 are used to explain the groupings of compounds and samples in the PCA. The loadings of each compound on PC1 and PC2 are shown in Figure 3.26a. PC1 and PC2 account for 23.2% and 17.7% of the variance in the data. Figure 3.26a shows that all sources "from prior knowledge" are well linked together except for the algae derived compounds. Terrestrial derived markers such as long chain fatty acids, long chain fatty alcohols, stigmasterol, β-sitosterol and ergosterol are negatively loaded on PC1 while short chain fatty alcohols and fatty acids (marine markers) have positive loadings. Branched fatty alcohols and fatty acids that are often used as bacterial indicators, together with coprostanol and epicoprostanol, are clustered at the bottom of the diagram with negative loadings on PC2. The algae group (fatty acid compounds) have 18:2ω6 and 18:3ω3 projected towards the terrestrial group. This may indicate that these compounds have higher plant origin rather than algae. Sources may be allocated to compounds on the basis of their position in the loadings plot. For example campesterol, st12 and 18:0 acid can be grouped with the terrestrial compounds, whereas cholestanol, C17, C18, st10 and st11 with the bacterial group. All the 16:1's (16:1ω7, 16:1ω9 and 16:1ω11), st14 and 17:0 acid may have marine and/or algal origin.
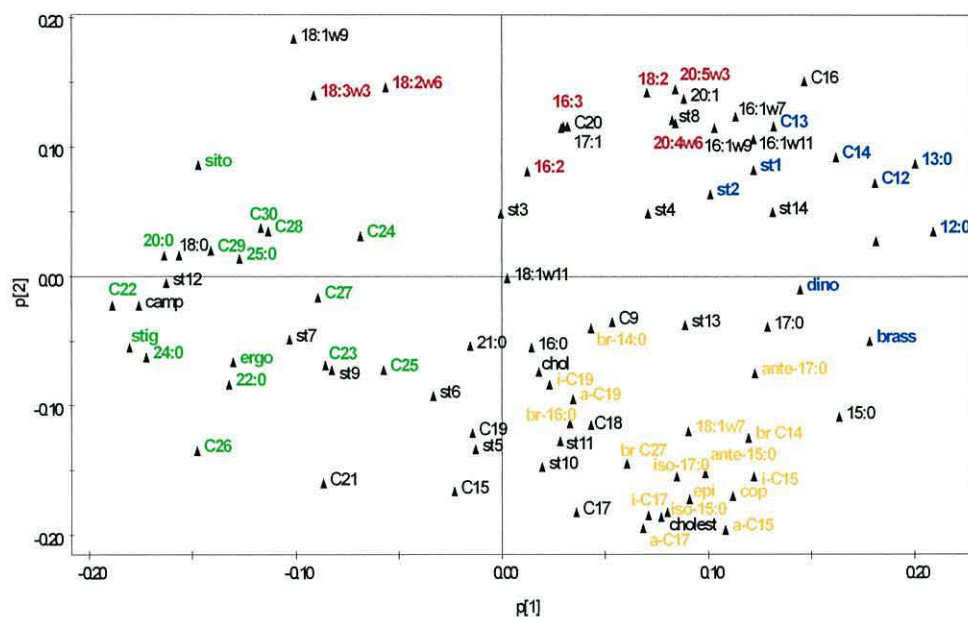
Figure 3.26a: The loadings for each compound on PC1 and PC2 in the PCA model

The plot of scores (Figure 3.26b) shows that site 1, 2 and 4 are true marine samples dominated with marine derived markers. Site 3 is a marine site but more influenced by bacteria. Strong bacterial/sewage compounds dominated samples 5, 6, 7, 9 and 14. Meanwhile samples 8, 10, 11, 12, 13, 15, 16 and 22 have single source with mixed influence. Terrestrial derived markers are dominant in samples 17, 18, 19, 20, 21, 23, 24 and 25.
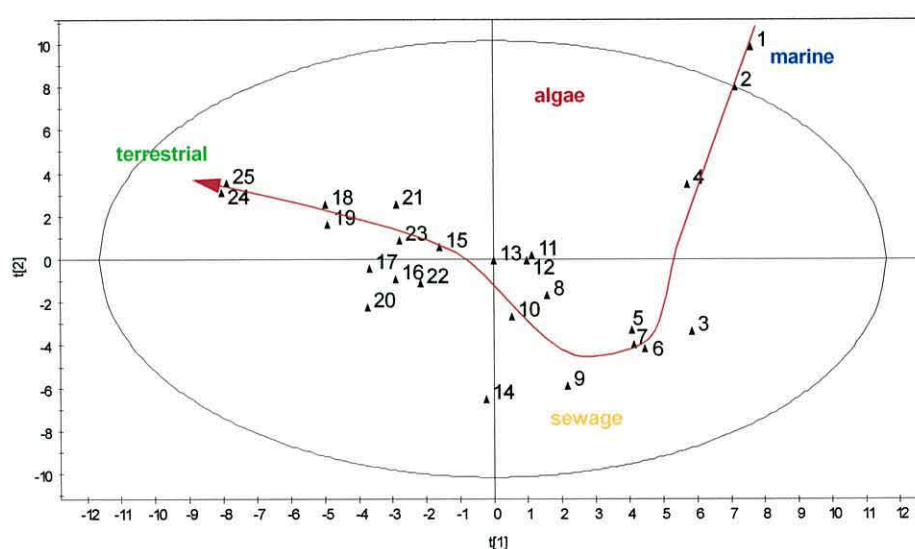


Figure 3.26b: The PCA scores for the first two components

## 3.4.2 Partial least squares (PLS) path modelling

The technique requires two blocks of data: those signatures representing the 100% condition (the $X$-block variables) and the data to be characterised (the $Y$-block data). The choice of signature samples was investigated, and based on the PCA analysis carried out with the mixed compounds, three sets of signatures were chosen. The upstream sites above the tidal limit (S18, S24 and S25) were used as $X$-block in a PLS model to characterise the terrestrial biomarker signatures and sites S1, S2 and S4 to characterise the marine signatures throughout the Mawddach estuary. Meanwhile, to characterise the sewage/bacterial signatures, sites S6, S9 and S14 were used as the $X$-block. The remaining sites were used as $Y$-block variables.

Figure 3.27a shows the comparison of the loadings on the first principal component in the $X$-block (t1) and $Y$-block (u1) for PLS when S18, S24 and S25 were used as terrestrial signatures ($X$-block). The projections were basically linear with no compound was significantly distant from the line of agreement (line 1:1). Only long chain alcohols such as $C_{27}$, $C_{28}$, $C_{29}$ and $C_{30}$ can be seen to be distant. These compounds are also terrestrial derived markers.



Figure 3.27a: Comparison of t1 and u1 projections for PLS with S18, S24 and S25 as terrestrial signatures
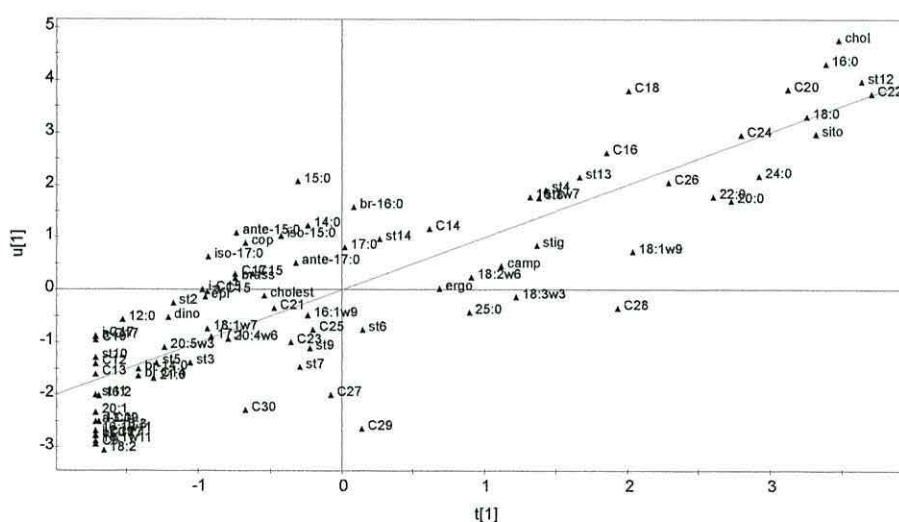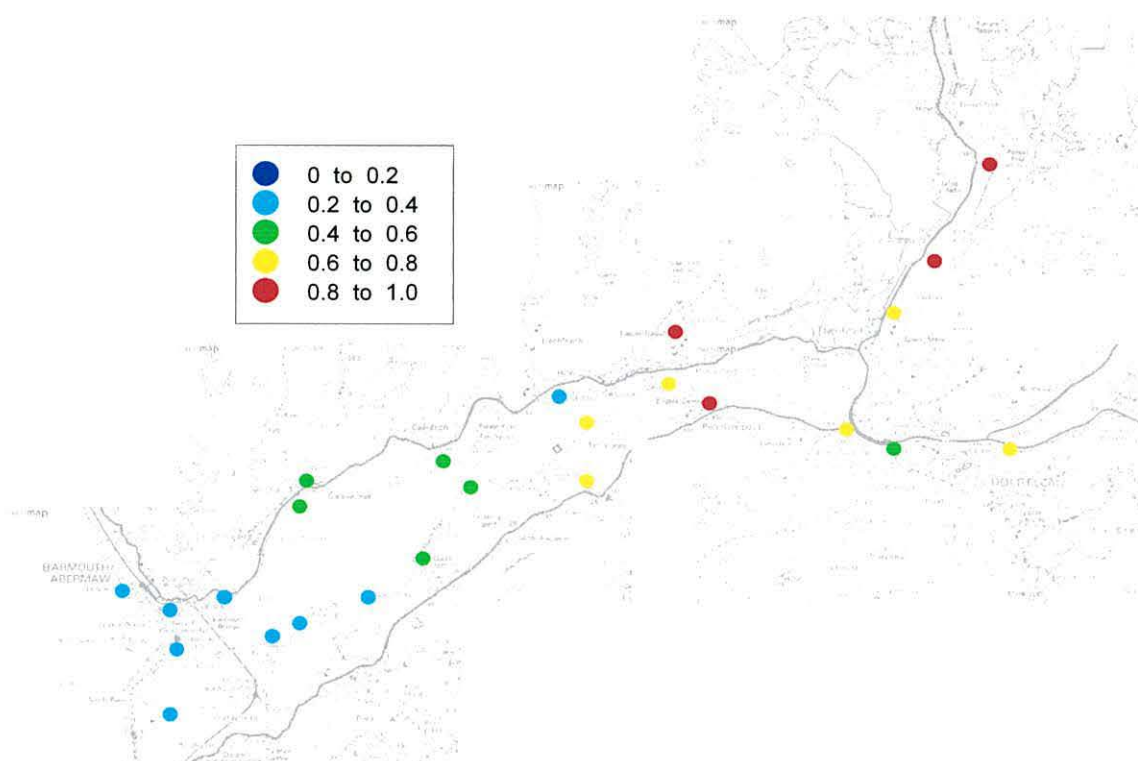
Figure 3.27b: A classed posting map showing the contribution of terrestrial signatures from a PLS model

With PLS, the contribution of signatures at each site can be determined. Figure 3.27b shows the contribution of S18, S24 and S25 (*X*-block) to the rest of the sampling sites. The terrestrial signature could explain almost 20 to 40% of the variance in the marine. Therefore, PLS confirms the contribution of terrestrial material seaward, with the decrease in riverine contribution offshore.

S1, S2 and S4 were chosen to characterise the marine contribution throughout the Mawddach Estuary. The plot of the *X*-block (t1) and *Y*-block (u1) projections (Figure 3.28a) was similar with the plot using S18, S24 and S25 as signatures. It was essentially linear with marine derived compounds such as st1, 12:0 acid and C12 fatty alcohol having the greatest distant from the 1:1 line. The percentage contribution of marine signatures to the other sampling sites is shown in Figure 3.28b. The marine signatures decrease towards those sites principally influenced by terrestrial and sewage/bacterial markers.
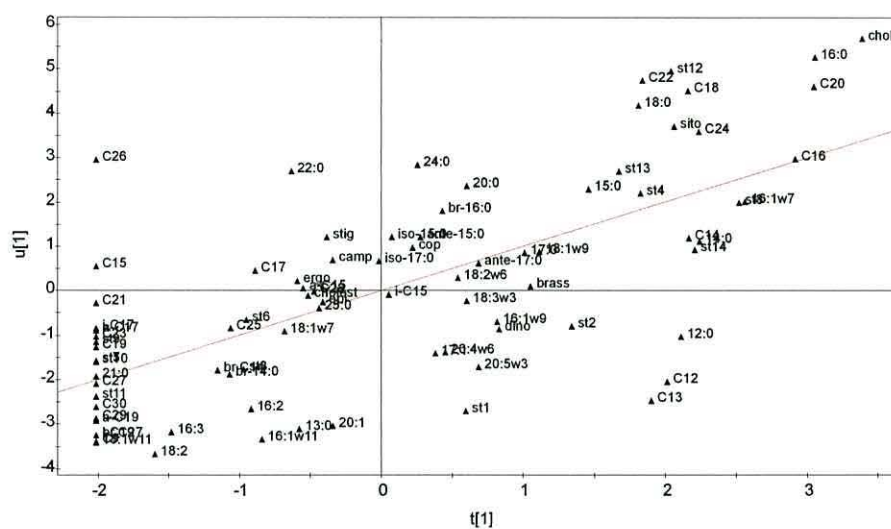
Figure 3.28a: Comparison of t1 and u1 projections for PLS with S1, S2 and S4 as marine signatures



Figure 3.28b: A classed posting map showing the contribution of marine signatures from a PLS model

To characterise the sewage (bacteria) signatures, S6, S9 and S14 were chosen to be the *X*-block. Figure 3.29a shows the plot of the *X*-block (t1) and *Y*-block (u1) with the line of agreement showing similar results with the terrestrial and marine signatures. No compound was significantly distant from line 1:1. Meanwhile, Figure 3.29b shows the contribution of the signatures to the remaining sampling sites. Site S5 illustrates the similarity with the signatures with having 80 - 100% of the signature contribution. This may be due to its location, where sewage compounds might be transported to S5 via tidal exchange. S1, the marine site is considered the "cleanest" site having 0 - 20% of the sewage signatures transported to this site. Meanwhile the above tidal limit sampling sites (S24 and S25) also have a small contribution of the sewage signature. The conclusions derived from Figure 3.29a are different from those derived from Figure 3.16. Figure 3.29a is based on the whole signatures not just a single compound (in this case, sewage biomarkers) and reflects different environmental behaviour.



Figure 3.29a: Comparison of t1 and u1 projections for PLS with S6, S9 and S14 as sewage signatures

Figure 3.29b: A classed posting map showing the contribution of sewage signatures from a PLS model

Most molecular markers are common to several types of living organisms, both terrestrial and marine (Saliot *et al.*, 1991; Colombo *et al.*, 1997), showing that some markers have multiple origins. For example, brassicasterol is a major sterol found in diatom (Laureillard and Saliot, 1993; Colombo *et al.*, 1997), but brassicasterol may be present in some higher plants (Volkman, 1986). Therefore, there will be some overlap between each of the signatures (*X*-block). Table 3.7 shows the overlap matrix for PLS. For instance, 27% of the marine signatures occurred within samples that high in bacterial derived markers and 31% in terrestrial derived markers, respectively. Thirty one percent (31%) of bacterial signatures are overlapped in marine and terrestrial samples. Meanwhile samples with marine and bacterial markers have 29% and 31% of terrestrial signatures.

Table 3.7: Overlap matrix for PLS in the Mawddach Estuary

| | X-block | | |
|---|---|---|---|
| | Marine | Bacteria | Terrestrial |
| Marine | x | 0.31 | 0.29 |
| Bacteria | 0.27 | x | 0.31 |
| Terrestrial | 0.31 | 0.31 | x |

Figure 3.30a shows the fit diagram illustrating that the marine signatures give good explanation with the sea sites (S1, S2 and S4) and terrestrial signatures are best at the river end. Sewage derived compounds are higher in the middle samples with very little on the coasts and in the rivers. Since the total fit is above 1.0, there must be some degree of overlap (as shown and discussed above). Therefore normalisation is used. When normalised (Figure 3.30b), the same result is observed but everything is scaled to fit 100%. Hence, the contribution of each source at each site is easier to observe. For example, 70% marine derived markers are occurred at site S1 and 15% each is terrestrial and sewage compound. Meanwhile, for site S24, 68% is terrestrial derived compounds, 17% and 15% are associated with sewage and marine markers respectively. Sixty three percent (63%) of sewage/bacterial derived markers are occurred within site S14, 22% is terrestrial markers and 15% is associated with marine compounds.
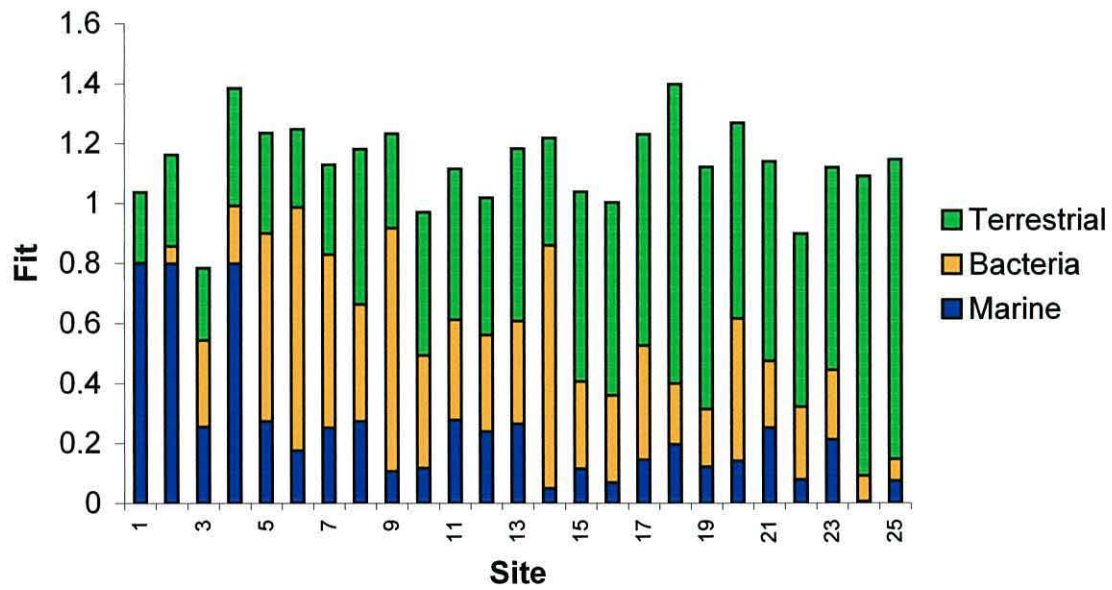
Figure 3.30a: The fit diagram
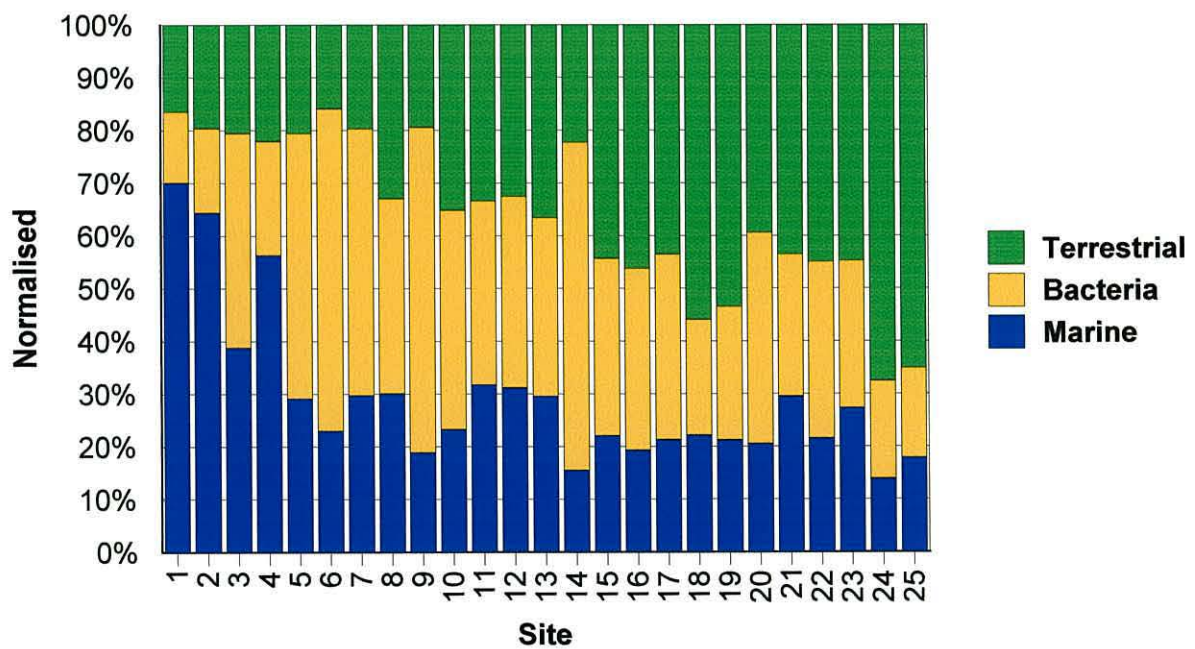


Figure 3.30b: The normalised fit diagram

Contribution (distance to mean projection) of three different sites in the Mawddach Estuary is shown in Figure 3.31. Only the fatty acids group was used to see the score contribution as fatty alcohols and sterols also showed similar results. S1 has the high loadings of the short chain fatty acids (marine markers) and polyunsaturated fatty acids (algal derived markers). All the 16:1's such as $16:1\omega7$, $16:1\omega9$ and $16:1\omega11$ also with high loadings showing similar results with the PCA. These compounds may have marine and algae origin. Bacterial compounds such as branched chain fatty acids have high loadings within S14. Meanwhile, S25 (above the tidal limit) as shown in Figure 3.31 has high loadings of long chain fatty acids, 24:0 and 25:0, which are terrestrial markers as well as the polyunsaturated fatty acids (algae derived markers).
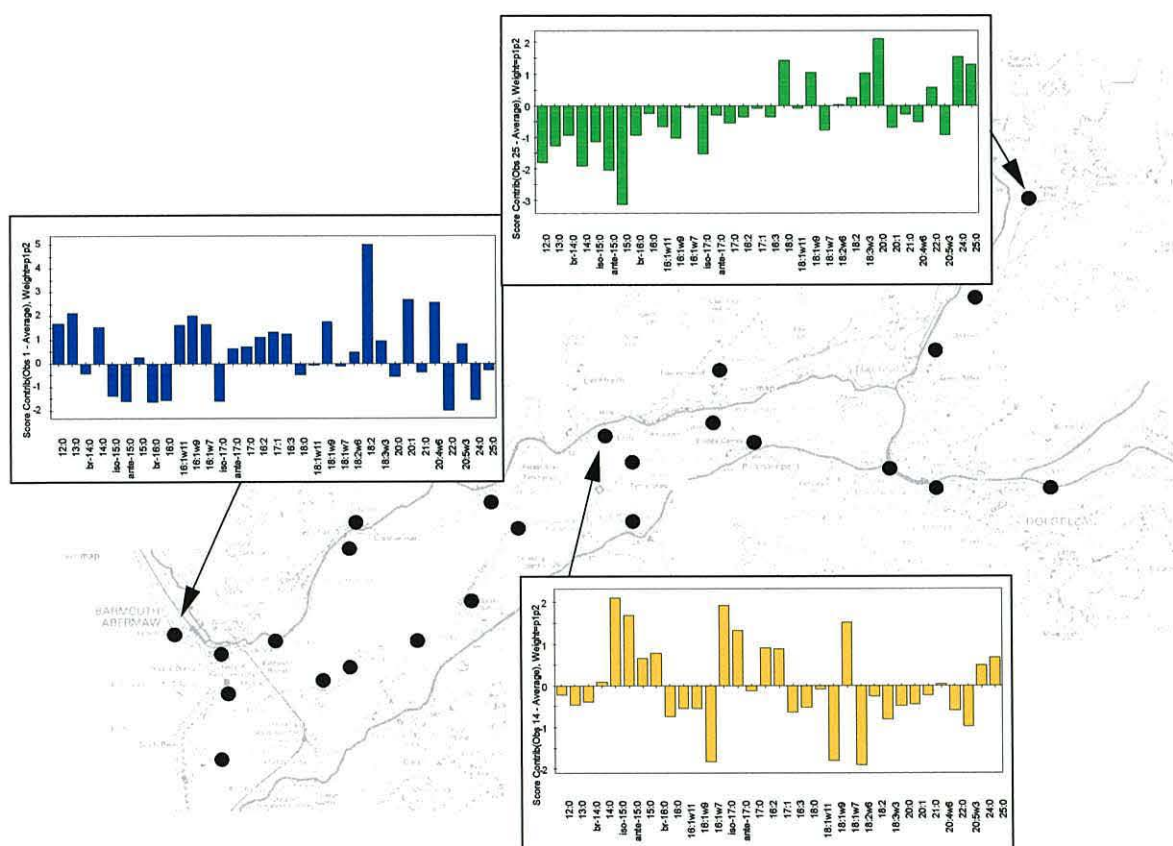


Figure 3.31: Contribution of S1, S14 and S25 in the PLS model

### 3.4.3 Factor analysis

All the data used for factor analysis was obtained by subtracting each observation from the mean and dividing by standard deviation. All the compounds were used for the analysis, but when the factor cannot be extracted, compounds with high percentage of coefficient of variation will be rejected. Before performing factor analysis, "initial extraction" was carried out first to determine the optimal number of factors to extract. There are several methods that can be used to do this. In this study, the Kaiser-Guttman rule was chosen. This method states that the number of factors to be extracted should be equal to the number of factors having an eigenvalue (variance) greater than 1.

### 3.4.3.1 Fatty acids

Factor analysis with four factors (according to the Kaiser-Guttman rule) with Maximum Likelihood method and Varimax rotation was carried out. In the set of fatty acids, four Varimax rotated factors accounted for 77.38% of the total variance in the data. Table 3.8 shows the rotated factor matrix, eigenvalues, percentage of variance and cumulative percentage of variance of four factors.

The first eigenvalue is 5.8, which accounts for 34.10% of the total variance and this constitutes the first and main factor. Meanwhile, the second factor accounts for 18.23% of the total variance with eigenvalue of 3.10. The third and fourth eigenvalues are 2.94 and 1.32 and these accounts for 17.31% and 7.74%, respectively of the total variance.

The first factor is characterised by high loadings of branched chain fatty acids (*iso*-15:0, *iso*-17:0 and *anteiso*-17:0) and the monounsaturated 18:1ω7 fatty acid. Therefore this factor represents bacterial inputs. The second factor, which accounts for 18.23% of the total variance, is mainly associated with high loading of monounsaturated fatty acids such as 16:1ω9, 16:1ω7, 17:1 and polyunsaturated acid 18:2ω6. This factor accounts for the algae derived fatty acids as these compounds can be found in marine algae. Therefore this factor represents marine input. Factor 3 (which accounts for 17.31% of the total variance) is characterised by long chain fatty acids, such as 20:0, 22:0, 24:0 and 25:0 fatty acids. High loading of these compounds relates this factor with terrestrial inputs. Meanwhile, factor 4 is associated with other bacterial inputs as branched fatty acids have high loadings from this analysis. Factor 4 accounts for 7.74% of the total variance.

Table 3.8: Rotated factor matrix for fatty acids

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| 14:0 | 0.084 | 0.082 | -0.399 | 0.141 |
| *iso* 15:0 | 0.430 | -0.192 | 0.035 | 0.869 |
| *ante* 15:0 | 0.446 | 0.109 | 0.026 | 0.887 |
| 15:0 | 0.331 | 0.347 | -0.200 | 0.597 |
| 16:1w9 | 0.059 | 0.568 | -0.045 | 0.059 |
| 16:1w7 | -0.064 | 0.340 | -0.107 | -0.138 |
| *iso* 17:0 | 0.911 | -0.146 | 0.133 | 0.275 |
| *ante* 17:0 | 0.897 | 0.125 | 0.018 | 0.354 |
| 17:0 | 0.446 | 0.187 | -0.080 | 0.123 |
| 17:1 | 0.279 | 0.845 | 0.032 | 0.203 |
| 18:0 | 0.056 | 0.556 | 0.721 | 0.198 |
| 18:1w7 | 0.863 | 0.233 | 0.098 | 0.286 |
| 18:2w6 | 0.145 | 0.780 | 0.225 | -0.076 |
| 20:0 | -0.093 | 0.223 | 0.798 | -0.070 |
| 22:0 | 0.046 | 0.541 | 0.516 | 0.182 |
| 24:0 | 0.293 | -0.245 | 0.851 | 0.125 |
| 25:0 | 0.289 | -0.099 | 0.455 | 0.433 |
| Eigenvalue | 5.797 | 3.099 | 2.942 | 1.316 |
| % of variance | 34.099 | 18.228 | 17.309 | 7.742 |
| Cumulative % of variance | 34.099 | 52.327 | 69.636 | 77.378 |

### 3.4.3.2 Fatty alcohols

Factor analysis of fatty alcohols in the Mawddach Estuary has also been carried out. The analysis generated six factors which together account for 79.29% of variance. The rotated factor matrix, eigenvalue, percentage of variance and cumulative percentage of variance of 6 factors are given in Table 3.9.

The first factor, which accounted for 25.84% of the variance, consists of high loading of branched chain fatty alcohols such as *br*-C14, *iso*-C15, *anteiso*-C15, *iso*-C17 and *anteiso*-C17. Branched fatty alcohols are associated with bacterial inputs. Meanwhile factor 2, which accounted for 20.80% of the variance, represents terrestrial derived fatty alcohols. This factor consists of long chain fatty alcohols (such as $C_{20}$, $C_{21}$, $C_{22}$, $C_{23}$, $C_{24}$, $C_{28}$ and $C_{30}$). Factor 3 (which accounts for 13.0% of the total variance) is mainly associated with high loading of short chain fatty alcohols ($C_{12}$, $C_{13}$, $C_{14}$ and $C_{16}$), phytol and monounsaturated 20:1. Therefore this factor represents marine input. Factors 4-6 are

characterised by the mixture of terrestrial, marine and bacterial derived fatty alcohols and together these factors account for 17.68%.

Table 3.9: Rotated factor matrix for fatty alcohols

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 |
|---|---|---|---|---|---|---|
| C12 | 0.035 | -0.329 | 0.725 | -0.443 | -0.037 | 0.156 |
| C13 | -0.091 | -0.075 | 0.897 | -0.131 | 0.002 | -0.044 |
| *br* C14 | 0.772 | 0.023 | 0.071 | 0.070 | -0.111 | -0.038 |
| C14 | -0.103 | -0.163 | 0.502 | 0.049 | -0.063 | 0.549 |
| *iso*-C15 | 0.781 | -0.019 | 0.039 | 0.430 | 0.092 | 0.236 |
| *ante*-C15 | 0.734 | -0.213 | -0.056 | 0.045 | -0.034 | 0.113 |
| C15 | 0.234 | 0.051 | -0.102 | 0.485 | 0.422 | 0.147 |
| C16 | -0.259 | 0.498 | 0.440 | 0.296 | 0.528 | -0.005 |
| *iso*-C17 | 0.834 | 0.020 | 0.070 | 0.090 | 0.252 | -0.271 |
| *ante*-C17 | 0.927 | -0.048 | -0.007 | 0.066 | 0.140 | -0.171 |
| C17 | 0.462 | -0.004 | -0.126 | 0.175 | 0.838 | -0.193 |
| C18 | 0.114 | -0.221 | -0.191 | 0.789 | 0.119 | -0.159 |
| C19 | 0.203 | 0.236 | 0.194 | 0.808 | 0.139 | -0.045 |
| C20 | 0.044 | 0.944 | 0.034 | 0.056 | 0.074 | -0.176 |
| C21 | 0.176 | 0.599 | -0.120 | 0.464 | 0.110 | -0.001 |
| C22 | -0.159 | 0.880 | -0.308 | 0.093 | 0.097 | 0.134 |
| C23 | -0.042 | 0.412 | -0.255 | 0.335 | 0.245 | 0.073 |
| C24 | -0.148 | 0.451 | -0.291 | 0.292 | 0.381 | 0.015 |
| C26 | 0.123 | 0.204 | -0.327 | 0.695 | 0.061 | 0.174 |
| C27 | -0.096 | 0.110 | -0.400 | -0.034 | 0.108 | 0.010 |
| C28 | -0.223 | 0.763 | -0.358 | -0.265 | -0.098 | 0.026 |
| C30 | -0.317 | 0.356 | -0.395 | -0.064 | 0.539 | 0.533 |
| Phytol | 0.019 | -0.113 | 0.666 | -0.159 | 0.113 | 0.024 |
| 20:1 | 0.049 | 0.154 | 0.420 | 0.261 | 0.533 | 0.103 |
| Eigenvalue | 6.202 | 4.992 | 3.114 | 2.037 | 1.473 | 1.211 |
| % of variance | 25.840 | 20.802 | 12.976 | 8.489 | 6.137 | 5.047 |
| Cumulative % of variance | 25.840 | 46.642 | 59.618 | 68.108 | 74.244 | 79.292 |

### 3.4.3.3 Sterols

Factor analysis of sterols in the Mawddach samples gives six factors describing 79.71% of the data variability. The rotated factor matrix, eigenvalues, percentage of variance and cumulative percentage of variance of six factors are given in Table 3.10.

Table 3.10: Rotated factor matrix for sterols

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 |
|---|---|---|---|---|---|---|
| cop | 0.858 | -0.078 | 0.007 | 0.444 | 0.083 | -0.119 |
| epi | 0.726 | -0.354 | -0.141 | -0.013 | 0.209 | 0.352 |
| st2 | 0.255 | -0.214 | 0.770 | -0.024 | -0.152 | -0.138 |
| st3 | 0.316 | 0.085 | 0.039 | -0.049 | -0.063 | -0.060 |
| chol | 0.298 | 0.118 | 0.323 | -0.320 | 0.128 | 0.037 |
| cholest | 0.800 | 0.009 | 0.210 | 0.045 | 0.398 | -0.078 |
| brass | 0.606 | -0.263 | 0.283 | 0.288 | -0.113 | 0.164 |
| st4 | -0.262 | -0.201 | 0.828 | 0.046 | 0.026 | -0.044 |
| st5 | 0.571 | -0.156 | -0.326 | -0.187 | 0.102 | -0.053 |
| ergo | 0.070 | 0.810 | -0.245 | 0.100 | -0.125 | -0.038 |
| st6 | 0.883 | 0.131 | 0.080 | -0.174 | -0.103 | 0.102 |
| camp | -0.082 | 0.892 | -0.153 | -0.159 | -0.034 | 0.052 |
| st7 | -0.055 | 0.341 | -0.317 | -0.158 | 0.094 | -0.092 |
| stig | 0.000 | 0.866 | -0.268 | -0.137 | -0.023 | -0.223 |
| st8 | -0.109 | -0.227 | 0.897 | 0.237 | 0.033 | 0.269 |
| st9 | -0.115 | 0.087 | -0.447 | -0.050 | 0.344 | -0.061 |
| st10 | 0.137 | -0.233 | -0.062 | -0.193 | 0.905 | 0.056 |
| sito | -0.093 | 0.654 | -0.063 | -0.283 | -0.320 | 0.007 |
| st12 | 0.034 | 0.572 | -0.063 | -0.001 | 0.071 | 0.348 |
| dino | 0.321 | -0.315 | 0.816 | 0.070 | 0.167 | -0.314 |
| st14 | -0.002 | -0.255 | 0.314 | 0.895 | -0.188 | 0.026 |
| Eigenvalue | 6.045 | 3.944 | 2.496 | 1.694 | 1.355 | 1.206 |
| % of variance | 28.786 | 18.780 | 11.884 | 8.068 | 6.452 | 5.741 |
| Cumulative % of variance | 28.786 | 47.565 | 59.449 | 67.518 | 73.969 | 79.710 |

Factor 1 describing 28.78% of the total variance. It is characterised by a high loading of sewage and marine derived sterols such as coprostanol, epicoprostanol, cholestanol, st6 and brassicasterol. It appears that this factor represents sewage and marine inputs. Factor 2 corresponds to 18.78% of the data variability of the model. It is characterised by high loading of ergosterol, campesterol, β-sitosterol and st12. This result relates this factor to terrestrial inputs. Meanwhile factor 3 explains 11.88% of the variance. Compounds such as st2, st4, st8 and dinosterol have high loadings in the model, and these compounds are associated with marine inputs. Factors 4-6 is characterised by the mixture of sources and together these factors account for 20.26%.

### 3.4.3.4 Mixed compounds

Factor analysis with seven factors was carried out, with the Maximum Likelihood method and Varimax rotation. Table 3.11 shows the factor structure matrix, eigenvalues, percentage of variance and cumulative percentage of variance.

Table 3.11: Rotated factor matrix for mixed compounds

| Variable | Factor1 | Factor2 | Factor3 | Factor4 | Factor5 | Factor6 | Factor7 |
|---|---|---|---|---|---|---|---|
| 15:0 | -0.06 | -0.74 | -0.09 | -0.08 | 0.40 | 0.06 | -0.25 |
| 16:1w7 | -0.18 | 0.07 | -0.14 | -0.37 | 0.55 | 0.01 | 0.37 |
| 17:0 | -0.09 | -0.33 | -0.04 | 0.04 | 0.61 | -0.10 | 0.09 |
| 18:0 | 0.22 | 0.01 | -0.89 | 0.15 | 0.34 | 0.00 | -0.13 |
| 20:0 | 0.40 | 0.12 | -0.66 | 0.24 | 0.01 | 0.03 | 0.02 |
| 22:0 | 0.07 | -0.08 | -0.79 | 0.09 | 0.04 | 0.06 | 0.04 |
| 24:0 | 0.26 | 0.07 | -0.36 | 0.80 | 0.06 | 0.03 | 0.16 |
| 25:0 | 0.07 | 0.01 | -0.17 | 0.63 | 0.24 | -0.29 | -0.15 |
| C14 | -0.43 | 0.12 | -0.09 | 0.07 | 0.45 | -0.06 | 0.13 |
| C16 | 0.18 | 0.21 | -0.25 | -0.05 | 0.01 | 0.66 | 0.16 |
| C17 | -0.09 | -0.53 | -0.13 | -0.01 | -0.37 | 0.70 | -0.27 |
| C18 | 0.10 | -0.12 | -0.10 | 0.06 | -0.15 | 0.06 | -0.66 |
| C20 | 0.70 | 0.08 | -0.16 | 0.29 | 0.02 | 0.46 | 0.41 |
| C22 | 0.49 | 0.35 | -0.26 | 0.55 | -0.28 | 0.29 | 0.27 |
| C24 | 0.23 | 0.09 | -0.51 | 0.34 | -0.20 | 0.34 | -0.12 |
| C20:1 | -0.03 | 0.14 | 0.07 | -0.09 | -0.09 | 0.65 | -0.12 |
| cop | 0.02 | -0.90 | 0.15 | -0.09 | -0.11 | -0.15 | 0.00 |
| chol | 0.06 | -0.07 | 0.04 | -0.17 | -0.29 | 0.15 | 0.02 |
| cholest | -0.08 | -0.81 | -0.04 | -0.01 | -0.03 | -0.15 | -0.12 |
| brass | -0.07 | -0.63 | 0.12 | -0.61 | 0.13 | 0.06 | 0.21 |
| ergo | 0.95 | -0.02 | -0.22 | 0.06 | 0.00 | -0.01 | -0.10 |
| camp | 0.67 | 0.16 | -0.44 | 0.09 | -0.36 | 0.03 | -0.03 |
| stig | 0.73 | 0.10 | -0.30 | 0.34 | -0.29 | -0.02 | -0.19 |
| sito | 0.51 | 0.30 | -0.40 | 0.25 | -0.40 | -0.16 | 0.49 |
| Eigenvalue | 3.49 | 3.18 | 3.04 | 2.38 | 1.97 | 1.95 | 1.44 |
| % of variance | 14.6 | 13.3 | 12.7 | 9.9 | 8.2 | 8.1 | 6 |
| Cumulative % of variance | 14.6 | 27.9 | 40.6 | 50.5 | 58.7 | 66.8 | 72.6 |

Significant loadings were identified by underlining the values greater than or equal to 0.30 in absolute value. In general, the larger the absolute values of the loading factor for a variable, the more important the variable is in interpreting the factor. In the Mawddach set, seven Varimax rotated factors accounted for 72.6% of the total variance in the data: 14.6%, 13.3%, 12.7%, 9.9%, 8.2%, 8.1% and 6% respectively.

The contribution of individual variable (compound) to end member's (source) total composition can be calculated by dividing the absolute value of the variable's loading for that factor by the sum of the absolute values of all the loadings for that factor. For example, the loading for saturated 20:0 acid is 0.321 and the sum of factor 1 loadings is 6.68. Therefore, 20:0 acid constitutes of 0.4/6.8=0.06 or 6% of the factor 1 end member. The composition of that factor's end member can be obtained by doing this calculation. The loadings in the factor pattern matrix are the projections of the variables onto the factor. Therefore a plot of the variables in relation of the factor (*e.g.* variables' contribution) is a convenient way of displaying relationships among the variables. Figure 3.32a, 3.33a, 3.34a, 3.35a and 3.36a show the percentage composition of factor 1, 2, 3, 4 and 5. The factor scores are important if the identification pattern among the subjects (samples) is an important aim. In this study, squared factor scores for subjects were used to determine the changes of factor contribution in the Mawddach Estuary, as shown in Figure 3.32b, 3.33b, 3.34b, 3.35b and 3.36b.

Factor 1 accounted for 14.6% of the variance in the data. It is loaded mainly with terrestrial derived sterols such as ergosterol, campesterol, stigmasterol and β-sitosterol (Figure 3.32a). The long chain alcohol, $C_{20}$, also had high loadings (composition) in factor 1. These compounds can be found in great abundance in higher plant. Therefore this factor represents **terrestrial inputs**. The change of factor 1 throughout the Mawddach Estuary is shown in Figure 3.32b. Samples 16, 23, 24 and 25 have the highest amounts of factor 1.
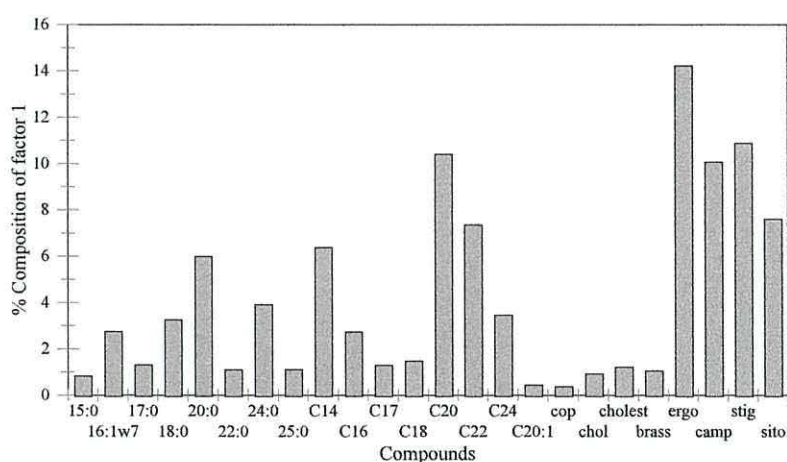


Figure 3.32a: Composition of factor 1 in sediments of Mawddach Estuary
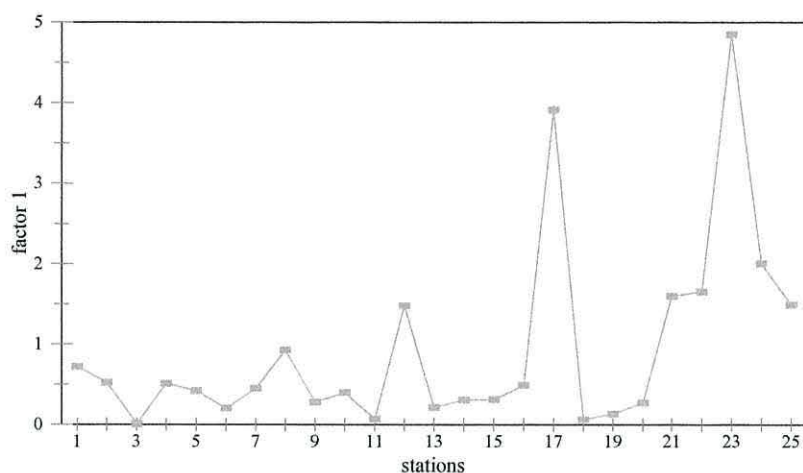
Figure 3.32b: Changes of factor 1 throughout the Mawddach Estuary

Factor 2 explained 13.3% of the total variance. It is composed mainly of odd chain length fatty alcohol and fatty acid (C17 and 15:0 acid) and sewage derived sterols like coprostanol and cholestanol (Figure 3.33a). High composition of factor 2 was also observed in other sterols such as brassicasterol and β-sitosterol. Coprostanol and cholestanol are present in sewage. Therefore this factor represents **sewage and bacterial inputs**. Station 3, 7 and 8 have the highest amount of this factor (Figure 3.33b).
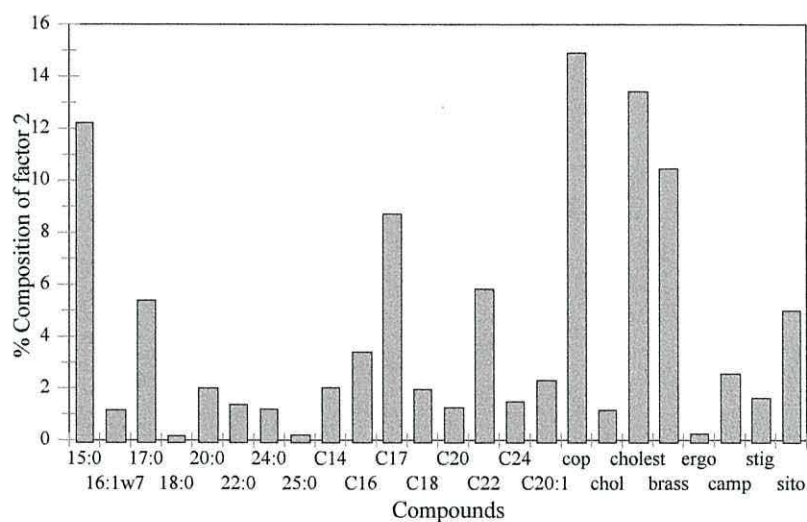


Figure 3.33a: Composition of factor 2 in sediments of Mawddach Estuary
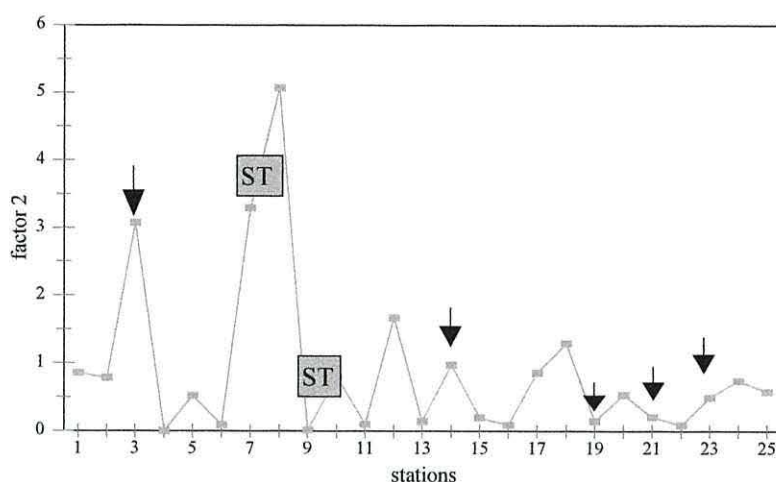
Figure 3.33b: Changes of factor 2 throughout the Mawddach Estuary

The variance was represented by factor 3 was 12.7%. Saturated 18:0 acid has the highest composition in factor 3. Long chain fatty acids (20:0, 22:0 and 24:0) and fatty alcohols such as $C_{24}$ have high composition in factor 3 (Figure 3.34a). β-sitosterol also had a high composition in factor 3. It can be seen that this factor also represents **terrestrial inputs**. Factor 3 is different with factor 1 which was also representing terrestrial inputs because factor 3 has high composition of fatty acids and fatty alcohols, while factor 1 is rich with terrestrial derived sterols. Stations 12 and 21 have the highest amounts of factor 3 (Figure 3.34b).
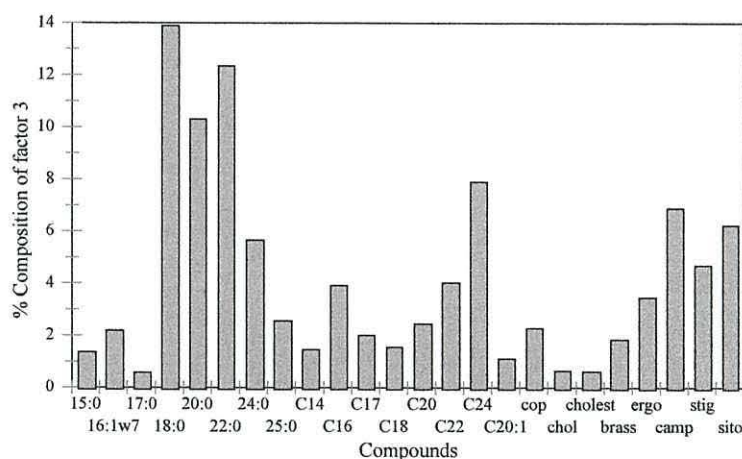


Figure 3.34a: Composition of factor 3 in sediments of Mawddach Estuary

98
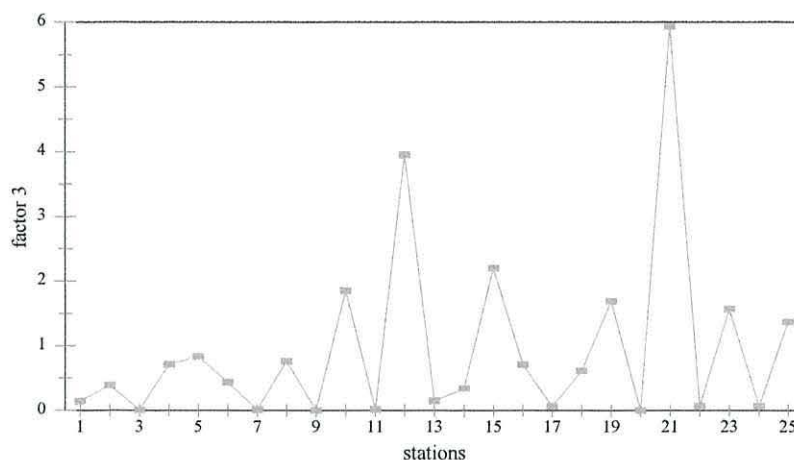
Figure 3.34b: Changes of factor 3 throughout the Mawddach Estuary

Factor 4 explained 9.9% of the total variance. It is mainly composed by the long chain fatty acids such as 24:0 and 25:0 acids and long chain fatty alcohol, $C_{22}$ (Figure 3.35a). These compounds are known to be present in the wax esters of higher plants were used as terrestrial indicators. Brassicasterol also had a high composition in factor 4. Figure 3.35b shows that station 12 and 14 have the highest amounts of factor 4.
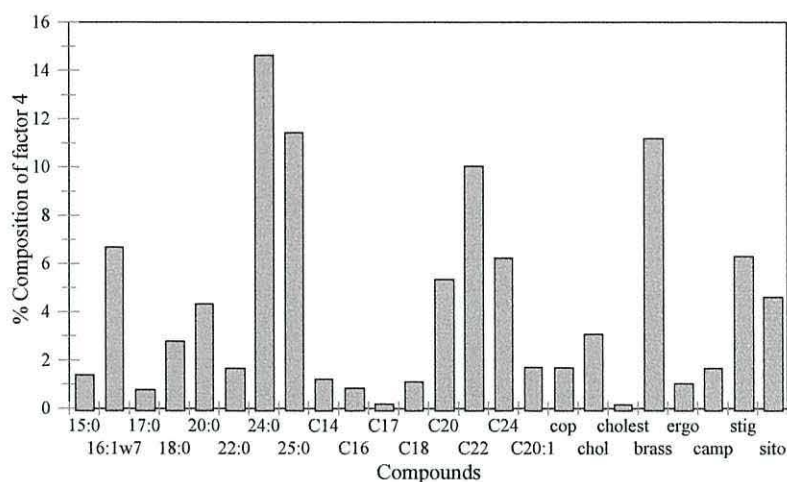


Figure 3.35a: Composition of factor 4 in sediments of Mawddach Estuary
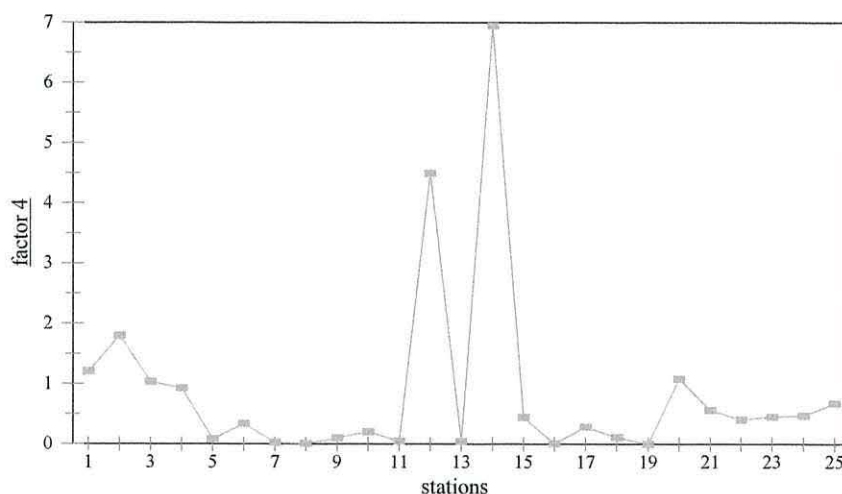
Figure 3.35b: Changes of factor 4 throughout the Mawddach Estuary

Factor 5 accounted for 8.2% of the variance in the data. It is loaded mainly by monounsaturated fatty acid 16:1ω7 and short chain fatty alcohols such as C14 (Figure 3.36a). These compounds can be found in great abundant in marine algae. However, brassicasterol and monounsaturated alcohol 20:1 which are indicators for algae especially diatoms have low composition in factor 5. Therefore this factor is not representing marine inputs. The change of factor 5 throughout the Mawddach Estuary is shown in Figure 3.36b. Only marine station 15 has the highest amount of factor 5.
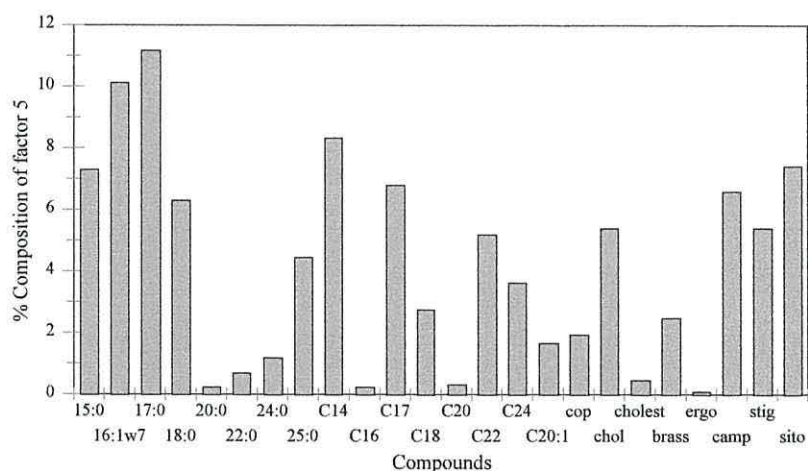


Figure 3.36a: Composition of factor 5 in sediments of Mawddach Estuary
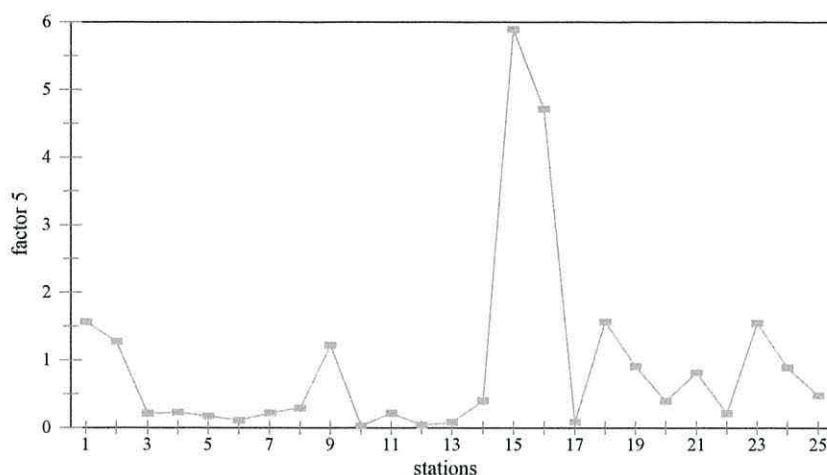
Figure 3.36b: Changes of factor 5 throughout the Mawddach Estuary

## 3.5 Discussion

Estuaries occupy a unique position between land and ocean for material exchange and biogeochemical processing of organic matter. The origin of organic matter in estuaries is complex, and sources include: a) *in situ* primary production, b) terrestrial material transported by river and groundwater flow, land runoff, and atmospheric deposition, c) anthropogenic sources such as wastewater treatment plants, industrial plants, etc., and d) material from the coastal ocean transported via tidal exchange (Mannino and Harvey, 1999). Fatty acids, fatty alcohols and sterols have been used to evaluate sources of organic matter in various depositional environments (Volkman, 1986; Volkman *et al.*, 1987; Wakeham and Lee, 1989; Yunker *et al.*, 1995; Mudge *et al.*, 1999). In this study, 25 surface samples were collected from the Mawddach Estuary to assess the spatial variability.

Long chain fatty acids and fatty alcohols are generally associated with the higher plants and are considered as indicator of terrigenous inputs (Fukushima and Ishiwatari, 1984; Saliot *et al.*, 1991; Carrie *et al.*, 1998). Long chain fatty acids and fatty alcohols were found in all samples collected. Higher concentrations of these compounds can be seen in the freshwater samples compared to the marine samples in the Mawddach Estuary. These results coincided with the terrestrial plant sterols ($C_{29}$ sterols). Long chain fatty acids and

fatty alcohols were also apparent in the grab samples. This was probably the result of the physical processes of dispersion and deposition of the riverine sediments combined with the selective diagenesis (Mudge and Norris, 1997). The same results were found from Conwy Bay (Mudge and Norris, 1997) and Peru sediments (Volkman *et al.*, 1987). These compounds originate from terrigenous sources judging from the high concentration of other biomarker indicative of vascular plant materials such as β-sitosterol (Volkman, 1986). Fukushima and Ishiwatari (1984) also found high concentration of long chain allochthonous materials in the surficial sediments of Tokyo Bay. These relatively high concentrations of long chain fatty acids and fatty alcohols in marine sediments were controlled by the input as well as by the degradation of organic matter (Westerhausen *et al.*, 1993).

Fukushima and Ishiwatari (1984) used the L/H ratio by assuming that chain length distributions may reflect input of fatty acids and fatty alcohols. The short/long ratios (similar to L/H ratio) for fatty acids and fatty alcohols in the Mawddach samples were both high in the marine samples and low in the freshwater samples (Figure 3.6 & Figure 3.10). These ratios together with the Alcohol Source Index, for example, $C_{24}/C_{14}$ ratio increased by a factor of 60 (Figure 3.11) from marine to freshwater samples strongly imply that freshwater samples (above the tidal limit) are rich with compounds derived from terrestrial plant sources. These results correspond with the SSIs, where SSIs were calculated to describe the degree of influence of terrestrial organic matter within marine sediments. The SSIs had consistently elevated ratios between the marine and freshwater samples in the Mawddach samples (Figure 3.14). But nearly all of the values are below 1.0 indicating dominance of the marine sterols in these areas.

Three sterols are often found in terrestrial higher plants; β-sitosterol, stigmasterol and campesterol (Laureillard and Saliot, 1993). Therefore, these compounds are commonly used as tracers of continentally derived organic matter into marine systems but these compounds also have planktonic sources (Volkman, 1986; Volkman *et al.*, 1998). These three sterols were found in all sediment samples. High relative amounts of β-sitosterol were previously reported for other sedimentary environments with mainly marine organisms contributing to the sedimentary organic matter (Volkman *et al.*, 1987; Hinrichs

*et al.*, 1999). In the Mawddach Estuary, β-sitosterol, campesterol and stigmasterol concentrations were high in the freshwater samples and low in the marine samples (Figure 3.13). β-sitosterol predominated over the other two components. Ergosterol, which is the principal membrane sterol of the eumycetic fungi, was also found in the Mawddach samples and in the Conwy core. Mudge and Norris (1997) also found ergosterol in samples collected from the Conwy Estuary. Newell and Fell (1992) found ergosterol (731 μg g organic mass$^{-1}$) in a decaying mangrove leaf from the supratidal zone. Therefore, the presence of ergosterol in sedimentary material may be considered to be indicative of the presence of decaying terrestrial matter (Patterson *et al.*, 1992).

The fatty acids detected in all marine samples were predominantly marine in origin. Similar distributions have been observed in other marine systems (Nadjek, 1993; Carrie *et al.*, 1998; Mudge *et al.*, 1999). Short chain saturated fatty acids are considered to originate from algae (Carrie *et al.*, 1998; Rohjans *et al.*, 1998; Mudge *et al.*, 1999). The saturates were dominated by 14:0, 16:0 and 18:0 acids. Fatty acids such as 16:1ω7, 18:4ω3 and 20:5ω3 are derived primarily from phytoplankton as these fatty acids are major lipids in many species of diatoms (Volkman *et al.*, 1989; Dunstan *et al.*, 1994; Reitan *et al.*, 1994). The monounsaturated fatty acid, 16:1ω7 is diatom derived (Carrie *et al.*, 1998; Mudge *et al.*, 1999) and can be found in all samples in high concentration, especially in marine samples. In the Mawddach samples, high value of unsaturated fatty acids together with the short chain saturated fatty acids can be seen in marine samples while low concentrations were present in the freshwater samples (Figure 3.3). Similar results were observed by Goñi *et al.* (2000) at Beaufort Shelf, which was dominated by monounsaturated 16:1, followed by the short chain fatty acids (16:0, 14:0 and 12:0 acids). These compounds are not different from micro algae such as diatoms and green algae (Goñi and Hedges, 1995). Hence, the predominance of 16:1ω7, which contributes more than 10% of the total fatty acids in S1 and S2 likely, is indicative of a marine diatom source. The 16:0 and 14:0 fatty acids also have been observed to be important constituents in Laptev Sea sediments, where they have been attributed to microalgal inputs, mainly diatoms (Zegouagh *et al.*, 1996).

Short chain fatty alcohols tend to be formed by marine organisms and are often used as marine indicator (Eglinton and Hamilton, 1967; Grimalt and Albaiges, 1990; Mudge and Norris, 1997). These compounds were dominant in the marine samples and have low concentration at the freshwater samples from the Mawddach Estuary. The same results were found in the surface samples from the Conwy Estuary (Mudge and Norris, 1997).

Positive correlations were observed between some typical sterols from the algal group. Brassicasterol is a major sterol apart from cholesterol in the diatom group (Laureillard and Saliot, 1993). Another sterol, cholesta-5,22-dien-3β-ol has also been reported as a good biomarker for diatoms (Nichols *et al.*, 1993; Skerratt *et al.*, 1995). Dinosterol is a specific marker of dinoflagellates (Volkman, 1986) was found in all samples. All the compounds mentioned above have high concentrations in marine samples and low in freshwater samples. These results agree with those of the fatty acids and fatty alcohols and suggest that marine derived organic matter appears to dominate the marine samples and terrestrial organic matter dominates the freshwater samples. Cholesterol is present in many marine organisms and was found in all samples. However, in the Mawddach Estuary, there was no clear trend of its distribution throughout the estuary.

Branched fatty acids and fatty alcohols (principally *iso* and *anteiso* odd chain length compounds) are commonly encountered in bacterial lipids (Parkes, 1987; Saliot *et al.*, 1991; Thoumelin *et al.*, 1997). Therefore these compounds are useful indicators of bacterial lipid contribution or bacterial biomass. The odd/even ratios of fatty acids have a similar distribution with the percentage of branched fatty acids demonstrating their link with bacterial biomass. The same results were found with samples from the Mawddach Estuary (Figure 3.5 and Figure 3.3). Mudge *et al.* (1998) found similar distribution of branched fatty acids and the odd/even ratios in the Ria Formosa lagoon, Portugal. In the Mawddach samples, high concentration of branched fatty acids and fatty alcohols can be seen at sampling sites situated near the sewage discharge points. The ratio of the branched fatty alcohols to the even carbon chain length precursor (Figure 3.9) suggests bacterial production in the lower to middle estuary and coincident with the percentage of branched fatty acids indicate the presence of bacteria in the sewage discharges. The fatty acid, $18:1\omega7$ that was usually associated with cell walls has also been used as bacterial biomarker in sediments (Claustre *et al.*, 1989; Saliot *et al.*, 1991; Thoumelin *et al.*, 1997;

Mudge *et al.*, 1995; Mudge *et al.*, 1998). In the Mawddach samples, concentrations of this fatty acid were strongly correlated with branched fatty acids and the total concentration of odd chain fatty acids.

Coprostanol has been used as a sewage tracer in a variety of environments (Venkatesan and Kaplan, 1990; Nichols *et al.*, 1993; Jeng and Han, 1994; Leeming and Nichols, 1998). Coprostanol is produced in the intestine of mammals by bacterial transformation of cholesterol. Therefore it present in sewage contaminated waters and sediments. The coprostanol/cholesterol ratios have been used to indicate the relative abundance of sewage in sediments (Grimalt and Albaiges, 1990; Nichols and Espey, 1991; Jeng and Han, 1996; Mudge and Bebianno, 1997; Mudge and Norris, 1997). The coprostanol/cholesterol ratios (Figure 3.16) were consistent with sewage inputs around the Mawddach Estuary. The ratios have greater value at sites near the sewage discharge points.

Principal component analysis provides a comprehensive approach to better elucidate the origins (e.g. terrestrial, marine/algal, bacteria, sewage) of the biomarker being measured in this study. Principal component analysis was carried out on individual chemical group as well as on the mixture of fatty acid, fatty alcohol and sterol compounds. In this study PCA carried out using the proportion data, added 0.001 (log transformed) tends to separate the fatty acids according to their geochemical source. PCA separates the fatty acids by primary source: marine, bacteria and terrestrial plants. Polyunsaturated fatty acids, short chain acids and 16:1ω7 acid principally have a marine and algal source in the Mawddach Estuary (Figure 3.23a). Branched fatty acids clearly cluster together. Bacteria are the major source of these compounds (Parkes, 1987; Killops and Killops, 1993). 18:1ω7, which is also abundant in bacteria, was grouped with the branched fatty acids reinforcing their link to the bacterial biomass. PCA for fatty acid compounds also separates the samples according to the geochemical source of the compounds. In the Mawddach Estuary, PCA clearly separates the freshwater samples and the marine samples (Figure 3.23b). Bacterial derived compounds influenced the samples collected near the sewage discharge points. PC1 separates the marine and bacterial fatty acids from the long chain compounds. Again the saturated 16:0 acid was not grouped with any other compounds.

In PCA models for fatty alcohols, PCA also clearly separates the compounds into 3 groups: terrestrial, marine and bacterial. Wax esters profiles in terrestrial plants and marine zooplankton are different. Therefore, the fatty alcohols from these waxes can be used to differentiate marine and terrigenous inputs (Sargent *et al.*, 1977; Cranwell and Volkman, 1981; Yunker *et al.*, 1995). Short chain fatty alcohols ($C_{14}$ to $C_{18}$) primarily have an origin in zooplankton wax esters. In the PCA model (Figure 3.24a), biomarkers of a terrestrial, higher plant source project on the left side of the PCA plot, with branched fatty alcohols on the right side. Meanwhile, biomarkers of marine source (short chain fatty alcohols) project on the lower right side of the PCA plot. It is clear that PCA separate the marine samples from the freshwater samples in the Mawddach Estuary (Figure 3.24b).

In the Mawddach samples, PCA makes a clear separation between the marine sterols, bacterial sterols and the higher plant sterols. Based on the PCA model, 24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22-dien-3β-ol, brassicasterol and dinosterol, have principally marine sources in the Mawddach Estuary. These compounds are the strongest indicators of marine sterol input. Cholesterol, which is present in many marine organisms, was grouped with the sewage derived sterols. However, cholesterol is also present in sewage discharges (Volkman, 1986) and in this system there was no clear trend of cholesterol distribution throughout the estuary. PCA suggests that β-sitosterol, stigmasterol, campesterol and ergosterol have terrigenous origin in the Mawddach Estuary (Figure 3.25a). S1 and S2, which are marine samples from the Mawddach Estuary were dominated by the marine sterols while sewage derived compounds were associated with the samples collected near the sewage discharge points and septic tanks. Samples collected above the tidal limit contain most of the terrestrial plant sterols.

PCA was conducted on the mix compounds of fatty acids, fatty alcohols and sterols. In the Mawddach Estuary, PCA showed the compounds separation from marine and bacterial derived compounds to the terrestrially derived organic matter. In the PCA projection, biomarkers of a terrestrial source project on the left side of the PCA plot with biomarkers of marine source project on the right side. Biomarkers of algal source (polyunsaturated fatty acids) project on the upper side of the PCA plot, with bacterial source compounds project towards the bottom side.

PCA is an adequate method for comparing the biomarker distribution throughout the sampling sites in the Mawddach Estuary. The application of this method results in a direct correspondence between the loadings and biomarkers. PCA also allows clear differentiation between samples. In PCA, it is not important that the data are normal and therefore it shows the robustness of this method. Another advantage of this method is its relative simplicity. For example, it is easy to see the contributions made by the attributes (compounds) to each component in PC.

The same compounds used for the mix PCA were used in the PLS model. The percentage of principal component in PLS is based on the biomarkers persisting in a sample (Yunker *et al.*, 1995). Some biomarker compounds may be degraded at different rates. However, the terrestrial fatty acids, fatty alcohols and sterols are more protected from degradation than the marine or bacterial derived organic matter (Kawamura and Ishiwatari, 1984; Grimalt and Albaiges, 1990; Yunker *et al.*, 1995). Samples collected from the Mawddach Estuary were used to characterise the terrestrial, marine and sewage/bacterial components. S1, S2 and S4 were used as the marine signatures to quantify the contribution of marine derived compounds, while S18, S24 and S25 were used as terrestrial signatures. S6, S9 and S14 were used to characterise the sewage biomarker signatures.

The separation between freshwater and marine samples provides a way to estimate the relative contribution of each end member to the rest of the samples. In the Mawddach Estuary, the samples collected above the tidal limit were used as the *X*-block (signatures) to characterise the terrestrial biomarker throughout the Mawddach Estuary. The percentage of the freshwater contribution decreases seaward in the Mawddach samples. These results reflect higher amounts of riverine organic matter in the freshwater samples and low in the marine samples. PLS confirms the control of the River Mawddach on the near shore with the decrease of terrestrial contribution in the marine samples. Meanwhile, the marine signatures are high at S1, S2 and S4 and decrease to approximately 20 - 40% at samples principally influenced by terrestrial and sewage markers. The sewage biomarkers also show a decrease at sampling sites influenced by terrestrial and marine derived markers. PLS indicates that very little sewage markers are present at S1. Both PLS and biomarkers indicate that marine, terrestrial and sewage derived compounds are present in all sampling sites. The long chain fatty alcohols, long chain fatty acids and higher plant sterols could

have a direct source in riverine sediments or an indirect source in resuspended bottom sediments. Here, the PLS results are supported by the PCA results and individual biomarker results.

Factor analysis is a generic term of statistical techniques concerned with the reduction of a set of observable variables in terms of a small number of latent factors. Factors are supposed to contain the essential information in a larger set of variables or objects. The use of factor analysis on single group compounds is very useful in the interpretation of the distribution of fatty alcohols, fatty acids and sterols in this study.

Factor analysis was carried out on samples from the Mawddach Estuary and was conducted on fatty acids, fatty alcohols, sterols and mixed compounds. Fatty acids, fatty alcohols, sterols and mixed compounds were extracted with 4, 3, 6 and 7 factors respectively. Bacterial derived compounds representing bacterial/sewage inputs became the main factor in fatty acids and fatty alcohols factor analysis and factor 2 with the mixed compounds. Meanwhile compounds that represent terrestrial input were obtained from every set of data. It became factor 1 with the mixed compound factor analysis, which means it explained the most information compared to the other factors. Marine derived compounds which representing marine and algal inputs were also observed from every factor analysis except for the mix compounds in this study. Marine input became the major factor with the sterol data but it became the 5th important factor with mixed compounds. Thus, all the data, from all four factor analyses carried out, is reduced to three major systems: bacterial/sewage, terrestrial and marine.

Factor analysis and principal component analysis have similar purpose, which is to reduce the original variables into fewer composite variables, called factors or principal components. Principal component analysis is used to find optimal ways of combining variables into a small number of subsets, while factor analysis may be used to identify the structure underlying such variables. In factor analysis, interest centres mainly on the common factor, where factor analysis analyses only the common variance of the observed variables. Meanwhile principal component analysis considers the total variance and makes no difference between common variance. Hence, it is generally used in a descriptive fashion (Krzanowski and Marriott, 1995).

## 3.6 Conclusions

The marine samples are characterised by relatively high concentration of short chain fatty alcohols, fatty acids and marine origin sterols. This can be seen from the results of ASIs and SSIs. The freshwater samples are characterised by an increasing dominance of terrestrially derived materials with high concentrations of long chain fatty alcohols and fatty acids. These results are also corresponded with the SSIs such as ergosterol/cholesterol index. Samples that are near the sewage discharge points have high concentration of bacterial input fatty acids and fatty alcohols. These samples are dominated by sewage derived materials as shown by coprostanol/cholesterol ratio. Multivariate analysis overcomes the difficulty that biomarkers often are not specific. With a multimarker approach, biomarkers may be clearly interpreted. PCA confirmed the above observations with PC1 and PC2 indicating biogeochemical sources. Three distinct regions in PC diagrams could be identified; terrestrial markers are clearly separated from bacterial/sewage and marine markers. PCA showed clearer compound separations using the proportion data, added 0.001 (log transformed) compared to the raw data. PLS modelling demonstrated the contribution of terrestrial, marine and sewage/bacterial derived compounds to the Mawddach Estuary. The results show that PLS modelling can be used quantitatively to see the contribution or transportation of compounds from one environment to another. Several steps have to be done before factor analysis can be performed. Factor analysis carried out with the Mawddach samples reduced the data set into three significant sources: marine, bacterial/sewage and terrestrial.

**CHAPTER 4: CORE SAMPLES FROM CONWY ESTUARY**

**4.1 Introduction**

Diagenesis is the processes affecting the organic matter in sediment that takes place prior to deposition and during the early stages of burial under condition of relatively low temperature and pressure (Killops and Killops, 1993). Biological, chemical and physical processes are responsible for diagenetic transformation. Microbial activity is one of the major agents altering sedimentary organic matter during early stages of diagenesis near the sediment-water interface where more biochemically labile compounds are consumed, leaving behind the more biochemically stable materials and varieties of alteration products (Wakeham and Ertel, 1988). The quantity and quality of organic matter preserved in sediments varies greatly depending on the nature of material delivered to the sediment and on the depositional environment. The diagenesis of organic matter generally proceeds by aerobic decomposition in the upper few centimetres of the sediment followed by anaerobic processes below the depth at which all oxygen has been consumed.

Core samples were collected and analysed from Conwy Estuary in North Wales to study the geochemistry and distribution of organic matter in sediment. The Conwy is the largest estuary of the North Wales coast. Its source is from the mountainous area of Snowdonia, draining an area of $\approx 660$ km$^2$. This estuary is an excellent area to study the biochemistry of sedimentary organic matter as it rich with terrestrial plants. Depth profiles of fatty alcohols, sterols and fatty acids may indicate the depth to which the organic matter may be subjected to diagenesis. A 50 cm core was collected at longitude $3^o$ 48' 46"W and latitude $53^o$ 16' 58"N (Figure 4.1) using a hand held corer. The core was cut at 2 cm interval and the fractions were refrigerated until further use.

Multivariate statistical analysis such as Principal Component Analysis (PCA) is invaluable in analysing the distribution and geochemistry of biomarkers (Yunker *et al.*, 1995; Mudge *et al.*, 1999). Fatty alcohols, sterols and fatty acids will be used as variables to carry out these analyses.
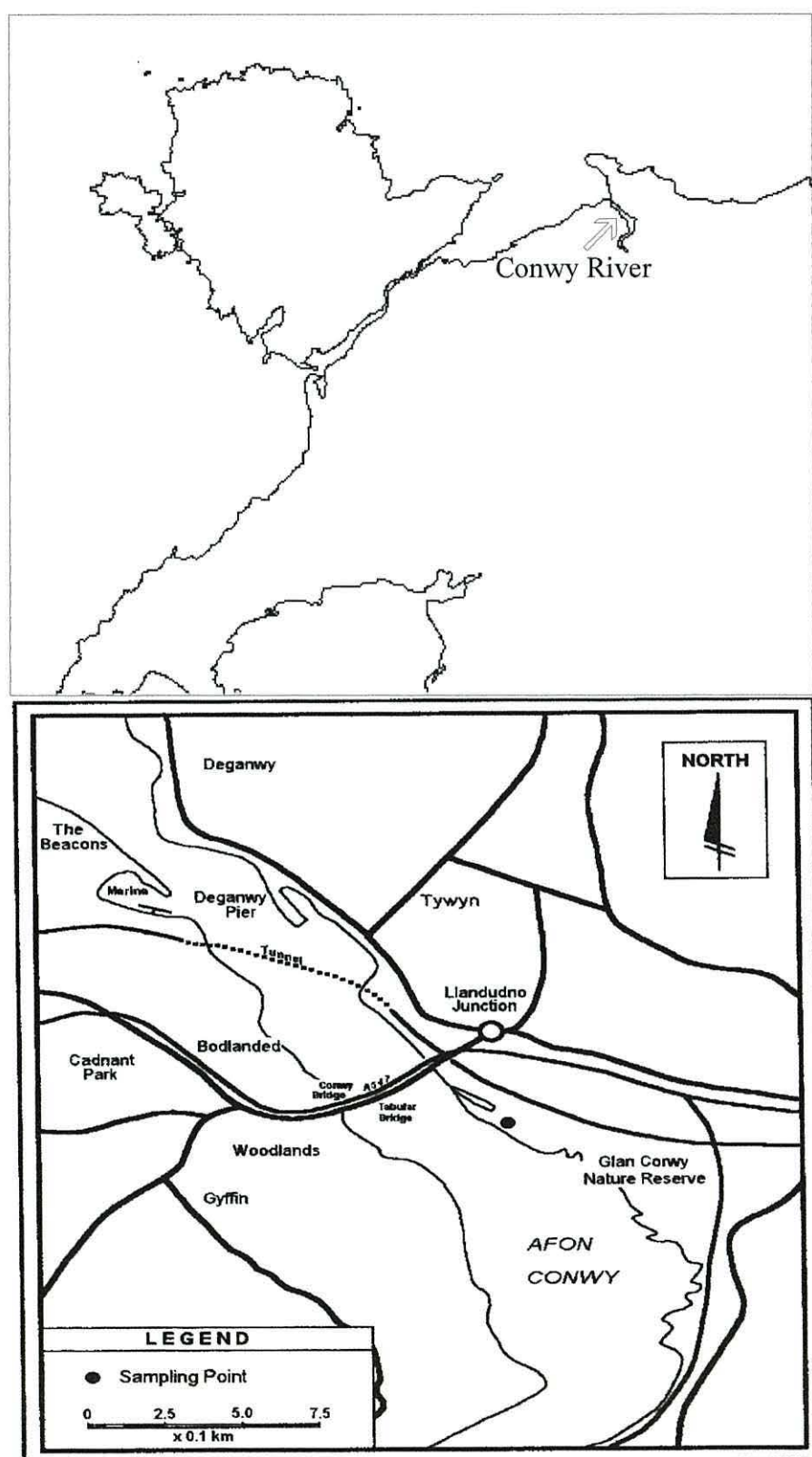
Figure 4.1: Location of sampling site

## 4.2 Results

It has been shown that biomarker profiles can identify terrigenous and marine derived inputs potentially. All the profiles of biomarkers maximise at the surface. This is consistent with inputs, which derive in the first instance from the water column whether the input sources are terrigenous, or not.

### 4.2.1 Fatty acids

Thirty-two fatty acids with chain lengths up to 25:0 were identified and quantified (Appendix 4). Saturated 16:0 was the major fatty acid found. 14:0-22:0 fatty acids are typical of phytoplankton. Fatty acids 14:0, 16:0, 16:1$\omega$7 and 20:5 are abundant in phytoplankton while 16:0, 18:1$\omega$9, 18:0 often predominate in zooplankton. Diatoms are rich in 14:0, 16:0 and 16:1 fatty acids (Wakeham and Lee, 1989; Hama, 1991; Reitan *et al.*, 1994). The longer chain fatty acids ($C_{24+}$) are characteristic of terrestrial and higher plant organic matter (Currie and Johns, 1989) even though they also contain 16:0-18:0 fatty acids (Matsuda and Koyama, 1977).

Profiles of percentage of saturated, polyunsaturated, monounsaturated and branched fatty acids are shown in Figure 4.2a, b, c and d. Polyunsaturated, monounsaturated and branched fatty acids decrease with depth. Similar patterns have been reported by Lajat *et al.* (1990). The decrease is dependent on chemical structure and thus on the stability of the molecule, where short chain fatty acids degrade more rapidly than long chain compounds, and polyunsaturated fatty acids degrade quicker than monounsaturated fatty acids. When polyunsaturated and monounsaturated fatty acids degrade, they become saturated fatty acids. This explains the increase of saturated fatty acids down the core as shown in Figure 4.2a. The change of monounsaturated fatty acids is greater than the polyunsaturated fatty acids.

Within the fatty acid compounds, correlation was generally high, with 78% of the compound pairs showing an *r* value greater than 0.5 (Table 4.1). Short chain fatty acids such as 14:0, which is abundant in marine phytoplankton correlated most strongly with 13:0, 16:0 and 18:0 saturated acids, with *r* values of 0.95, 0.97 and 0.93 respectively. Monounsaturated 16:1$\omega$7 acid, correlated strongly with short chain saturated acids like 12:0, 13:0, 14:0 and 16:0 acids, with *r* values of 0.91, 0.93, 0.96 and 0.98 respectively.

Table 4.1: Coefficients of correlation between fatty acids in the Conwy core

| | 12:0 | 13:0 | br14:0 | 14:0 | iso15:0 | ante15:0 | 15:0 | br16:0 | 16:0 | 16:1ω11 | 16:1ω9 | 16:1ω7 | iso17:0 | 16:1ω5 | ante17:0 | 17:0 | 16:2 | 17:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13:0 | 0.82*** | | | | | | | | | | | | | | | | | |
| br14:0 | 0.85*** | 0.91*** | | | | | | | | | | | | | | | | |
| 14:0 | 0.86*** | 0.95*** | 0.86*** | | | | | | | | | | | | | | | |
| iso15:0 | 0.83*** | 0.81*** | 0.90*** | 0.79*** | | | | | | | | | | | | | | |
| ante15:0 | 0.83*** | 0.87*** | 0.97*** | 0.85*** | 0.96*** | | | | | | | | | | | | | |
| 15:0 | 0.82*** | 0.82*** | 0.83*** | 0.79*** | 0.81*** | 0.84*** | | | | | | | | | | | | |
| br16:0 | 0.84*** | 0.94*** | 0.90*** | 0.95*** | 0.83*** | 0.88*** | 0.90*** | | | | | | | | | | | |
| 16:0 | 0.85*** | 0.95*** | 0.86*** | 0.97*** | 0.80*** | 0.85*** | 0.81*** | 0.96*** | | | | | | | | | | |
| 16:1ω11 | 0.34 | 0.61** | 0.69*** | 0.54** | 0.44 | 0.62** | 0.36 | 0.54** | 0.50* | | | | | | | | | |
| 16:1ω9 | 0.86*** | 0.76*** | 0.82*** | 0.80*** | 0.75*** | 0.80*** | 0.82*** | 0.83*** | 0.81*** | 0.51* | | | | | | | | |
| 16:1ω7 | 0.91*** | 0.93*** | 0.88*** | 0.96*** | 0.85*** | 0.88*** | 0.86*** | 0.97*** | 0.98*** | 0.47 | 0.88*** | | | | | | | |
| iso17:0 | 0.95*** | 0.87*** | 0.92*** | 0.91*** | 0.89*** | 0.91*** | 0.84*** | 0.90*** | 0.88*** | 0.51* | 0.88*** | 0.93*** | | | | | | |
| 16:1ω5 | 0.79*** | 0.79*** | 0.93*** | 0.79*** | 0.80*** | 0.89*** | 0.76*** | 0.83*** | 0.78*** | 0.77*** | 0.90*** | 0.83*** | 0.88*** | | | | | |
| ante17:0 | 0.87*** | 0.90*** | 0.88*** | 0.94*** | 0.86*** | 0.88*** | 0.83*** | 0.96*** | 0.96*** | 0.48 | 0.84*** | 0.98*** | 0.91*** | 0.83*** | | | | |
| 17:0 | 0.90*** | 0.78*** | 0.89*** | 0.83*** | 0.82*** | 0.86*** | 0.84*** | 0.88*** | 0.83*** | 0.53** | 0.95*** | 0.90*** | 0.93*** | 0.94*** | 0.89*** | | | |
| 16:2 | 0.78*** | 0.83*** | 0.91*** | 0.75*** | 0.89*** | 0.89*** | 0.84*** | 0.83*** | 0.76*** | 0.55** | 0.72*** | 0.79*** | 0.85*** | 0.80*** | 0.79*** | 0.78*** | | |
| 17:1 | 0.87*** | 0.65*** | 0.81*** | 0.71*** | 0.84*** | 0.83*** | 0.85*** | 0.80*** | 0.72*** | 0.34 | 0.87*** | 0.82*** | 0.88*** | 0.84*** | 0.84*** | 0.93*** | 0.78*** | |
| 16:3 | 0.84*** | 0.70*** | 0.81*** | 0.70*** | 0.87*** | 0.85*** | 0.86*** | 0.75*** | 0.71*** | 0.31 | 0.82*** | 0.80*** | 0.83*** | 0.80*** | 0.78*** | 0.87*** | 0.81*** | 0.91*** |
| 18:0 | 0.88*** | 0.86*** | 0.83*** | 0.93*** | 0.80*** | 0.82*** | 0.73*** | 0.88*** | 0.92*** | 0.51** | 0.89*** | 0.94*** | 0.93*** | 0.84*** | 0.92*** | 0.90*** | 0.73*** | 0.78*** |
| 16:3 | 0.88*** | 0.84*** | 0.90*** | 0.88*** | 0.88*** | 0.92*** | 0.81*** | 0.90*** | 0.88*** | 0.55** | 0.89*** | 0.93*** | 0.92*** | 0.89*** | 0.94*** | 0.91*** | 0.80*** | 0.88*** |
| 18:1ω9 | 0.34 | 0.52** | 0.34 | 0.43 | 0.27 | 0.31 | 0.65*** | 0.54** | 0.53** | 0.11 | 0.47 | 0.50* | 0.34 | 0.31 | 0.42 | 0.40 | 0.38 | 0.34 |
| 18:1ω7 | 0.93*** | 0.83*** | 0.86*** | 0.90*** | 0.86*** | 0.85*** | 0.79*** | 0.88*** | 0.89*** | 0.38 | 0.89*** | 0.95*** | 0.96*** | 0.83*** | 0.94*** | 0.93*** | 0.77*** | 0.87*** |
| 18:2ω6 | 0.84*** | 0.78*** | 0.79*** | 0.79*** | 0.76*** | 0.76*** | 0.85*** | 0.83*** | 0.82*** | 0.35 | 0.89*** | 0.88*** | 0.84*** | 0.80*** | 0.82*** | 0.90*** | 0.72*** | 0.82*** |
| 18:2 | 0.06 | 0.37 | 0.49 | 0.23 | 0.29 | 0.42 | 0.10 | 0.26 | 0.22 | 0.84*** | 0.11 | 0.16 | 0.20 | 0.48 | 0.19 | 0.21 | 0.43 | 0.06 |
| 18:3ω3 | 0.73*** | 0.78*** | 0.68*** | 0.77*** | 0.59** | 0.64** | 0.83*** | 0.84*** | 0.84*** | 0.29 | 0.75*** | 0.86*** | 0.68*** | 0.65*** | 0.82*** | 0.76*** | 0.60** | 0.68*** |
| 20:0 | 0.74*** | 0.79*** | 0.60** | 0.89*** | 0.57** | 0.59** | 0.62** | 0.80*** | 0.90*** | 0.23 | 0.68*** | 0.87*** | 0.73*** | 0.52** | 0.84*** | 0.65*** | 0.48 | 0.53** |
| 21:0 | 0.41 | 0.67*** | 0.37 | 0.71*** | 0.32 | 0.37 | 0.45 | 0.64** | 0.74*** | 0.18 | 0.36 | 0.64** | 0.41 | 0.25 | 0.61** | 0.32 | 0.30 | 0.17 |
| 22:0 | 0.50* | 0.65*** | 0.40 | 0.72*** | 0.37 | 0.40 | 0.43 | 0.63** | 0.75*** | 0.12 | 0.38 | 0.66*** | 0.46 | 0.26 | 0.66 | 0.35 | 0.31 | 0.24 |
| 22:1ω11 | 0.83*** | 0.80*** | 0.83*** | 0.83*** | 0.90*** | 0.88*** | 0.87*** | 0.90*** | 0.87*** | 0.34 | 0.83*** | 0.92*** | 0.86*** | 0.78*** | 0.94*** | 0.86*** | 0.79*** | 0.88*** |
| 20:5ω3 | 0.65*** | 0.84*** | 0.91*** | 0.80*** | 0.78*** | 0.89*** | 0.70*** | 0.85*** | 0.79*** | 0.86*** | 0.71*** | 0.78*** | 0.78*** | 0.90*** | 0.81*** | 0.78*** | 0.81*** | 0.68*** |
| 24:0 | 0.64** | 0.71*** | 0.54** | 0.75*** | 0.57** | 0.54** | 0.56** | 0.70*** | 0.81*** | 0.21 | 0.62** | 0.76*** | 0.66*** | 0.50* | 0.72*** | 0.60** | 0.50* | 0.46 |
| 25:0 | 0.62** | 0.66*** | 0.48 | 0.75*** | 0.50* | 0.49 | 0.49 | 0.67*** | 0.78*** | 0.15 | 0.48 | 0.71*** | 0.61** | 0.37 | 0.70*** | 0.48 | 0.42 | 0.40 |

| | 16:3 | 18:0 | 16:3 | 18:1ω9 | 18:1ω7 | 18:2ω6 | 18:2 | 18:3ω3 | 20:0 | 21:0 | 22:0 | 22:1ω11 | 20:5ω3 | 24:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18:0 | 0.74*** | | | | | | | | | | | | | |
| 16:3 | 0.80*** | 0.88*** | | | | | | | | | | | | |
| 18:1ω9 | 0.44 | 0.39 | 0.30 | | | | | | | | | | | |
| 18:1ω7 | 0.83*** | 0.96*** | 0.91*** | 0.35 | | | | | | | | | | |
| 18:2ω6 | 0.89*** | 0.87*** | 0.78*** | 0.61** | 0.89*** | | | | | | | | | |
| 18:2 | 0.11 | 0.16 | 0.22 | -0.10 | 0.08 | 0.06 | | | | | | | | |
| 18:3ω3 | 0.70*** | 0.72*** | 0.71*** | 0.70*** | 0.74*** | 0.83*** | 0.05 | | | | | | | |
| 20:0 | 0.51** | 0.85*** | 0.69*** | 0.47 | 0.81*** | 0.71*** | -0.06 | 0.78*** | | | | | | |
| 21:0 | 0.20 | 0.56** | 0.41 | 0.57** | 0.46 | 0.44 | 0.004 | 0.67*** | 0.86*** | | | | | |
| 22:0 | 0.23 | 0.60** | 0.46 | 0.39 | 0.55** | 0.43 | -0.04 | 0.66*** | 0.90*** | 0.94*** | | | | |
| 22:1ω11 | 0.88*** | 0.83*** | 0.91*** | 0.44 | 0.90*** | 0.84*** | 0.08 | 0.79*** | 0.73*** | 0.48 | 0.53** | | | |
| 20:5ω3 | 0.62** | 0.72*** | 0.84*** | 0.27 | 0.69*** | 0.61** | 0.65*** | 0.59** | 0.49 | 0.36 | 0.32 | 0.72*** | | |
| 24:0 | 0.51* | 0.80*** | 0.58** | 0.60** | 0.74*** | 0.69*** | -0.01 | 0.67*** | 0.86*** | 0.76*** | 0.76*** | 0.64** | 0.41 | |
| 25:0 | 0.36 | 0.70*** | 0.52** | 0.40 | 0.65*** | 0.51** | 0.01 | 0.63** | 0.89*** | 0.84*** | 0.90*** | 0.59** | 0.37 | 0.88*** |

| * | p < 0.05 |
|---|---|
| ** | p < 0.01 |
| *** | p < 0.001 |

Meanwhile with polyunsaturated 16:3, 18:2$\omega$6, 18:3$\omega$3, 20:5$\omega$3, 16:1$\omega$7 showed *r* values of 0.93, 0.98, 0.86 and 0.78 respectively. Branched fatty acids have good correlations with each other. For example, *anteiso*-15:0 correlated strongly with *iso*-15:0, *iso*-17:0 and *anteiso*-17:0 with *r* values of 0.96, 0.91 and 0.88 respectively. 18:1$\omega$7 acid has strong positive correlations with branched fatty acids such as *iso*-15:0, *anteiso*-15:0, *iso*-17:0 and *anteiso*-17:0. Correlation coefficients between 18:1$\omega$7 and these acids are 0.86, 0.85, 0.96
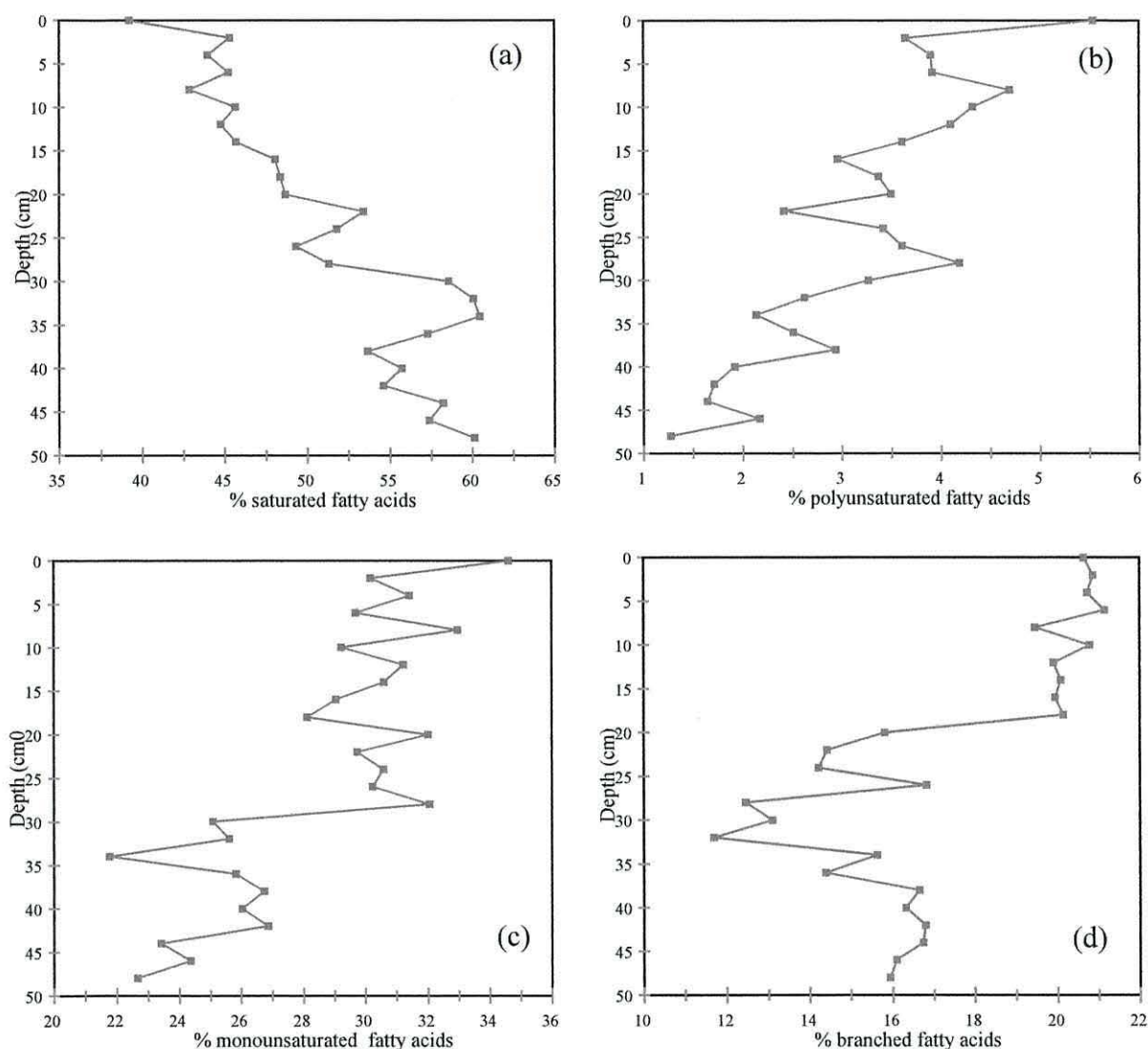


Figure 4.2: Plots of percentage of (a) saturated, (b) polyunsaturated, (c) monounsaturated, and (d) branched fatty acids with depth in the Conwy core.

and 0.94 respectively. Long chain saturated 24:0 acid correlated strongly with 25:0 acids with *r* value of 0.88.

Branched fatty acids, principally *iso* and *anteiso* odd chain compounds are found in significant abundance. These compounds are known to be present in bacteria (Wakeham and Ertel, 1988; Wakeham and Canuel, 1990; Mudge *et al.*, 1998). Figure 4.2d shows that branched fatty acids decrease down the core at 18-20 cm, suggesting that microbial activity is high between the surface and 18-20 cm. It may be caused by an increased availability of food. The profile of odd/even ratio (Figure 4.3) shows a similar distribution as the
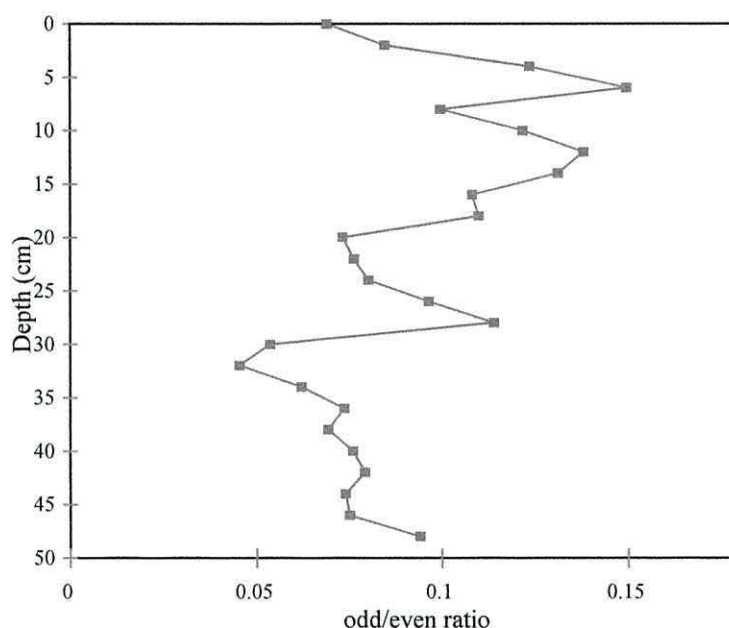


Figure 4.3: Change in the odd/even ratio of fatty acids throughout the Conwy core

percentage of branched fatty acids (*r*=0.66, p<0.001) supporting their link to bacterial biomass. Odd chain fatty acids are synthesised by bacteria generally while even numbered fatty acids are the products of the Kreb's Cycle where the chain is elongated in 2 carbon sub-units (Parkes, 1987). Most of the fatty acids occurring naturally in plants and animals have predominantly even numbers of carbon atoms because they are effectively formed from acetyl ($C_2$) units, which are derived from glucose in the presence of various enzymes, co-enzymes and carrier proteins (Killops and Killops, 1993). This is also known as citric acid cycle, where the propyl units were added by bacteria to form *iso* and *anteiso* branched

116

compounds. In all organisms except bacteria, the Kreb's Cycle is carried out in the matrix of intracellular structures called mitochondria.

The short/long ratios document the importance of marine derived organic matter. The profile of short/long ratio ($\sum$ 12:0-20:0)/($\sum$ 21:0-25:0) with depth is shown in Figure 4.4, where the ratios decrease with depth, showing that the short chain fatty acids were degraded more rapidly than the long chain compounds.
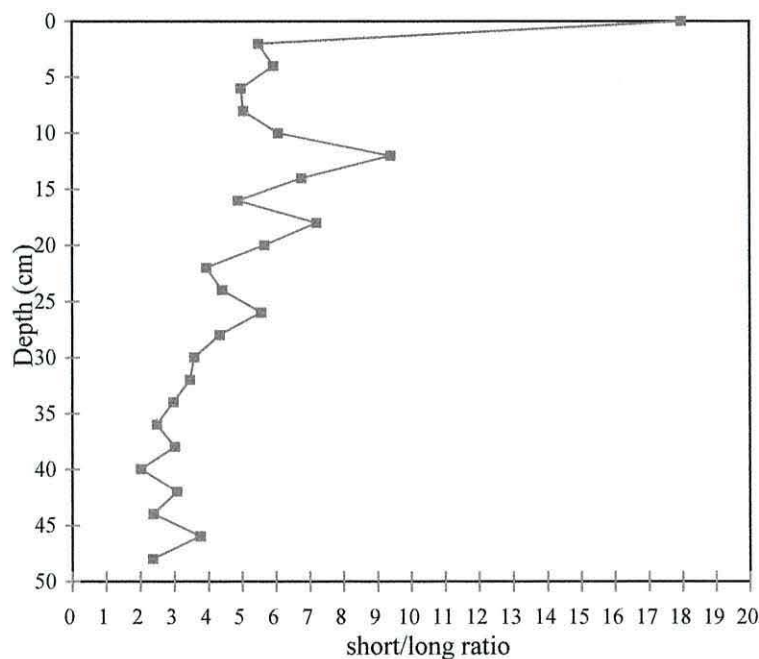


Figure 4.4: Change in the short/long ratio of fatty acids along the Conwy core

Another fatty acid, 18:1ω7 is also used as biomarker for bacteria activity (Bradshaw *et al.*, 1991). Figure 4.5 shows the profile of 18:1ω7/18:0 ratios throughout the core. The profile is similar with profiles of percentage of branched fatty acids ($r$=0.50, p<0.05) and odd/even ratio ($r$=0.62, p<0.01) reinforcing its link to bacterial activity.
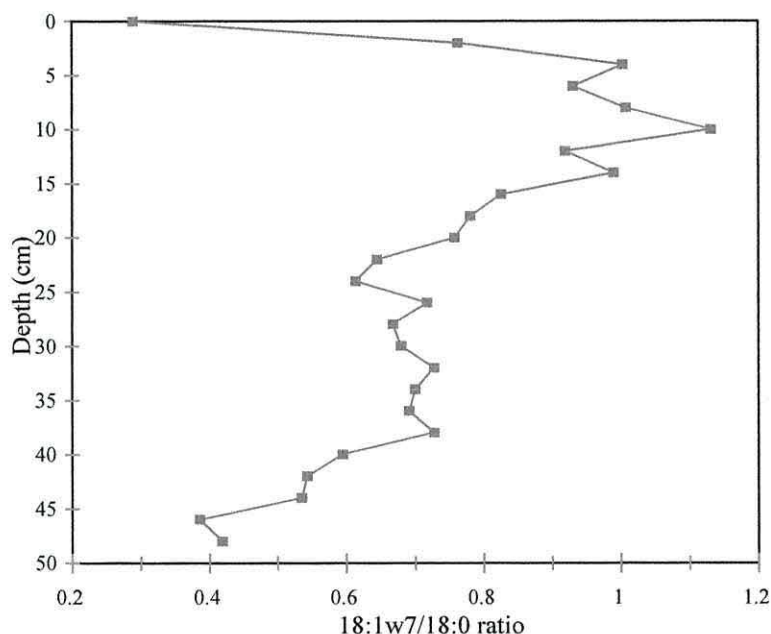
Figure 4.5: Profile of 18:1ω7/18:0 ratios with depth in the Conwy core

### 4.2.2 Fatty alcohols

Normal alcohols were found in the carbon range from 14 through 29 with a maximum at $C_{16}$ and a secondary maximum at $C_{20}$ (Appendix 5). Phytol and an unsaturated $C_{20}$ were also present. Short chain alcohols are often used as marine indicators, while longer chain alcohols are indicators of terrestrial plant waxes (Mudge and Norris, 1997; Mudge and Lintern, 1999). The distribution between long and short chain fatty alcohols changes between depth with more short chain fatty alcohols near the surface and longer chain fatty alcohols at the bottom of the core. Figure 4.6 shows the mean chain length of fatty alcohols throughout the core. The chain length increases down the core.
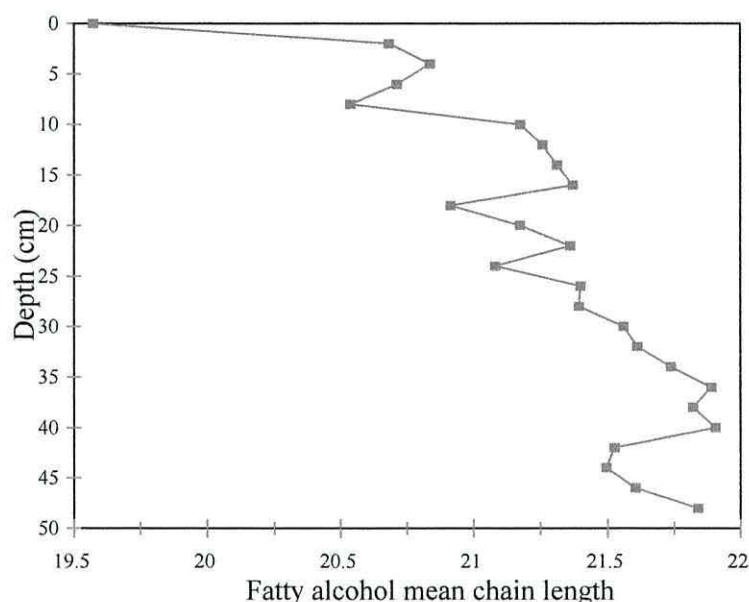
Figure 4.6: Profile of fatty alcohol mean chain length with depth in the Conwy core

Compared to fatty acids, correlation within the fatty alcohols found in the Conwy Estuary was low. Only 42% of the compound pairs have an $r$ value greater than 0.5 (Table 4.2). Saturated $C_{16}$ was prominent in the upper section of the core correlated most strongly with zooplankton monounsaturated fatty alcohol (20:1) with $r$ value of 0.97. With $C_{14}$ and $C_{22}$, $C_{16}$ showed $r$ values of 0.67 and 0.86 respectively. Branched fatty alcohols, such as *anteiso*-$C_{15}$ were correlated strongly with *iso*-$C_{15}$, *iso*-$C_{17}$ and *anteiso*-$C_{17}$ with $r$ values of 0.92, 0.70 and 0.63 respectively. *Anteiso*-$C_{15}$ was also correlated strongly with odd chain length fatty alcohols such as $C_{15}$ and $C_{17}$ with $r$ values of 0.92 and 0.86 respectively. Interestingly $C_{18}$ was not strongly correlated with short chain fatty alcohols but was correlated strongly with long chain compounds. For example, with $C_{20}$, $C_{24}$ and $C_{26}$ saturated alcohols; the correlation coefficients were 0.93, 0.89 and 0.78 respectively. $C_{22}$ has positive correlations with both short and long chain fatty alcohols. Meanwhile $C_{24}$ correlated strongly with $C_{18}$, $C_{20}$, $C_{25}$, $C_{26}$ and $C_{27}$ with $r$ values of 0.89, 0.85, 0.79, 0.78 and 0.62 respectively. Phytol has positive correlations with short chain alcohols such as

Table 4.2: Coefficients of correlation between fatty alcohols in the Conwy core

| | C14 | isoC15 | anteC15 | C15 | C16 | isoC17 | anteC17 | C17 | C18 | isoC19 | C19 | C20 | C21 | C22 | C23 | C24 | C25 | C26 | C27 | C28 | C29 | Phytol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| isoC15 | 0.73** | | | | | | | | | | | | | | | | | | | | | |
| anteC15 | 0.82** | 0.92** | | | | | | | | | | | | | | | | | | | | |
| C15 | 0.72** | 0.99** | 0.92** | | | | | | | | | | | | | | | | | | | |
| C16 | 0.67** | 0.96** | 0.88** | 0.97** | | | | | | | | | | | | | | | | | | |
| isoC17 | 0.49 | 0.47 | 0.70** | 0.46 | 0.47 | | | | | | | | | | | | | | | | | |
| anteC17 | 0.43 | 0.38 | 0.63* | 0.36 | 0.38 | 0.88** | | | | | | | | | | | | | | | | |
| C17 | 0.83** | 0.94** | 0.86** | 0.93** | 0.90** | 0.36 | 0.27 | | | | | | | | | | | | | | | |
| C18 | -0.11 | -0.10 | -0.21 | -0.08 | 0.01 | -0.34 | -0.41 | -0.01 | | | | | | | | | | | | | | |
| isoC19 | 0.70** | 0.60* | 0.80** | 0.59* | 0.62* | 0.86** | 0.79** | 0.52* | -0.30 | | | | | | | | | | | | | |
| C19 | 0.18 | 0.55 | 0.42 | 0.59* | 0.67** | 0.10 | -0.06 | 0.45 | 0.46 | 0.21 | | | | | | | | | | | | |
| C20 | -0.16 | -0.07 | -0.24 | -0.05 | 0.02 | -0.44 | -0.52* | -0.01 | 0.93** | -0.36 | 0.53* | | | | | | | | | | | |
| C21 | 0.33 | 0.44 | 0.23 | 0.43 | 0.48 | -0.23 | -0.35 | 0.57* | 0.67** | -0.11 | 0.56* | 0.68** | | | | | | | | | | |
| C22 | 0.57* | 0.81** | 0.65** | 0.82** | 0.86** | 0.13 | 0.02 | 0.83** | 0.46 | 0.30 | 0.77** | 0.48 | 0.82** | | | | | | | | | |
| C23 | 0.46 | 0.44 | 0.38 | 0.44 | 0.44 | 0.05 | -0.08 | 0.49 | 0.66** | 0.17 | 0.50* | 0.64* | 0.78** | 0.74** | | | | | | | | |
| C24 | -0.32 | -0.40 | -0.48 | -0.38 | -0.31 | -0.52* | -0.54* | -0.30 | 0.89** | -0.49 | 0.21 | 0.85** | 0.52* | 0.16 | 0.56* | | | | | | | |
| C25 | -0.52* | -0.28 | -0.44 | -0.25 | -0.16 | -0.47 | -0.56* | -0.35 | 0.72** | -0.50* | 0.53* | 0.82** | 0.40 | 0.20 | 0.32 | 0.75** | | | | | | |
| C26 | -0.04 | -0.26 | -0.31 | -0.25 | -0.14 | -0.44 | -0.45 | -0.07 | 0.78** | -0.37 | 0.21 | 0.73** | 0.65** | 0.28 | 0.50* | 0.78** | 0.55* | | | | | |
| C27 | -0.62* | -0.47 | -0.62* | -0.44 | -0.34 | -0.61* | -0.63* | -0.50* | 0.49 | -0.59* | 0.33 | 0.62* | 0.20 | -0.06 | 0.03 | 0.62* | 0.87** | 0.44 | | | | |
| C28 | -0.36 | -0.47 | -0.46 | -0.47 | -0.39 | -0.19 | -0.26 | -0.40 | 0.39 | -0.34 | 0.09 | 0.45 | 0.12 | -0.12 | 0.02 | 0.48 | 0.51* | 0.56* | 0.48 | | | |
| C29 | -0.51* | -0.38 | -0.52* | -0.36 | -0.30 | -0.50* | -0.51* | -0.41 | 0.20 | -0.49 | 0.18 | 0.38 | 0.08 | -0.13 | -0.11 | 0.39 | 0.68** | 0.22 | 0.86*** | 0.39 | | |
| Phytol | 0.70** | 0.68** | 0.84** | 0.66** | 0.64* | 0.85** | 0.80** | 0.62* | -0.37 | 0.82** | 0.07 | -0.47 | -0.05 | 0.33 | 0.18 | -0.57* | -0.63* | -0.39 | -0.74** | -0.37 | -0.62* | |
| 20:1 | 0.62* | 0.96** | 0.86** | 0.97** | 0.97** | 0.40 | 0.31 | 0.88** | 0.02 | 0.56* | 0.71** | 0.06 | 0.46 | 0.86** | 0.43 | -0.30 | -0.11 | -0.20 | -0.29 | -0.40 | -0.24 | 0.55* |

\* $p < 0.01$
\*\* $p < 0.001$

$C_{14}$ and $C_{16,}$ as well as with monounsaturated 20:1 with $r$ values of 0.70, 0.64 and 0.55 respectively.
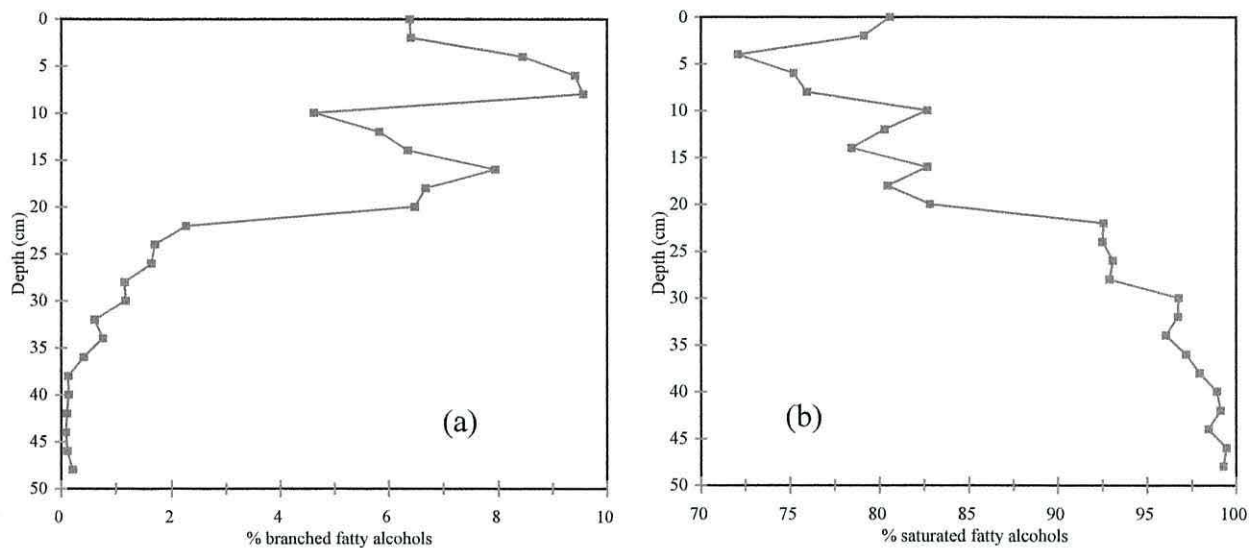


Figure 4.7: Profiles of (a) percentage of branched and (b) saturated fatty alcohols with depth in the Conwy core

Percentage of branched fatty alcohols decreases significantly from 22-24 cm down the core (Figure 4.7a). This result corresponds to percentage of saturated fatty alcohols, which started to increase from the same depth (Figure 4.7b). Branched fatty alcohols are associated with bacterial inputs (Parkes, 1987) and the sub-surface maxima at 4-10 cm probably reflect an availability of food source without differentiating between a marine, *in situ* or terrigenous origin.

Fukushima and Ishiwatari (1984) used the L/H ratio on the assumption that chain length distributions may reflect the input of fatty alcohols from particular source organisms. The short/long ratio $(\sum C_{14}\text{-}C_{20})/(\sum C_{21}\text{-}C_{29})$ is below 1.0 throughout the core except at 0-2 cm and 8-10 cm (Figure 4.8). The ratios indicate that the longer chain compounds were more predominant than the shorter chain compounds. This may be due to the degradation of the shorter length compounds. This is supported by the percentage of branched fatty alcohols, which is high at the 0-20 cm depth showing high microbial activity.
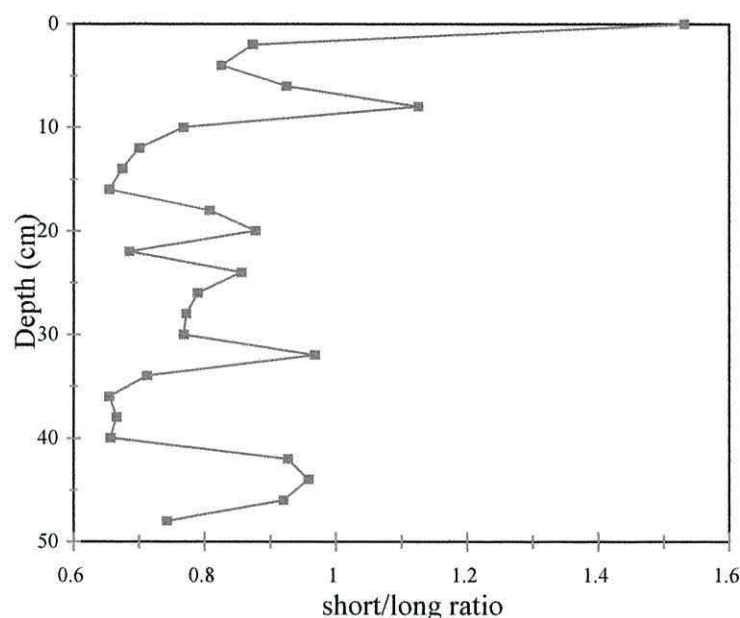
Figure 4.8: Profile of short/long ratio of fatty alcohols throughout the Conwy core

Alcohol Source Index (ASI), which is an index proposed to describe the degree of influence of terrestrial organic matter with marine sediments (Mudge and Norris, 1997) was calculated as:

$$ASI = \frac{\text{concentration of terrestrial fatty alcohol}}{\text{concentration of marine fatty alcohol}}$$

It may be hypothesised that the ratio would increase with depth. $C_{14}$ and $C_{16}$ were proposed as the marine fatty alcohols, whilst $C_{22}$ and $C_{24}$ could be assumed to be of terrestrial origin. $C_{16}$ appears to be the strongest marine marker. For example, the $C_{24}/C_{16}$ ratio increased by a factor of 49, whilst $C_{24}/C_{14}$ ratio increased by a factor of 28. $C_{24}$ appears to be the strongest terrestrial marker. The $C_{24}/C_{14}$ ratio increases by a factor of 28, whilst $C_{22}/C_{14}$ increased by a factor of 2. Figure 4.9 illustrates all the four ratios with depth in the Conwy core.
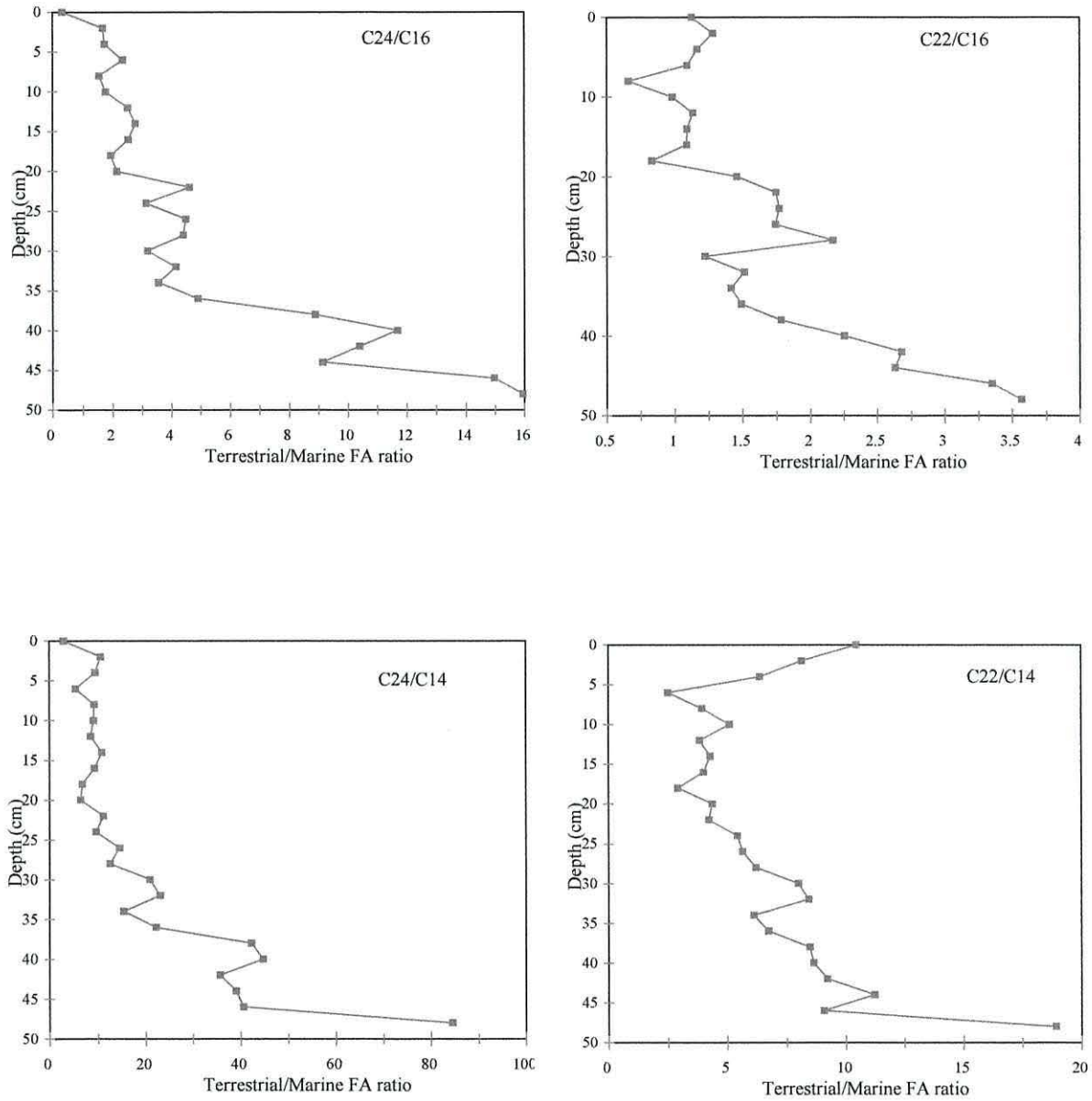
Figure 4.9: Profile of ASIs throughout the Conwy core

**4.2.3 Sterols**

Sterols were also quantified from each sample down the core (Appendix 6). A total of 23 different sterols and stanols were quantified (Table 4.3). The major sterol found was cholesterol, which can be derived from the faeces and carcasses of fish and zooplankton (Gagosian *et al.*, 1983). Cholesterol is sometimes a major sterol in phytoplankton including cyanobacteria (Killops and Killops, 1993). Cholesterol concentration decreases with increasing depth (Figure 4.10). The concentration of other marine sterols show similar trend (Appendix 6) with depth, but sterols from higher plants apparently survives.
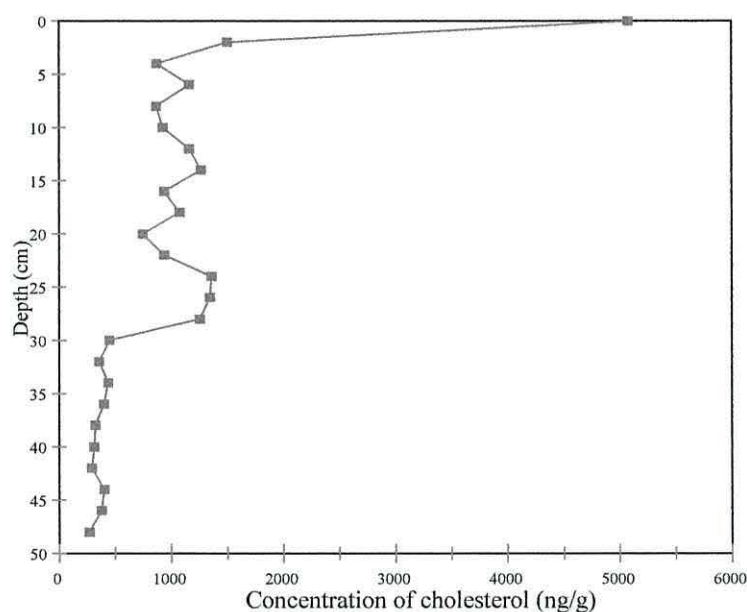


Figure 4.10: Profile of cholesterol concentration with depth in the Conwy core

Within the sterols, only 45% of compound pairs showed an *r* value greater than 0.5 (Table 4.4). Cholesterol, which appeared to be more prominent in the top samples correlated most strongly with coprostanol, 24-norcholesta-5,22(E)-3β-ol, cholestanol, cholesta-5,22(E)-dien-3β-ol and epicoprostanol with *r* values of 0.96, 0.95, 0.93 and 0.92 respectively. These compounds are abundant in marine organisms and in sewage discharges. Within the sewage derived compounds, coprostanol correlated strongly with epicoprostanol and cholestanol with *r* values of 0.88 and 0.87 respectively. The higher plant sterol, β-sitosterol, was strongly correlated with marine, sewage and terrestrial derived compounds

in this study. Meanwhile ergosterol has strong positive correlations with other higher plant sterols. For example, correlation coefficients between ergosterol and campesterol, stigmasterol and β-sitosterol were 0.92, 0.93 and 0.62 respectively.

Table 4.3: Trivial and systematic names of the sterols identified in the Conwy core

| Abbreviation | Systematic name |
| --- | --- |
| st1 | 24-nor-cholesta-5,22(E)-3β-ol |
| st2 | 24-nor-5α(H)-cholest-22(E)-en-3β-ol |
| cop | 5β-cholestan-3β-ol (coprostanol) |
| epi | 5β-cholestan-3α-ol (epicoprostanol) |
| st3 | cholesta-5,22(E)-dien-3β-ol |
| st4 | 5α-(H)-cholest-22(E)-en-3β-ol |
| chol | cholest-5-en-3β-ol (cholesterol) |
| cholest | 5α(H)-cholestan-3β-ol (cholestanol) |
| brass | 24-methylcholesta-5,22-dien-3β-ol (brassicasterol) |
| st5 | 24-methyl-5α(H)-cholest-22(E)-en-3β-ol |
| st6 | 5α(H)-cholest-7-en-3β-ol |
| ergo | ergosta-5,7,22(E)-trien-3β-ol (ergosterol) |
| st7 | 24-methylenecholest-5-en-3β-ol |
| camp | 24-methylcholest-5-en-3β-ol (campesterol) |
| st8 | 24-methyl-5α(H)-cholestan-3β-ol |
| stig | 24-ethylcholesta-5,22(E)-dien-3β-ol (stigmasterol) |
| st9 | 24-ethyl-5α(H)-cholest-22(E)-en-3β-ol |
| st10 | 4α,24-dimethyl-5α(H)-cholest-22(E)-en-3β-ol |
| st11 | 23,24-dimethylcholest-5-en-3β-ol |
| sito | 24-ethylcholest-5-en-3β-ol (sitosterol) |
| st12 | 24-ethyl-5α(H)- cholestan-3β-ol |
| dino | 4α,23,24-trimethyl-5α(H)-cholest-22(E)-en-3β-ol (dinosterol) |
| st13 | 4α,24-dimethyl-5α(H)- cholestan-3β-ol |

The major sterols found in higher plants are β-sitosterol, stigmasterol and campesterol (Volkman, 1986; Laureillard and Saliot, 1993). Another sterol, ergosterol is found in terrestrial decomposing fungi (Weete, 1973). All the four sterols had similar profiles throughout the core with a high concentration at the surface. This high concentrations may be from the fresh organic material from terrestrial plants and algal and decrease rapidly due to diagenetic processes.

Table 4.4: Coefficients of correlation between sterols in the Conwy core

| | st1 | st2 | cop | epi | st3 | st4 | chol | cholest | brass | st5 | st6 | ergo | st7 | camp | st8 | stig | st9 | st10 | st11 | sito | st12 | st13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| st2 | 0.76** | | | | | | | | | | | | | | | | | | | | | |
| cop | 0.96** | 0.72** | | | | | | | | | | | | | | | | | | | | |
| epi | 0.85** | 0.71** | 0.88** | | | | | | | | | | | | | | | | | | | |
| st3 | 0.80** | 0.67** | 0.83** | 0.97** | | | | | | | | | | | | | | | | | | |
| st4 | 0.54* | 0.81** | 0.52* | 0.47 | 0.41 | | | | | | | | | | | | | | | | | |
| chol | 0.95** | 0.76** | 0.96** | 0.95** | 0.92** | 0.55* | | | | | | | | | | | | | | | | |
| cholest | 0.84** | 0.77** | 0.87** | 0.95** | 0.89** | 0.60* | 0.93** | | | | | | | | | | | | | | | |
| brass | 0.63* | 0.41 | 0.74** | 0.72** | 0.68** | 0.27 | 0.69** | 0.64* | | | | | | | | | | | | | | |
| st5 | 0.09 | 0.54* | 0.09 | 0.37 | 0.40 | 0.29 | 0.23 | 0.40 | 0.01 | | | | | | | | | | | | | |
| st6 | 0.62* | 0.54* | 0.66** | 0.87** | 0.93** | 0.31 | 0.78** | 0.74** | 0.50* | 0.48 | | | | | | | | | | | | |
| ergo | -0.17 | 0.05 | -0.14 | -0.16 | 0.23 | 0.06 | -0.00 | 0.21 | -0.07 | 0.55* | 0.30 | | | | | | | | | | | |
| st7 | -0.24 | -0.14 | -0.21 | -0.19 | 0.28 | -0.10 | -0.04 | 0.08 | -0.05 | 0.33 | 0.42 | 0.52* | | | | | | | | | | |
| camp | 0.10 | 0.27 | 0.10 | 0.41 | 0.47 | 0.24 | 0.27 | 0.47 | 0.05 | 0.65** | 0.49 | 0.92** | 0.50* | | | | | | | | | |
| st8 | 0.13 | 0.17 | 0.19 | 0.50* | 0.59* | 0.08 | 0.33 | 0.47 | 0.22 | 0.53* | 0.66** | 0.77** | 0.54* | 0.79** | | | | | | | | |
| stig | -0.13 | 0.04 | -0.07 | 0.24 | 0.30 | -0.02 | 0.07 | 0.29 | 0.01 | 0.58* | 0.34 | 0.93** | 0.54* | 0.92** | 0.76** | | | | | | | |
| st9 | 0.67** | 0.58* | 0.69** | 0.91** | 0.96** | 0.35 | 0.82** | 0.81** | 0.54* | 0.49 | 0.97** | 0.36 | 0.43 | 0.57* | 0.71** | 0.40 | | | | | | |
| st10 | -0.16 | -0.09 | -0.14 | 0.22 | 0.30 | -0.05 | 0.01 | 0.11 | -0.02 | 0.35 | 0.44 | 0.45 | 0.90** | 0.48 | 0.51* | 0.48 | 0.48 | | | | | |
| st11 | 0.08 | 0.12 | 0.14 | 0.49 | 0.56* | 0.04 | 0.30 | 0.40 | 0.23 | 0.41 | 0.62* | 0.63* | 0.82** | 0.67** | 0.62* | 0.71** | 0.64* | 0.75** | | | | |
| sito | 0.57* | 0.53* | 0.61* | 0.82** | 0.85** | 0.36 | 0.71 | 0.81 | 0.53* | 0.51* | 0.80** | 0.62* | 0.31 | 0.76** | 0.84** | 0.62* | 0.87** | 0.31 | 0.56* | | | |
| st12 | 0.64* | 0.40 | 0.71** | 0.52* | 0.51* | 0.28 | 0.65** | 0.51* | 0.62* | -0.05 | 0.42 | -0.04 | -0.40 | 0.04 | 0.17 | -0.02 | 0.40 | -0.36 | -0.04 | 0.48 | | |
| st13 | -0.13 | -0.18 | -0.07 | -0.05 | 0.11 | -0.14 | -0.02 | 0.04 | 0.04 | 0.12 | 0.12 | 0.48 | 0.37 | 0.37 | 0.44 | 0.49 | 0.15 | 0.23 | 0.42 | 0.31 | 0.16 | |
| dino | 0.55* | 0.52* | 0.54* | 0.77** | 0.80** | 0.26 | 0.62* | 0.69** | 0.40 | 0.49 | 0.78** | 0.43 | 0.45 | 0.57* | 0.60* | 0.43 | 0.82** | 0.42 | 0.64* | 0.76** | 0.12 | 0.22 |

| * | $p < 0.01$ |
|---|---|
| ** | $p < 0.001$ |
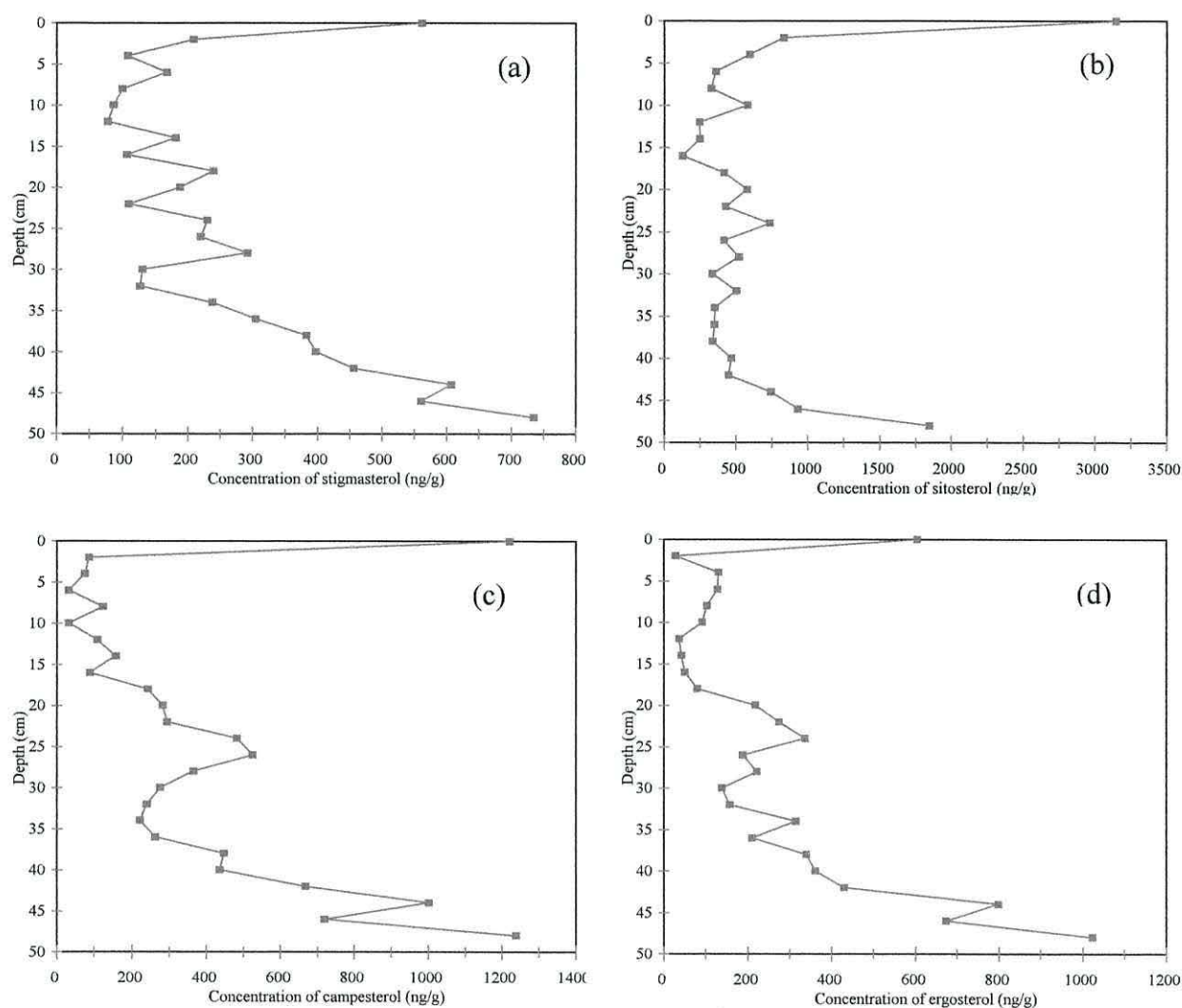
Figure 4.11: Sedimentary depth profiles of (a) stigmasterol, (b) β-sitosterol, (c) campesterol and (d) ergosterol in the Conwy core

decrease below that and started increasing consistently along the core (Figure 4.11a, b, c, d). The effects of diagenesis cannot be overlooked when comparing sediment deposited over an approximately 50 year's time span. The terrigenous sterols present in a plant wax or biopolymer are protected from bacterial degradation. This implies that marine sterols are degraded more rapidly than sterols derived from terrigenous sources in marine sediment as established by earlier studies (Gagosian *et al.*, 1983; Meyers *et al.*, 1984; Volkman *et al.*, 1987).

Sterol source index (SSI) was calculated by assuming cholesterol as marine sterol (Grimalt and Albaiges, 1990):

$$\text{Sterol Source Index} = \frac{\text{terrestrially derived sterol}}{\text{cholesterol}}$$

β-sitosterol, campesterol, stigmasterol and ergosterol were used to calculate this index. The indices were plotted with depth of the core (Figure 4.12). The ergosterol/cholesterol ratio appeared to be the strongest index, increasing by a factor of 32 between the surface and bottom samples. The β-sitosterol/cholesterol, stigmasterol/cholesterol and campesterol/cholesterol ratios increased by factors of 11, 25 and 19 respectively (not shown here). The ergosterol/cholesterol ratio was correlated strongly with $C_{24}/C_{16}$ alcohol
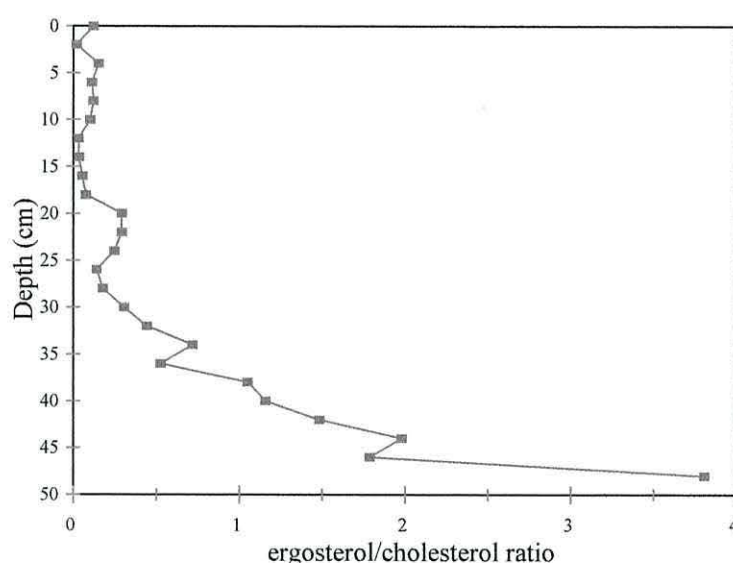


Figure 4.12: SSI profile in the Conwy core with ergosterol/cholesterol ratio

ratio ($r$=0.78, p<0.001) (Figure 4.13), and suggests that SSI can be used as an indicator of terrestrially derived sterols in marine sediment. Ergosterol is the principal sterol in the membrane of the eumycetic fungi, which constitute one of the main groups of mycelial microbial decomposers (Newell and Fell, 1992). Therefore the presence of ergosterol in sediment can be used as indicator of decaying terrestrial matter. Recent studies (Mudge and Norris, 1997; Mudge and Lintern, 1999) have shown that ergosterol can be used to

indicate terrestrial inputs in sediments. The large areas of woodland in this estuary catchment are probably the main source of this sterol.
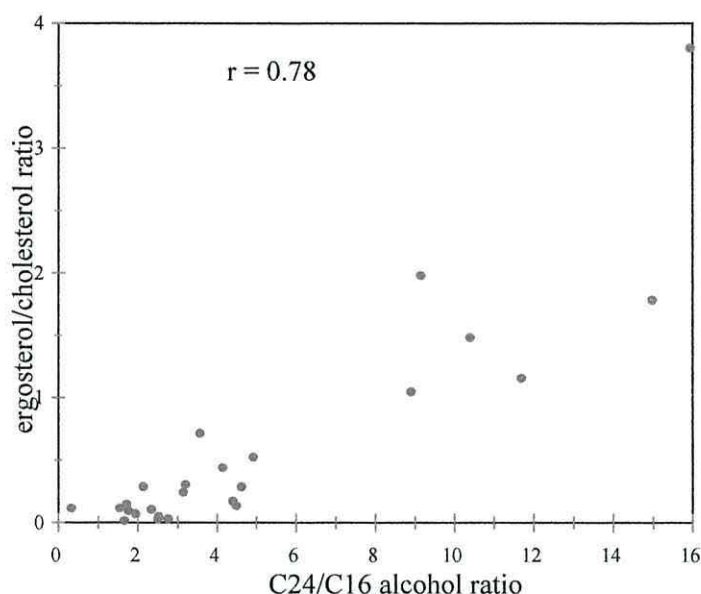


Figure 4.13: The correlation between ASI and SSI in the Conwy core

The River Conwy receives domestic sewage from Conwy town and Dwr Cymru Welsh Water sewage treatment at Trefriw and near Dolgarrog. There are also a number of discharges above the tidal limit at Llanrwst, Betws-y-Coed , Dolwyddelan and Penmachno. The estuary also potentially receives sewage from marine discharged material brought back to shore from the long sea outfall serving Llandudno. Coprostanol is produced in the intestines of mammals (including man) by enteric microbial reduction of cholesterol (Escalona *et al.*, 1980). Therefore the presence of this compound in marine sediments is considered a reliable indicator of sewage pollution (Venkatesan and Kaplan, 1990; Venkatesan and Mirsadeghi, 1992; Sherwin *et al.*, 1993). The coprostanol/cholesterol ratio is often used to indicate sources from sewage materials (Grimalt and Albaiges, 1990; Nichols and Espey, 1991; Mudge and Bebianno, 1997; Mudge and Norris, 1997; Mudge and Lintern, 1999; Mudge *et al.*, 1999). Figure 4.14 shows the profile of coprostanol/cholesterol ratio throughout the core. The ratio was low at the bottom of the core and increases upward from the depth of about 36-40 cm suggests an increasing input

of coprostanol from the past to the sampling time. The tourism in Conwy and Llandudno may have been greater in the Victorian era. However, the coprostanol/cholesterol ratio was low at the bottom of the core, showing that the coprostanol input was low during the past and the ratio increased upward. The sewage treatment was poor during the 1900's and became better in recent years. Therefore, the increase of coprostanol/cholesterol ratios might be because of an increase in population and tourist activities in Conwy and Llandudno.
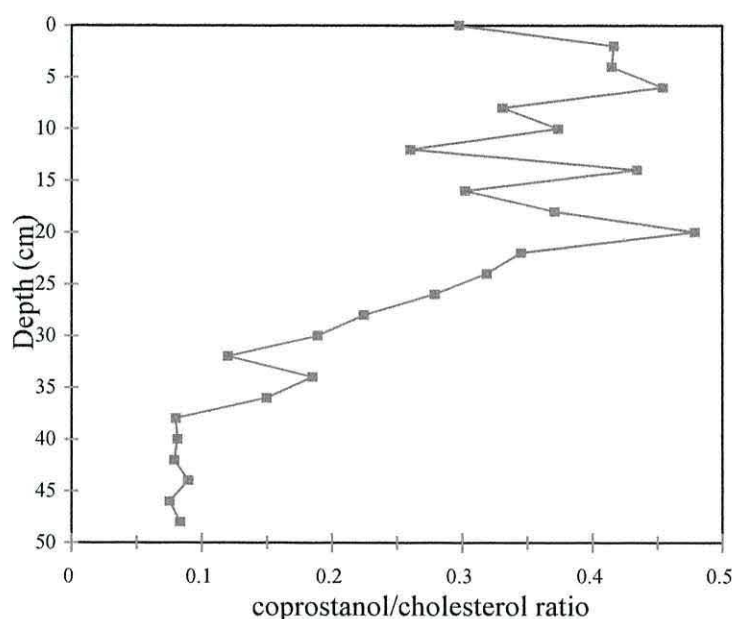


Figure 4.14: Profile of coprostanol/cholesterol ratio with depth throughout the Conwy core

## 4.3 Multivariate statistical analysis

### 4.3.1 Principal component analysis

Principal Component Analysis (PCA) was conducted on individual compound group as well as on mixtures of fatty alcohols, sterols and fatty alcohols.

### 4.3.1.1 Fatty acids

The loadings on PC1 (34.6%) and PC2 (14.8%) can be seen in Figure 4.15a. Compounds which are phytoplankton markers such as 16:1$\omega$7 with short chain marine derived fatty acids and bacterial markers such as branched or odd chain length compounds are loaded positively on PC1. On the other hand the long chain moieties are negatively loaded on PC1. From the score plot (Figure 4.15b), the upper part of the core contain the marine and bacterial derived fatty acids, while the bottom part of the core are influenced by terrestrial input fatty acids.
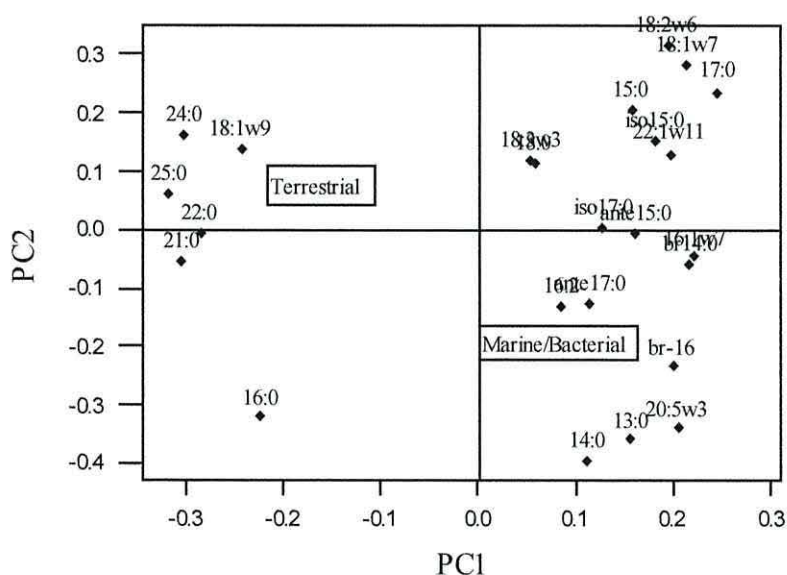


Figure 4.15a: Plot of the first two principal components after PCA of fatty acids in the Conwy core
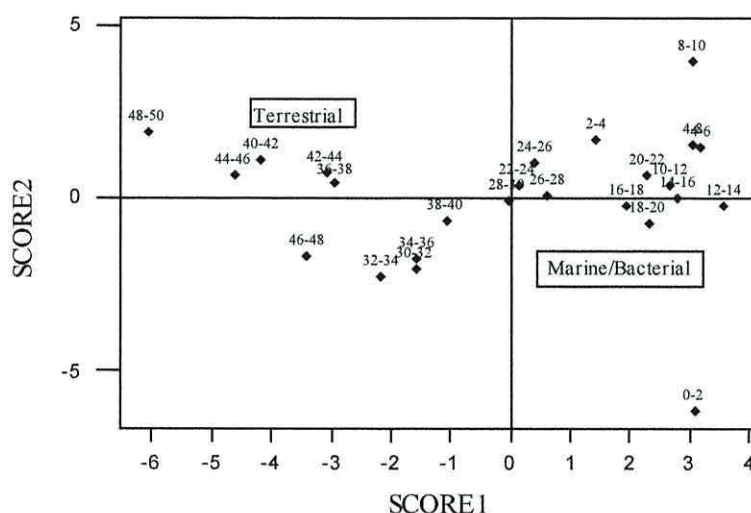
Figure 4.15b: Scores from PCA of fatty acids in the Conwy core

### 4.3.1.2 Fatty alcohols

The loading of each compound on principal component 1 (PC1) and principal component 2 (PC2) are shown in Figure 4.16a and the scores in Figure 4.16b. PC1 and PC2 account for 51.9% and 13.4% of the variance in the data. The data show that the terrestrial alcohols project on the right side with high positive loading, while bacterial derived compounds together with marine derived fatty alcohols clustered at the left side with negative loading. Short chain alcohols are often used as marine indicators and long chain alcohols are indicators for terrestrial plant (Mudge and Norris, 1997). The plot of scores (Figure 4.16b) shows that surface samples contain most of the compounds, corresponds with inputs, which derive in the first instance from the water column. Figure 4.16b also clearly distinguish the core with marine and bacterial input alcohols dominate at the depth of 2-30 cm and terrigenous fatty alcohols dominate the rest of the core. This supports the observations that the ASIs, gave values that indicated the predominance of terrigenous biomarkers at the bottom part of the core. The short/long ratio also shows the same results.
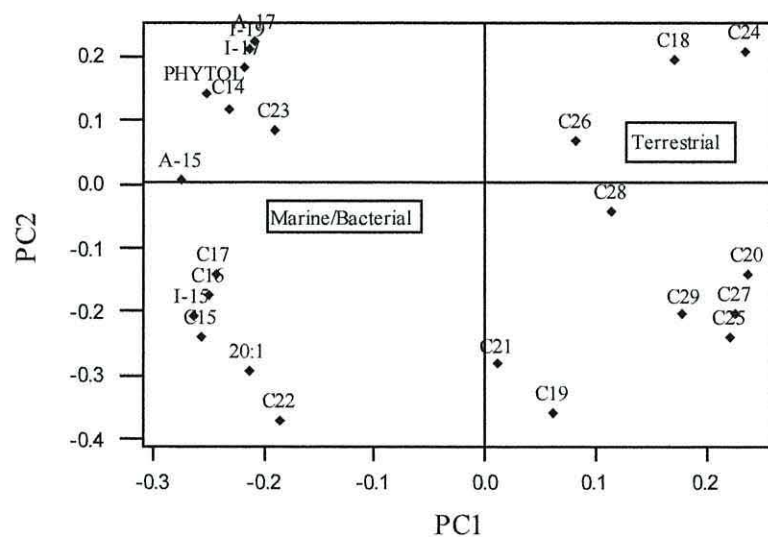
Figure 4.16a: Plot of the two first principal components after PCA of fatty alcohols in the Conwy core
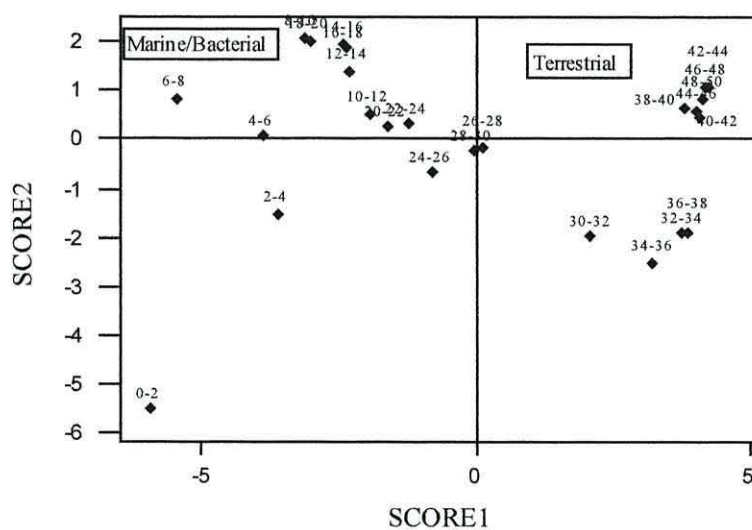


Figure 4.16b: Scores from PCA of fatty alcohols in the Conwy core

### 4.3.1.3 Sterols

Figure 4.17a illustrates the loadings of the sterols in PC1 and PC2, which account for 36.3% and 18.3% respectively, of the variance in the data. PC1 appears to correspond directly to source input with strong negative loadings representing terrigenous derived compounds (ergosterol have the most negative loading, stigmasterol and campesterol) and strong positive loadings representing sewage and marine inputs (coprostanol have the most positive loading, epicoprostanol, cholesterol, st1 and st2). Dinosterol, which is a biomarker for dinoflagellates, is loaded negatively with the terrestrial markers. These results correspond to the PCA applied to fatty acids and fatty alcohols where marine terrestrial gradient related to the depth of the core is identified. It is evident from Figure 4.17b that PC1 separate the core according to the geochemical source of the compounds with terrestrial derived compounds such as β-sitosterol, campesterol, stigmasterol and ergosterol dominating the lower part of the core and marine together with sewage biomarkers (cholesterol, brassicasterol, coprostanol, epicoprostanol, and cholestanol) appear to dominate the upper part of the core.
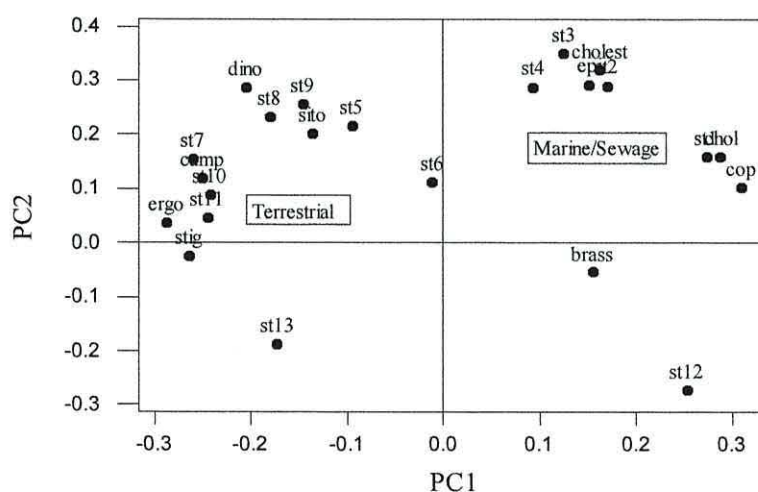


Figure 4.17a: Plot of the first two principal components after PCA of sterols in the Conwy core
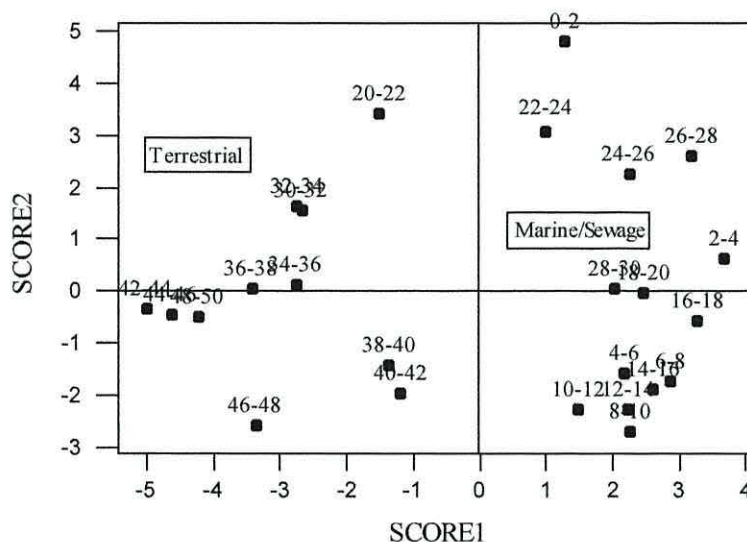
Figure 4.17b: Scores from PCA of sterols in the Conwy core

### 4.3.1.4 Mixed PCA

All data was used to perform PCA with mix compounds of fatty alcohols, fatty acids and sterols.

a) Raw data

Figure 4.18 shows the loadings of each compound, which separated them into three clear groups. All the fatty acids were clustered together with positive loadings, while terrestrial sterols and fatty alcohols were negatively loaded on PC1. Bacterial/sewage derived fatty alcohols and sterols were positively loaded on PC2. Since all the fatty acids are grouped together, the data was then transformed into proportion data.

135
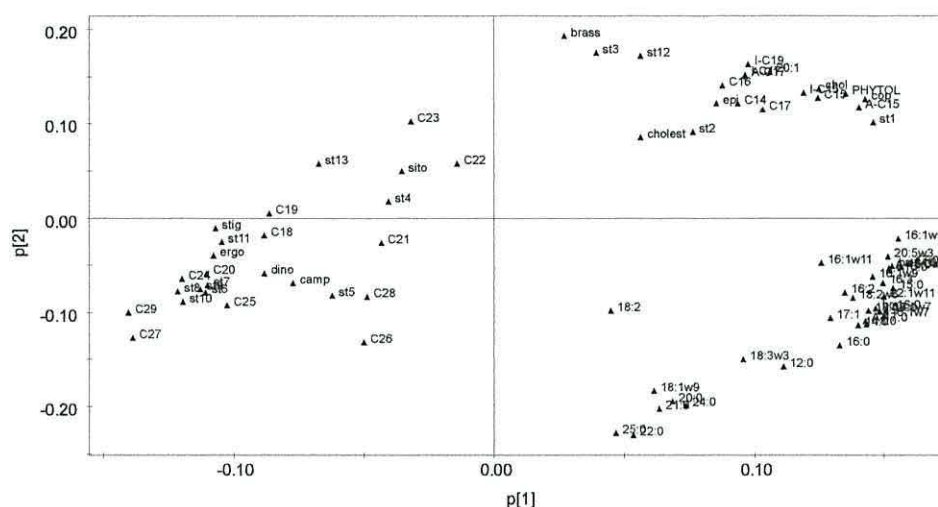
Figure 4.18: Plot of the first two principal components after mixed PCA in the Conwy core with raw data

b) Proportion data without transformation

The loadings of each compound are shown in Figure 4.19. PC1 and PC2 account for 36.7% and 13.6% of the variance in the data set, and reflect important geochemical features. Variables projecting on the left side with negative loadings of Figure 4.19 have a terrigenous source: long chain alcohols, long chain fatty acids and higher plant sterols (stigmasterol and ergosterol). Cholesterol with other type of sterols (st1, st2 and st12) and branched chain fatty alcohols and fatty acids, together with coprostanol are loaded positively on PC1. Cholesterol, st1 and st2 are principally marine sterols. Odd chain fatty acids and fatty alcohols including coprostanol are sewage and bacterial derived markers.
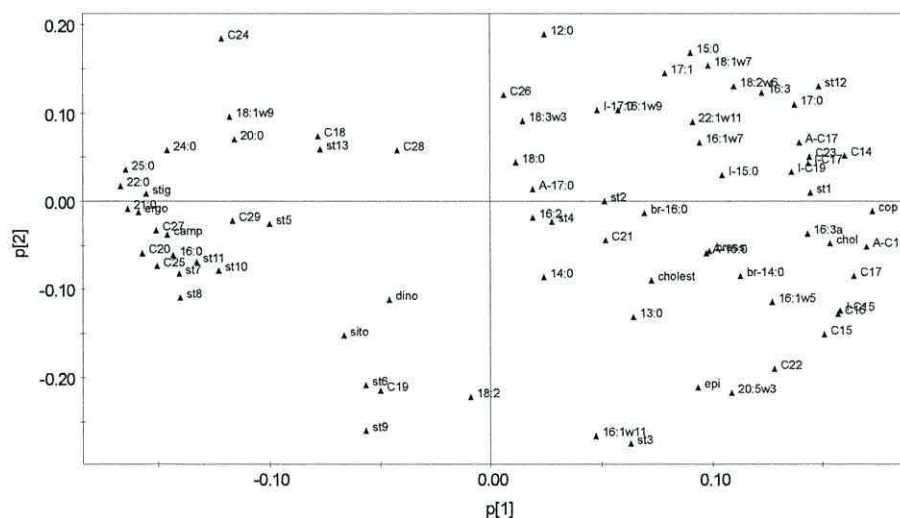


Figure 4.19: Plot of the first two principal components after mixed PCA in the Conwy core with proportion data (no transformation)

When the proportion data was then log transformed, the PCA did not show any advantage in doing so (Figure 4.20). PCA carried out with proportion data (added 0.001 log transformed) showed similar results with PCA performed with proportion data without transformation (Figure 4.21)



Figure 4.20: Plot of the first two principal components after mixed PCA in the Conwy core with proportion data (log transformed)



Figure 4.21: Plot of the first two principal components after mixed PCA in the Conwy core with proportion data added 0.001 (log transformed)

137

Since PCA that carried out with proportion data (no transformation) showed the "best" result, the plot of scores of the PCA is shown in Figure 4.22. The surface sample is totally different from the rest of the core. The upper part of the core (0-28cm) contains marine and bacterial derived organic matter while terrestrial input compounds influence the bottom part of the core.
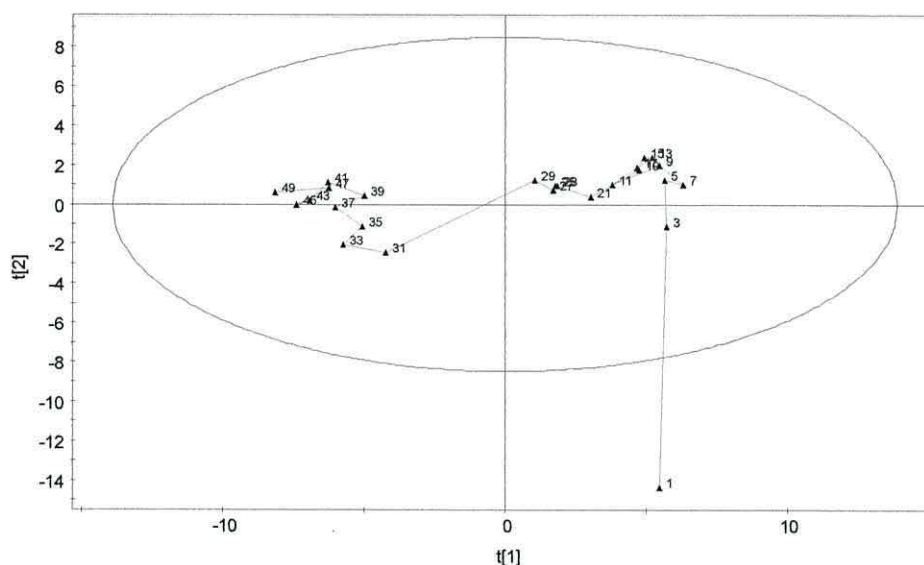


Figure 4.22: Scores from PCA of mixed variables in the Conwy core with proportion data (no transformation)

## 4.4 Discussion

Various marine, terrestrial and bacterial/sewage biomarkers including fatty alcohols, fatty acids and sterols were measured in a sediment core from Conwy Estuary to investigate their temporal variability. The degradation of organic matter in marine sediments can clearly be seen in the core sample from the Conwy Estuary. After sediment deposition at the sea bottom, diagenesis rapidly changes the composition and concentration of organic matter. Diagenetic processes that affect lipid distribution in marine sediments include particle reworking due to digestion of organic matter by benthic fauna, microbial decomposition and abiotic reactions (Sun and Wakeham, 1999). In the core samples, a decrease of short chain fatty acids and fatty alcohols and the increase of the longer chain moieties can be seen. Short chain compounds are less stable and disappear more rapidly

than the longer chain compounds. Therefore general decreasing of short chain fatty acids and fatty alcohols in these samples was probably caused by microbial and chemical degradation during early diagenesis. The increase of long chain compounds may be due to greater preservation of these compounds or greater contributions from terrestrial organic matter in the past.

Terrestrial inputs were mainly characterised by the presence of higher plant sterols (β-sitosterol, campesterol and stigmasterol), long chain fatty alcohols and long chain fatty acids. ASIs and SSIs were calculated to describe the degree of influence of terrestrial organic matter within sediments. In the Conwy core, both ASIs and SSIs increased with depth. Nearly all of the ASI ratios were > 1.0 indicating the dominance of terrestrial fatty alcohols in the core. The core site is near the large area of woodland in this estuary. Hence it might be affected by a direct terrestrial supply. Fluvial input from the River Conwy is an important process in the transport of terrestrial derived compounds into the Conwy Estuary.

β-sitosterol is among the major sterols of higher plants (Laureillard and Saliot, 1993). However, several recent studies (Pearson *et al.*, 2000; Hudson *et al.*, 2001; Matsumoto *et al.*, 2001) have found β-sitosterol to be of marine origin in certain sediments. β-sitosterol predominated over the other two components, stigmasterol and campesterol, which were also found in terrestrial higher plants. Another sterol, ergosterol was also found in the Conwy core. It is a sterol found in terrestrial decomposing fungi (Weete, 1973). The ergosterol/cholesterol ratio appeared to be the strongest SSI index, increased by a factor of 32 between the surface and bottom samples. Mudge and Norris (1997) observed similar distributions in their surface samples collected from the Conwy Estuary.

Brassicasterol has been frequently used as a biomarker of diatoms as it represents over 90% of the sterols in most species of this algal class (Volkman, 1986). Therefore it can describe temporal changes in marine inputs to the sediments provided it survives early diagenesis. Meanwhile, phytol, which is derived from the phytyl chain of chlorophyll, can be used to track down algal inputs through photosynthesis (Ishiwatari *et al.*, 1999). Brassicasterol and phytol show a down core decrease in the Conwy core, which could be

due to a preferential degradation of these compounds during early diagenesis. Terrigenous sterols such as β-sitosterol, campesterol and stigmasterol increase in concentration with depth. Therefore it is apparent that marine sterols have been subjected to more rapid degradation with depth while sterols from higher plants are more resistant. These results were similar to those observed with fatty acids and fatty alcohols.

The fatty acids collected from the Conwy core were predominantly marine in origin. Saturated 16:0 was the major fatty acid found. Short chain fatty acids (saturated and mono/polyunsaturated) compounds are commonly related to a marine source (Carrie *et al.*, 1998; Mudge *et al.*, 1998; Fahl and Stein, 1999). The polyunsaturated fatty acid 20:5ω3 was detected in the Conwy core. Green algae contain abundant C18 polyunsaturated fatty acids especially with bonds in the ω3 and ω6 positions (Volkman *et al.*, 1989). Relatively high concentrations of 18:3ω3 and 18:2ω6 polyunsaturated fatty acids were detected in the Conwy core. The 18:3ω3 fatty acid is typical of dinoflagellates (Colombo *et al.*, 1997). 16:1ω7 has strong correlation with polyunsaturated fatty acids such as 16:3, 18:2ω6, 18:3ω3, 20:5ω3 reinforcing their link to marine input. In the Conwy core, short chain saturated fatty acids and the unsaturated are the major components in the top samples but decrease in deep samples. Short chain fatty acids are less stable and degrade more rapidly than the long chain compounds, and unsaturated acids degrade more than the saturated compounds.

Significant bacterial activity was found in the core through the profile of percentage of branched fatty acids and branched fatty alcohols. The percentage of branched fatty acids and branched fatty alcohols decrease down the core from 22-24 cm and 18-20 cm respectively. These compounds are known to be present in bacteria (Parkes, 1987; Wakeham and Ertel, 1988; Wakeham and Canuel, 1990). This bacterial activity was also confirmed by 18:1ω7 and enhanced odd/even ratios of fatty acid biomarkers. The 18:1ω7 acid has good correlations with branched fatty acids (Table 4.1). The subsurface maxima for branched fatty alcohols and 18:1ω7/18:0 ratios, probably reflects an availability of food sources without differentiating between a marine, *in situ* or terrigenous origin. Therefore these compounds may be produced from post depositional processes carried out by the bacteria.

5β-coprostanol has been used to define the area affected by sewage particles because it is hydrophobic and is associated with sewage particles (Kelly, 1995). 5β-coprostanol is also relatively stable and has been used in sedimentary cores to reconstruct the pollution history (Venkatesan and Kaplan, 1990). Ratios of 5β-coprostanol/cholesterol in the Conwy core range from 0.08-0.48. It is lower than the range of 5β-coprostanol/cholesterol found in the Macao Estuary, southern China (Peng *et al.*, 2002). Ratios of 5β-coprostanol/cholesterol in the Macao Estuary were from 0.3-0.55. Higher range of 5β-coprostanol/cholesterol found here may related to population increase and industrial development in Taipa Island nearby the sampling site. The ratio of 5β-coprostanol/cholesterol was low at the bottom of the Conwy core (Figure 4.14) and started to increase upward from the depth of about 36-40 cm suggesting an increase input of 5β-coprostanol from that point onwards. This depth marks the time when high concentration of 5β-coprostanol began to be deposited in the Conwy Estuary.

Input of organic matter from the land to the ocean via rivers is an important component of the global carbon cycle. It is widely recognised that estuaries act as traps for particles and dissolved material from rivers and the coastal ocean. Major sources of organic matter in Conwy estuarine sediments include terrestrial vascular plants; phytoplankton produced in riverine and coastal waters as well as from the sewage outlets. The quantity and quality of organic matter in the uppermost sediment layers (surface) depend on the supply from different sources, like marine algae or terrestrial plants (Volkman *et al.*, 2000). Organisms like algae, protozoa and bacteria are additional source of organic matter (Harvey and Macko, 1997; Rütters *et al.*, 2002) and most of the early diagenetic transformation and degradation processes of organic matter in the intertidal are mediated by micro-organisms (Killops and Killops, 1993). Percentage of polyunsaturated, monounsaturated and branched fatty acids as well as percentage of branched fatty alcohols decrease down the Conwy core. The decrease in the ratios of short/long homologues of fatty acids and fatty alcohols are also observed with depth. These may be due to microbial degradation in diagenetic process. Meanwhile, percentage of saturated fatty acids and fatty alcohols increase down the core also suggesting the diagenesis of the polyunsaturated, monounsaturated and branched compounds.

Only principal component analysis (PCA) was carried out for the Conwy data. PCA was performed on individual chemical group as well as on the mixture of fatty acids, fatty alcohols and sterols. There are slight differences on the PCA result carried out with the Conwy data compared to the Mawddach Estuary. These can be seen in the single group as well as in the mixed group PCA. In the Conwy PCA the marine and bacterial (sewage) compounds are clustered together while in the Mawddach PCA marine and bacterial compounds were separated clearly. This may be because Mawddach samples were collected spatially and Conwy samples were temporal, where bacteria in the core samples degraded the incoming organic matter.

PCA separates the terrestrial derived fatty acids from the marine and bacterial derived compounds. Short chain and polyunsaturated fatty acids as well as $16:1\omega7$ monounsaturated acids principally have a marine source in the Conwy core. The saturated $16:0$ acids are a major fatty acid in phytoplankton and are ubiquitous in most marine organisms (Carrie *et al.*, 1998). This acid was isolated in the PCA model for the Conwy core. Hence, it is not included for source determination. Long chain fatty acids as well as $18:1\omega9$ were clustered together. $18:1\omega9$, monounsaturated acid is common in animals, higher plants and algae (Killops and Killops, 1993).

Similar results were observed from the PCA carried out with fatty alcohols where PCA separates variables into terrigenous and marine/bacterial sources. Fatty alcohols from wax esters profiles in terrestrial plants and marine zooplankton are different (Sargent *et al.*, 1977; Cranwell and Volkman, 1981; Yunker *et al.*, 1995). Short chain fatty alcohols together with the monounsaturated $20:1$ were clustered together. These compounds are produced abundantly by zooplankton (Sargent *et al.*, 1977; Graeve and Kattner, 1992). Another monounsaturated fatty alcohol, phytol, is a typical algal biomarker as it derives from the side chain of chlorophyll in almost all species of phytoplankton. Phytol was grouped with the marine and bacterial alcohols in the PCA model.

Among sterols, PCA also separates the terrigenous derived compounds from the sewage and marine sterols. Cholesterol, which is present in many marine organisms, was grouped with the marine and sewage derived sterols. Cholesterol is also present in sewage

142

discharges (Volkman, 1986). Therefore, cholesterol in this system can be derived from marine or sewage inputs. In the Conwy core, PCA suggests that ergosterol, stigmasterol and campesterol were the strongest indicators of the terrestrial plant sterols. However, dinosterol, which is a biomarker of dinoflagellates, is clustered with terrestrial markers showing that ergosterol, stigmasterol and campesterol might also originate from marine inputs.

PCAs that have been conducted using proportion data showed clearer separation of the compounds compared to the raw data in the mixed compounds of fatty acids, fatty alcohols and sterols. In the Conwy core, PCA showed the compound separation from the marine and bacterial derived compounds to the terrestrially derived organic matter. However, the separations were not really clear compared to the PCA carried out on the Mawddach data. Saturated 16:0 acid and C18 fatty alcohol were clustered together with the terrestrial derived compounds such as long chain fatty acids and fatty alcohols as well as the plant sterols: ergosterol, stigmasterol and campesterol. These compounds were abundant in marine organisms. Based on the PCA model, cholesterol with st1 (24-nor-cholesta-5,22(E)-3β-ol) and st2 (24-nor-5α(H)-cholest-22(E)-en-3β-ol) have principally marine sources in the Conwy core. Branched chain fatty acids and fatty alcohols including coprostanol are sewage and bacterial derived markers. These compounds are clustered together with marine derived compounds.

### 4.5 Conclusions

Diagenetic processes occur in the core sediment of Conwy Estuary. It can be seen from the profiles of biomarkers, which decrease with depth. The upper part of the core is characterised by marine and bacterial biomarkers with high concentration of short chain fatty alcohols, fatty acids and sterols. The lower part of the core is characterised by increasing dominance of terrestrially derived materials. This is clearly shown by SSIs (especially ergosterol/cholesterol ratio), ASIs and short/long ratio. It is likely that the inputs have not change much with time. Therefore, the change in signature was seen in the Conwy core and not a change in source. The coprostanol/cholesterol ratio shows that the sewage derived materials increase from bottom to top of the core suggesting the increasing input of sewage from past until now. PCA confirmed these observations with PC1 and PC2

indicated biogeochemical sources. PC diagrams show two separated regions. Therefore, terrestrial markers are clearly separated from marine and bacterial markers. Meanwhile, PCA conducted with proportion data without any transformation shows the clear separation of compounds. The upper part of the core contains the marine and bacterial (sewage) derived compounds while terrestrial input compounds influence the bottom part of the core.

**CHAPTER 5: GRAB SAMPLES FROM THE CLYDE SEA**

**5.1 Introduction**

Lipids diversity and specificity makes them useful compounds to study organic matter sources in marine environments. Therefore their characterisation, both at individual and group level, has been one of the main objectives in marine organic geochemistry (Wakeham and Lee, 1989). Fatty acids are among the most abundant biomarkers, and have been used as sediment biomarkers by many researchers (Scribe *et al.*, 1991, Colombo *et al.*, 1997; Mudge *et al.*, 1998; Carrie *et al.*, 1998). Sterols are among the most specific and diverse lipid biomarkers and have been successfully used as biomarkers (Volkman, 1986; Saliot *et al.*, 1991). Another group of compounds are fatty alcohols. Fatty alcohols in marine sediments are primarily derived from wax esters (Sargent *et al.*, 1977).

The analysis of fatty acids, fatty alcohols and sterols from the surface sediment samples of the Clyde Sea will be discussed in this chapter. The Clyde Sea is a deep, partially enclosed basin, on the west coast of Scotland. The Clyde Sea communicates with the adjacent shelf sea only via exchange flow over a relatively shallow sill (depth of 45m). The basin receives large inputs (60-70 $m^3s^{-1}$) (Poodle, 1986) of freshwater from the River Clyde and other river sources, which enters mainly through the sea loch system to the north and from rivers along the Ayrshire coast. Thirty-three samples were collected from the Firth of Clyde, Kilbrannan Sound, Gareloch, Loch Long, Loch Goil, Loch Striven, Loch Riddon and Loch Fyne in Scotland (Figure 5.1). A grab sampler from *RV Prince Madog* was used to collect these samples, which were then scraped into glass jars.

**5.2 Results**
**5.2.1 Fatty acids**

Fatty acids are often the most abundant lipid type in sediments because they are abundant in most organisms. Therefore they can be used to differentiate sources that contribute to the sedimentary lipid. A total of 35 fatty acids were identified (Appendix 7). 16:0 and 18:0 fatty acids were the most two abundant compounds found from the 33 sampling sites.
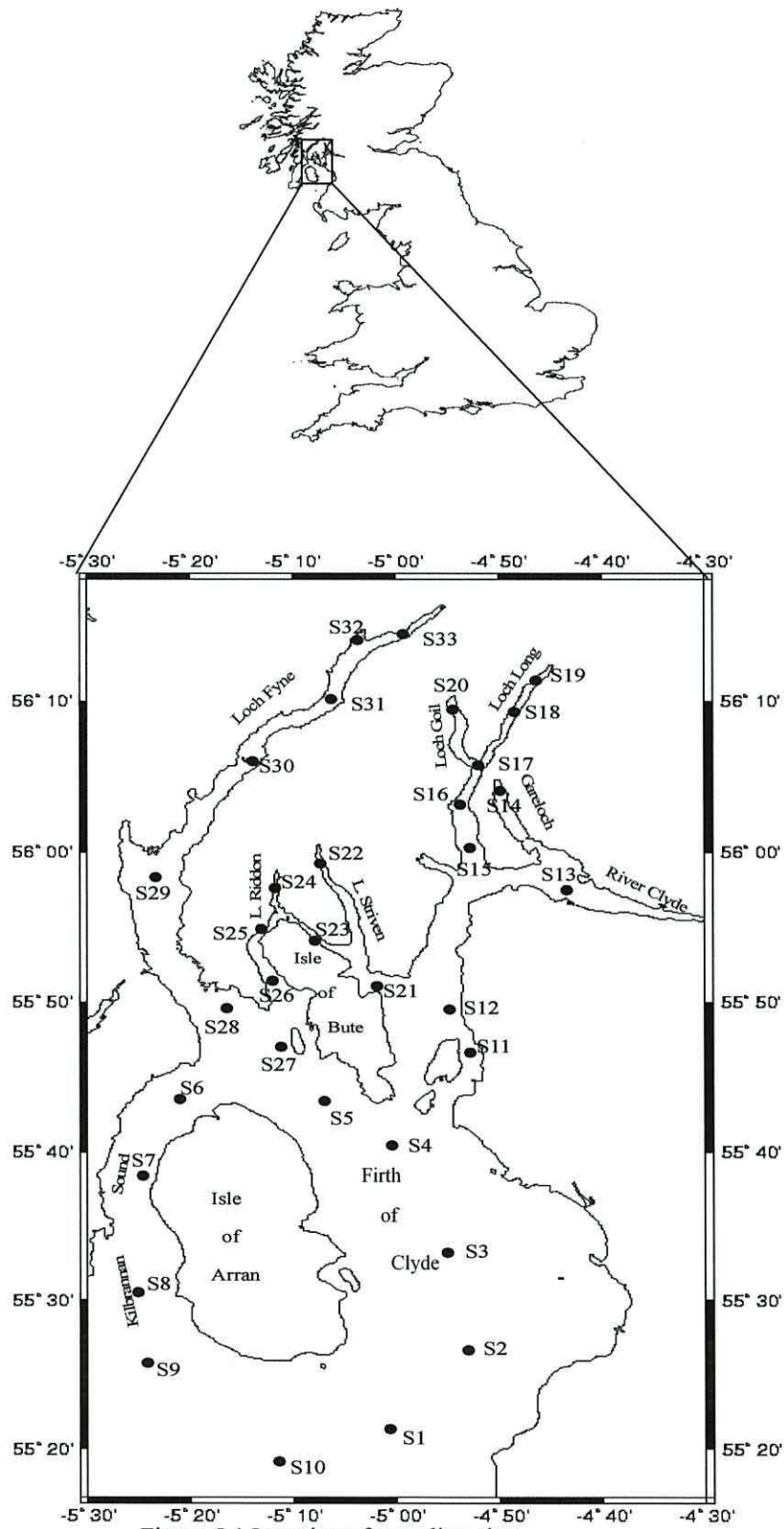
Figure 5.1 Location of sampling sites

Table 5.1: Coefficients of correlation between fatty acids in the Clyde Sea sediment samples

| | br12:0 | 12:0 | iso13:0 | ante13:0 | 13:0 | br14:0 | 14:0 | iso15:0 | ante15:0 | 15:0 | iso16:0 | 16:0 | 16:1 | 16:1ω9 | 16:1ω7 | iso17:0 | 16:1ω5 | ante17:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12:0 | 0.69** | | | | | | | | | | | | | | | | | |
| iso13:0 | 0.73** | 0.34 | | | | | | | | | | | | | | | | |
| ante13:0 | 0.76** | 0.48 | 0.78** | | | | | | | | | | | | | | | |
| 13:0 | 0.70** | 0.90** | 0.54* | 0.52 | | | | | | | | | | | | | | |
| br14:0 | 0.53* | 0.27 | 0.65** | 0.92** | 0.27 | | | | | | | | | | | | | |
| 14:0 | 0.39 | 0.63** | 0.21 | 0.27 | 0.67** | 0.16 | | | | | | | | | | | | |
| iso15:0 | 0.18 | 0.001 | 0.57** | 0.55* | 0.24 | 0.63** | 0.32 | | | | | | | | | | | |
| ante15:0 | 0.57** | 0.24 | 0.78** | 0.84** | 0.40 | 0.85** | 0.30 | 0.83** | | | | | | | | | | |
| 15:0 | 0.29 | 0.43 | 0.43 | 0.30 | 0.69** | 0.16 | 0.58** | 0.57** | 0.45 | | | | | | | | | |
| iso16:0 | 0.52* | 0.35 | 0.76** | 0.67** | 0.60** | 0.60** | 0.62** | 0.75** | 0.80** | 0.64** | | | | | | | | |
| 16:0 | -0.06 | 0.01 | 0.04 | -0.03 | 0.17 | -0.06 | 0.64** | 0.47 | 0.23 | 0.50* | 0.47 | | | | | | | |
| 16:1 | 0.03 | 0.46 | 0.25 | 0.23 | 0.60* | 0.16 | 0.53* | 0.51* | 0.30 | 0.68** | 0.49 | 0.43 | | | | | | |
| 16:1ω9 | 0.26 | 0.31 | 0.29 | 0.24 | 0.42 | 0.18 | 0.72** | 0.33 | 0.28 | 0.47 | 0.69** | 0.52* | 0.36 | | | | | |
| 16:1ω7 | 0.11 | 0.40 | -0.06 | 0.09 | 0.44 | 0.08 | 0.80** | 0.29 | 0.18 | 0.44 | 0.41 | 0.60** | 0.55* | 0.49 | | | | |
| iso17:0 | 0.20 | 0.04 | 0.58** | 0.61** | 0.20 | 0.72** | 0.19 | 0.77** | 0.79** | 0.36 | 0.69** | 0.17 | 0.33 | 0.26 | 0.05 | | | |
| 16:1ω5 | 0.54* | 0.57** | 0.62** | 0.56** | 0.74** | 0.45 | 0.71** | 0.63** | 0.68** | 0.70** | 0.88** | 0.43 | 0.61** | 0.62** | 0.55* | 0.60** | | |
| ante17:0 | 0.16 | -0.03 | 0.49 | 0.72** | 0.01 | 0.89** | -0.03 | 0.71** | 0.78** | 0.10 | 0.51* | 0.04 | 0.24 | 0.07 | 0.01 | 0.78** | 0.33 | |
| 17:0 | 0.25 | 0.20 | 0.49 | 0.61** | 0.20 | 0.75** | 0.32 | 0.68** | 0.70** | 0.20 | 0.60** | 0.28 | 0.37 | 0.38 | 0.19 | 0.66** | 0.48 | 0.77** |
| 16:2 | 0.01 | -0.03 | -0.09 | -0.09 | 0.01 | -0.08 | 0.33 | -0.03 | 0.01 | 0.03 | 0.18 | 0.39 | 0.06 | 0.33 | 0.53* | 0.01 | 0.19 | -0.03 |
| 17:1 | 0.30 | 0.44 | 0.52* | 0.43 | 0.63** | 0.34 | 0.48 | 0.61** | 0.52* | 0.73** | 0.71** | 0.30 | 0.67** | 0.55* | 0.24 | 0.50* | 0.71** | 0.30 |
| 18:0 | 0.09 | 0.37 | 0.28 | 0.01 | 0.53* | 0.01 | 0.45 | 0.24 | 0.20 | 0.61** | 0.49 | 0.36 | 0.55* | 0.51* | 0.18 | 0.30 | 0.52* | -0.00 |
| 18:1ω9 | 0.23 | 0.30 | 0.33 | 0.20 | 0.47 | 0.20 | 0.59** | 0.49 | 0.38 | 0.56** | 0.63** | 0.42 | 0.46 | 0.56** | 0.54* | 0.37 | 0.70** | 0.13 |
| 18:1ω7 | 0.17 | 0.31 | 0.40 | 0.34 | 0.54* | 0.32 | 0.65** | 0.75** | 0.60** | 0.76** | 0.79** | 0.62** | 0.71** | 0.58** | 0.57** | 0.56** | 0.80** | 0.37 |
| 18:2ω6 | 0.29 | 0.26 | 0.30 | 0.22 | 0.38 | 0.14 | 0.47 | 0.42 | 0.38 | 0.51* | 0.52* | 0.39 | 0.27 | 0.28 | 0.48 | 0.34 | 0.73** | 0.07 |
| 19:0 | 0.11 | 0.01 | 0.53* | 0.45 | 0.18 | 0.51* | 0.11 | 0.60** | 0.56** | 0.36 | 0.61** | 0.24 | 0.38 | 0.41 | -0.06 | 0.60** | 0.41 | 0.62** |
| 18:3ω3 | 0.56** | 0.45 | 0.36 | 0.29 | 0.44 | 0.14 | 0.37 | 0.14 | 0.29 | 0.35 | 0.33 | 0.23 | 0.04 | 0.15 | 0.28 | 0.03 | 0.52* | -0.06 |
| 20:0 | -0.01 | -0.11 | 0.38 | 0.37 | -0.02 | 0.51* | -0.08 | 0.44 | 0.44 | 0.06 | 0.37 | 0.06 | 0.17 | 0.12 | -0.19 | 0.62** | 0.19 | 0.62** |
| 20:1 | -0.03 | 0.05 | 0.21 | -0.00 | 0.22 | 0.02 | 0.26 | 0.25 | 0.17 | 0.35 | 0.43 | 0.22 | 0.28 | 0.36 | 0.20 | 0.40 | 0.51* | 0.09 |
| 22:0 | 0.12 | 0.04 | 0.41 | 0.41 | 0.17 | 0.43 | 0.08 | 0.46 | 0.41 | 0.38 | 0.37 | 0.18 | 0.19 | 0.30 | -0.18 | 0.42 | 0.16 | 0.46 |
| 22:1ω11 | 0.08 | 0.14 | 0.35 | 0.16 | 0.32 | 0.16 | 0.56** | 0.42 | 0.36 | 0.51* | 0.64** | 0.51* | 0.41 | 0.72** | 0.24 | 0.59** | 0.60** | 0.19 |
| 24:0 | 0.62** | 0.42 | 0.65** | 0.69** | 0.43 | 0.61** | 0.26 | 0.27 | 0.55* | 0.18 | 0.50* | 0.04 | 0.04 | 0.40 | -0.13 | 0.40 | 0.30 | 0.44 |
| 25:0 | -0.03 | -0.08 | 0.18 | 0.23 | -0.01 | 0.28 | 0.12 | 0.35 | 0.22 | 0.20 | 0.19 | 0.28 | 0.08 | 0.27 | -0.04 | 0.22 | -0.05 | 0.34 |
| 26:0 | -0.10 | -0.12 | 0.08 | 0.12 | -0.03 | 0.16 | 0.16 | 0.34 | 0.19 | 0.26 | 0.15 | 0.38 | 0.09 | 0.22 | 0.01 | 0.23 | -0.04 | 0.24 |
| 27:0 | -0.06 | -0.12 | 0.01 | 0.06 | -0.11 | 0.09 | 0.15 | 0.26 | 0.14 | 0.18 | 0.07 | 0.49 | -0.06 | 0.13 | 0.06 | 0.11 | -0.01 | 0.16 |
| 28:0 | -0.11 | -0.14 | 0.07 | 0.13 | -0.07 | 0.20 | 0.14 | 0.40 | 0.26 | 0.21 | 0.14 | 0.42 | 0.10 | 0.12 | 0.07 | 0.24 | -0.02 | 0.31 |

|        | 17:0 | 16:2 | 17:1 | 18:0 | 18:1ω9 | 18:1ω7 | 18:2ω6 | 19:0 | 18:3ω3 | 20:0 | 20:1 | 22:0 | 22:1ω11 | 24:0 | 25:0 | 26:0 | 27:0 |
|--------|------|------|------|------|--------|--------|--------|------|--------|------|------|------|---------|------|------|------|------|
| 16:2   | -0.02 |      |      |      |        |        |        |      |        |      |      |      |         |      |      |      |      |
| 17:1   | 0.47 | -0.11 |      |      |        |        |        |      |        |      |      |      |         |      |      |      |      |
| 18:0   | 0.21 | 0.01 | 0.57** |      |        |        |        |      |        |      |      |      |         |      |      |      |      |
| 18:1ω9 | 0.23 | 0.25 | 0.48 | 0.44 |        |        |        |      |        |      |      |      |         |      |      |      |      |
| 18:1ω7 | 0.46 | 0.15 | 0.72** | 0.56** | 0.71** |     |        |      |        |      |      |      |         |      |      |      |      |
| 18:2ω6 | 0.11 | 0.13 | 0.32 | 0.14 | 0.47 | 0.48 |        |      |        |      |      |      |         |      |      |      |      |
| 19:0   | 0.70** | -0.09 | 0.51* | 0.59** | 0.20 | 0.41 | 0.08 |      |        |      |      |      |         |      |      |      |      |
| 18:3ω3 | -0.01 | 0.03 | 0.13 | 0.06 | 0.27 | 0.20 | 0.77** | -0.03 |    |      |      |      |         |      |      |      |      |
| 20:0   | 0.64** | -0.11 | 0.16 | 0.42 | 0.09 | 0.16 | -0.08 | 0.85** | -0.15 |   |      |      |         |      |      |      |      |
| 20:1   | 0.07 | 0.18 | 0.21 | 0.42 | 0.43 | 0.42 | 0.52* | 0.30 | 0.25 | 0.23 |     |      |         |      |      |      |      |
| 22:0   | 0.54* | -0.22 | 0.40 | 0.41 | 0.03 | 0.22 | -0.07 | 0.77** | -0.05 | 0.65** | 0.06 | |         |      |      |      |      |
| 22:1ω11 | 0.40 | 0.26 | 0.49 | 0.60* | 0.50* | 0.60** | 0.32 | 0.50* | 0.06 | 0.36 | 0.66** | 0.40 |    |      |      |      |      |
| 24:0   | 0.52* | -0.10 | 0.35 | 0.28 | 0.09 | 0.21 | -0.12 | 0.48 | 0.10 | 0.39 | -0.05 | 0.64** | 0.38 |   |      |      |      |
| 25:0   | 0.39 | -0.08 | 0.16 | 0.03 | -0.03 | 0.19 | -0.19 | 0.34 | -0.20 | 0.25 | -0.08 | 0.76** | 0.31 | 0.59* |  |      |      |
| 26:0   | 0.22 | -0.02 | 0.13 | 0.07 | 0.02 | 0.26 | -0.14 | 0.18 | -0.18 | 0.12 | -0.04 | 0.62** | 0.34 | 0.50* | 0.93** |  |      |
| 27:0   | 0.16 | -0.03 | -0.05 | -0.06 | -0.01 | 0.20 | 0.15 | 0.04 | 0.20 | 0.001 | 0.01 | 0.44 | 0.23 | 0.34 | 0.76** | 0.84** |  |
| 28:0   | 0.23 | 0.02 | 0.12 | 0.01 | -0.01 | 0.32 | -0.09 | 0.11 | -0.12 | 0.06 | -0.11 | 0.49 | 0.23 | 0.42 | 0.82** | 0.94** | 0.84** |

\*    $p < 0.01$
\*\*  $p < 0.001$

Correlation analysis was carried out for all the compounds. Only 29% of compound pairs showing correlation coefficient ($r$) value greater than 0.5 within the fatty acids (Table 5.1). Short chain fatty acids such as 14:0 saturated acid, correlated strongly with 12:0, 13:0 and 16:0 saturated fatty acids, with $r$ values of 0.63, 0.67 and 0.64 respectively. 14:0 also has strong positive correlation with monounsaturated 16 carbon acids. For example, with 16:1ω9, 16:1ω7, and 16:1ω5, 14:0 had $r$ values of 0.72, 0.80 and 0.71 respectively. Branched *iso*-15:0 showed strong correlation with *anteiso*-15:0, *iso*-17:0, *anteiso*-17:0 and 18:1ω7 with $r$ values of 0.83, 0.77, 0.71 and 0.75 respectively. The polyunsaturated acid, 18:3ω3 correlated most strongly with 18:2ω6 with an $r$ value of 0.77. The saturated fatty acid, 22:0, correlated most strongly with other long chain saturated fatty acids such as 24:0, 25:0 and 26:0 with $r$ values of 0.64, 0.76 and 0.62 respectively.

Algae are a major source of fatty acids in most marine sedimentary environments. Homologous series of short chain saturated fatty acids ($<C_{20}$) are considered to originate from algae (Carrie *et al.*, 1998; Rohjans *et al.*, 1998). 16:0 and 18:0 fatty acids are commonly assigned to planktonic sources (Volkman *et al.*, 1998). Polyunsaturated fatty acids are normally associated with phytoplankton. Chlorophyta (green algae) contain abundant $C_{16}$ and $C_{18}$ polyunsaturated fatty acids especially with the positional isomers ω3 and ω6 (Volkman *et al.*, 1989; Carrie *et al.*, 1998).

Figure 5.2a and 5.2b show the distribution of the percentage of polyunsaturated and monounsaturated fatty acids throughout the sampling sites. The distribution of these fatty acids is similar to each other ($r=0.60$, p<0.01). A high percentage of monounsaturated fatty acids (>20% of total fatty acids) can be seen at S1, S2, S3 and S10 while S1 and S10 also have high value of polyunsaturated fatty acids. Short chain saturated fatty acids and unsaturated fatty acids have high concentration at S1, S2, S3 and S10, which are situated at the open seas.
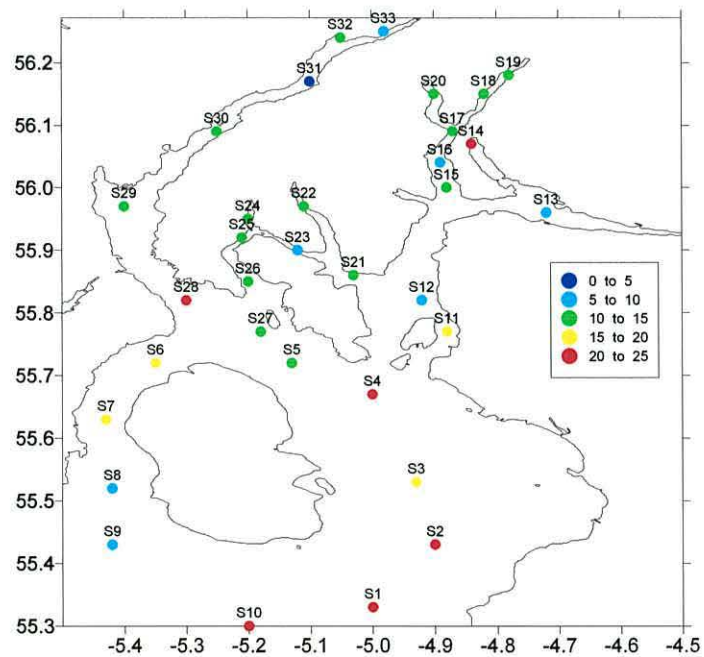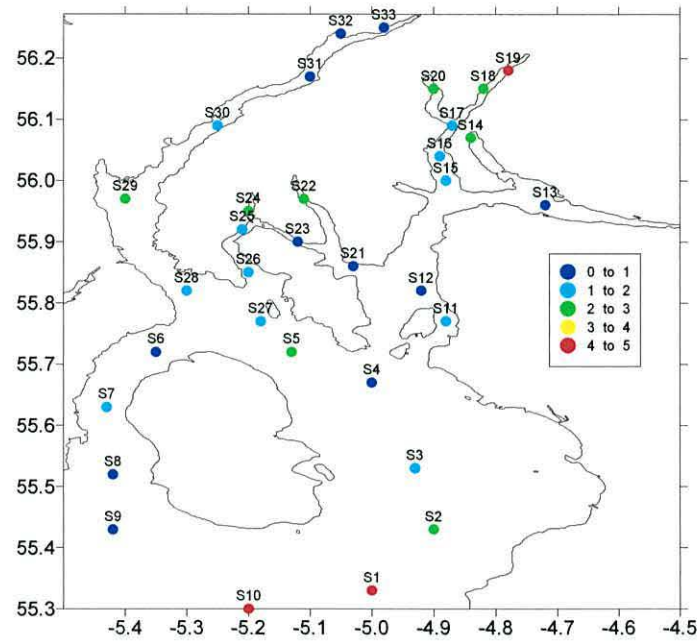
Figure 5.2: Distribution of (a) polyunsaturated and (b) monounsaturated fatty acids throughout the surface sediment of the Clyde Sea

Monounsaturated fatty acids, 16:1ω5, 16:1ω7 and 16:1ω9, are the 16-carbon monounsaturated fatty acids were identified in this study. 16:1ω7 is a diatom derived fatty acid (Skerratt *et al.*, 1995; Carrie *et al.*, 1998; Mudge *et al.*, 1998). The ratio between 16:1ω7 and 16:0 fatty acids has been used as an indicator for diatoms (Skerratt *et al.*, 1995; Mudge *et al.*, 1998). Figure 5.3 shows that the 16:1ω7/16:0 ratios are high at the open sea sampling sites.
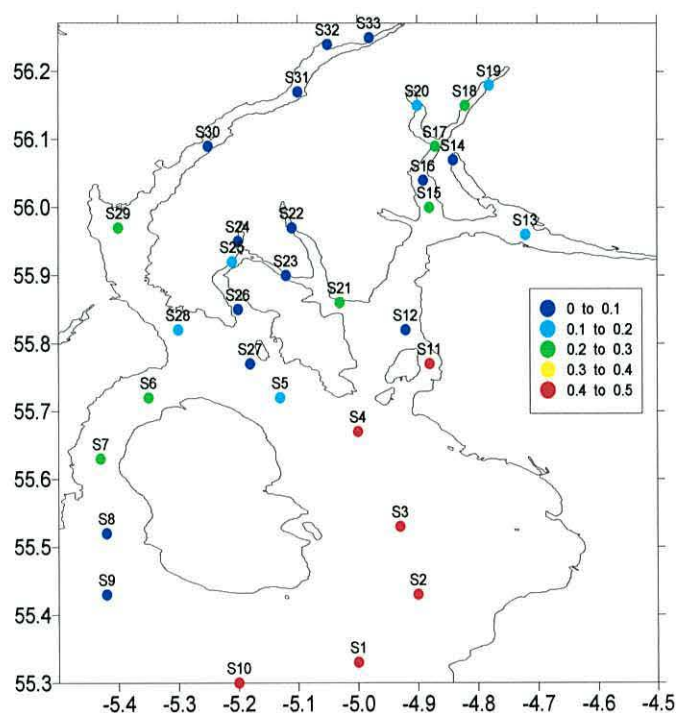


Figure 5.3: Distribution of 16:1ω7/16:0 ratios throughout the surface sediment of the Clyde Sea

Branched fatty acids (principally *iso* and *anteiso* odd chain length compounds) are commonly encountered in bacterial lipids (Parkes, 1987; Thoumelin *et al.*, 1997). Therefore these acids are useful indicators of bacterial lipid contribution (Thoumelin *et al.*, 1997; Carrie *et al.*, 1998). The distribution of percentage of branched fatty acids is shown in Figure 5.4. S13, which is situated at the mouth of River Clyde, has the highest percentage of branched fatty acids. This sampling site is near the area with a high population, therefore the high percentage of branched fatty acids may be related to the

151

sewage discharges. Low percentages of polyunsaturated and monounsaturated fatty acids were observed at sites with a high percentage of branched fatty acids, indicating those areas with bacterial biomass. The odd/even ratios (Figure 5.5) show a similar distribution ($r$=0.69, p<0.001) as the percentage of branched fatty acids associating their link with bacterial biomass.
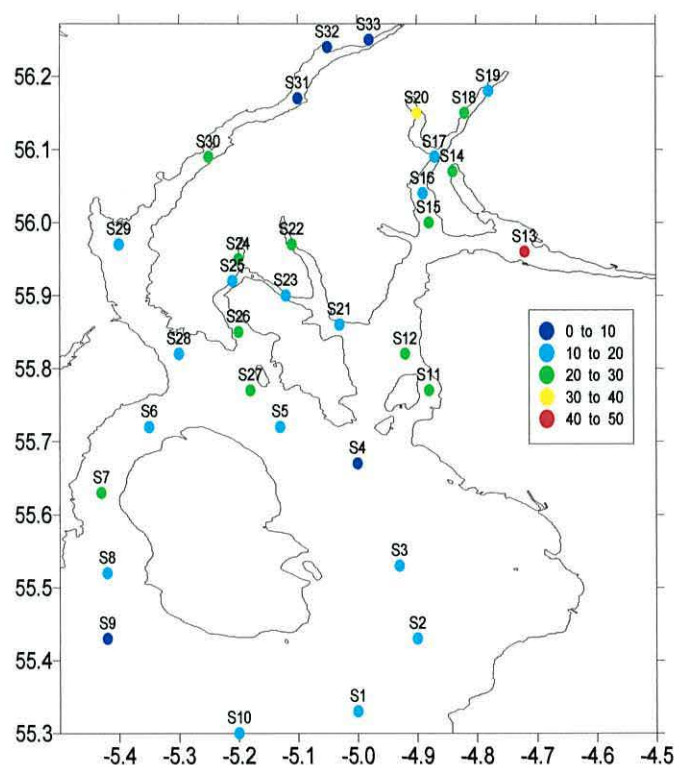


Figure 5.4: Distribution of percentage of branched fatty acids throughout the surface sediment of the Clyde Sea

$18:1\omega7$ is the dominant $C_{18}$ compound in bacteria and has been used as bacterial marker in sediments (Saliot *et al.*, 1991; Thoumelin *et al.*, 1997; Mudge *et al.*, 1998). In these organisms $18:1\omega7/18:1\omega9$ is generally greater than 1 (Parkes and Taylor, 1983; Thoumelin *et al.*, 1997). The value of $18:1\omega7/18:1\omega9$ ratios were greater than 1 in 20 sampling sites with the highest value is at S12, but these ratios were poorly correlated to percentage of branched fatty acids ($r$=0.22, p<0.05). This may be due to different bacterial classes occurred in different sampling sites.

152
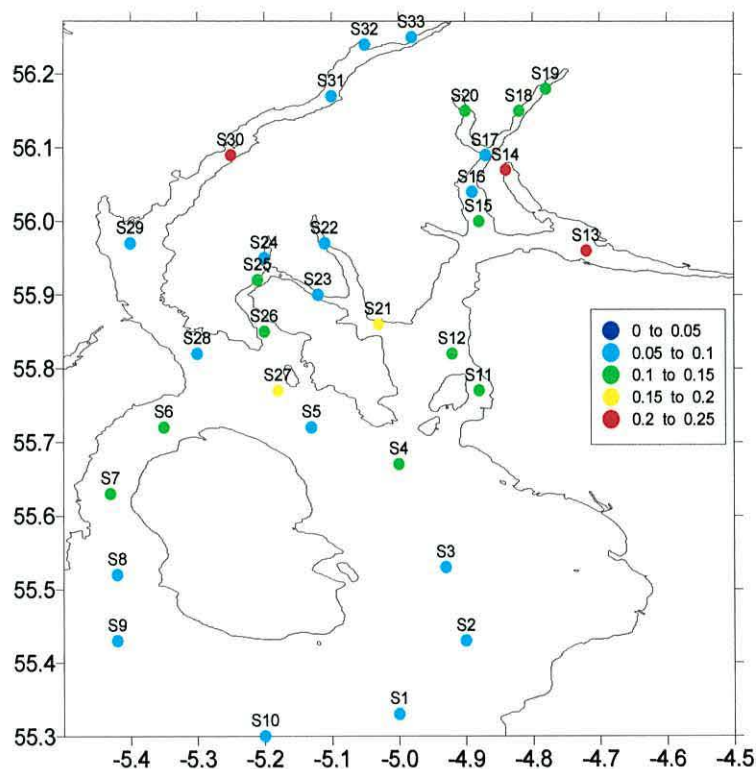
Figure 5.5: Distribution of odd/even ratios of fatty acids throughout the surface sediment of the Clyde Sea

Long chain fatty acids are generally considered as terrestrial plant biomarkers in sediments as they occur in surface waxes of higher plants (Thoumelin *et al.*, 1997; Mudge *et al.*, 1998; Volkman *et al.*, 1998). Fatty acids with chain lengths up to 28:0 were quantified in the sediment samples. The most abundant fatty acids with more than twenty carbon atoms were the saturated 24:0 and 26:0. Figure 5.6 shows the distribution of short/long ratios $(\sum 12:0\text{-}20:0)/(\sum 21:0\text{-}28:0)$ with the highest values at S1 and S10. In general, the ratios were larger than 1 indicating that the short chain compounds were more predominant in all samples.
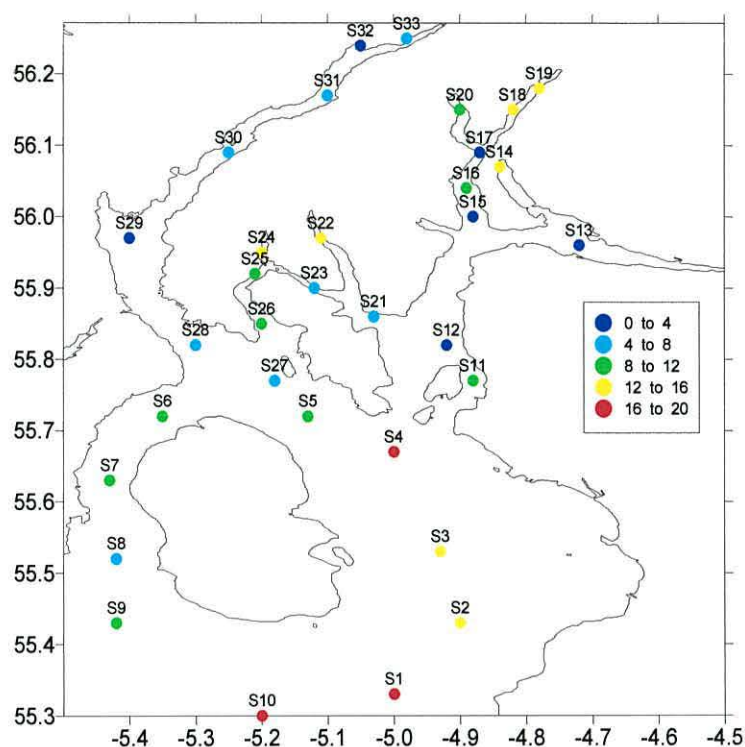
Figure 5.6: Distribution of short/long ratios of fatty acids throughout the surface sediment of the Clyde Sea

## 5.2.2 Fatty alcohols

Other than fatty acids and sterols, fatty alcohols were also identified from the sediment samples. Fatty alcohols in marine sediments are primarily derived from wax esters, which are supplied by a variety of organisms. The primary sources of wax esters in marine environments are thought to be from marine zooplankton and terrestrial plants (Fukushima and Ishiwatari, 1984; Mudge and Norris, 1997). Twenty-nine saturated and monounsaturated alcohols were found (Appendix 8).

In general, correlation within the fatty alcohol group was low, with only 12% of compound pairs showing an $r$ value greater than 0.5 (Table 5.2). $C_{16}$, which is abundant in marine samples, correlated with $C_{11}$, $C_{13}$, $C_{14}$ and $C_{24}$, with $r$ values of 0.60, 0.52, 0.52 and 0.54 respectively. Branched fatty alcohols such as *iso*-$C_{17}$ correlated strongly with *anteiso*-$C_{17}$, *iso*-$C_{15}$ and *anteiso*-$C_{15}$, with correlation coefficient of 0.95, 0.62 and 0.51 respectively.

Table 5.2: Coefficients of correlation between fatty alcohols in the Clyde Sea sediment samples

| | $C_{11}$ | $C_{12}$ | $C_{13}$ | $C_{14}$ | $isoC_{15}$ | $anteC_{15}$ | $C_{15}$ | $C_{16}$ | $isoC_{17}$ | $anteC_{17}$ | $C_{17}$ | $C_{18}$ | $C_{19}$ | $C_{20}$ | $brC_{21}$ | $C_{21}$ | $C_{22}$ | $brC_{23}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_{12}$ | 0.79** | | | | | | | | | | | | | | | | | |
| $C_{13}$ | 0.51* | 0.41 | | | | | | | | | | | | | | | | |
| $C_{14}$ | 0.36 | 0.20 | 0.20 | | | | | | | | | | | | | | | |
| $isoC_{15}$ | -0.13 | 0.12 | 0.46 | 0.18 | | | | | | | | | | | | | | |
| $anteC_{15}$ | -0.15 | -0.18 | 0.44 | -0.22 | 0.65** | | | | | | | | | | | | | |
| $C_{15}$ | 0.49 | 0.40 | 0.56** | 0.35 | 0.24 | -0.11 | | | | | | | | | | | | |
| $C_{16}$ | 0.60** | 0.49 | 0.52* | 0.52* | 0.18 | -0.06 | 0.47 | | | | | | | | | | | |
| $isoC_{17}$ | -0.02 | -0.03 | 0.64** | 0.27 | 0.62** | 0.51* | 0.32 | 0.23 | | | | | | | | | | |
| $anteC_{17}$ | -0.06 | -0.08 | 0.62** | 0.24 | 0.65** | 0.50* | 0.30 | 0.17 | 0.95** | | | | | | | | | |
| $C_{17}$ | 0.14 | 0.06 | 0.53* | 0.54* | 0.68** | 0.20 | 0.47 | 0.36 | 0.69** | 0.75** | | | | | | | | |
| $C_{18}$ | 0.53* | 0.47 | 0.37 | 0.21 | -0.09 | -0.12 | 0.64** | 0.40 | -0.06 | -0.06 | 0.01 | | | | | | | |
| $C_{19}$ | 0.22 | 0.20 | 0.20 | 0.02 | 0.32 | 0.32 | 0.36 | 0.07 | -0.12 | -0.14 | 0.10 | 0.52* | | | | | | |
| $C_{20}$ | 0.10 | 0.06 | 0.34 | 0.08 | 0.13 | 0.22 | 0.24 | 0.15 | 0.03 | 0.01 | 0.15 | 0.52* | 0.60** | | | | | |
| $brC_{21}$ | -0.05 | -0.15 | 0.05 | 0.06 | 0.18 | 0.19 | -0.02 | 0.05 | -0.14 | -0.10 | 0.08 | 0.30 | 0.54* | 0.42 | | | | |
| $C_{21}$ | 0.06 | -0.11 | 0.08 | 0.11 | 0.36 | 0.37 | 0.08 | 0.16 | 0.14 | 0.10 | 0.21 | 0.29 | 0.57* | 0.48 | 0.51* | | | |
| $C_{22}$ | -0.14 | -0.28 | 0.09 | 0.21 | 0.54* | 0.32 | 0.10 | 0.25 | 0.33 | 0.36 | 0.46 | 0.14 | 0.31 | 0.33 | 0.34 | 0.75** | | |
| $brC_{23}$ | 0.47 | 0.33 | 0.14 | 0.19 | -0.04 | 0.02 | 0.17 | 0.30 | -0.04 | -0.09 | 0.10 | 0.21 | 0.28 | 0.03 | 0.24 | 0.31 | 0.15 | |
| $C_{23}$ | -0.20 | -0.32 | 0.06 | 0.09 | 0.36 | 0.38 | 0.14 | 0.21 | 0.41 | 0.37 | 0.28 | 0.22 | 0.25 | 0.32 | 0.22 | 0.64** | 0.77** | 0.13 |
| $C_{24}$ | 0.03 | 0.02 | 0.50* | 0.36 | 0.64** | 0.23 | 0.37 | 0.54* | 0.55* | 0.60** | 0.75** | -0.02 | 0.02 | 0.24 | -0.02 | 0.15 | 0.50* | 0.001 |
| $C_{25}$ | -0.15 | 0.22 | -0.12 | -0.01 | -0.04 | 0.09 | -0.20 | 0.23 | 0.03 | -0.02 | -0.14 | -0.02 | -0.16 | 0.03 | -0.23 | -0.02 | 0.02 | -0.01 |
| $C_{26}$ | -0.15 | -0.16 | -0.02 | 0.17 | 0.18 | 0.02 | -0.001 | 0.36 | 0.18 | 0.26 | 0.28 | -0.01 | -0.19 | 0.12 | -0.02 | 0.16 | 0.41 | -0.03 |
| $C_{28}$ | -0.10 | -0.07 | -0.01 | 0.17 | 0.13 | -0.09 | 0.01 | 0.36 | 0.07 | 0.18 | 0.29 | -0.12 | -0.31 | -0.8 | -0.19 | -0.07 | 0.22 | -0.10 |
| 16:1 | 0.15 | 0.06 | 0.45 | 0.18 | 0.53* | 0.27 | 0.22 | 0.16 | 0.18 | 0.33 | 0.62** | -0.00 | 0.30 | 0.19 | 0.28 | 0.18 | 0.18 | 0.12 |
| 18:1 | 0.06 | 0.03 | 0.26 | 0.27 | 0.41 | 0.01 | 0.18 | 0.12 | 0.29 | 0.50* | 0.67** | -0.16 | -0.12 | -0.16 | -0.04 | -0.07 | 0.15 | -0.00 |
| Phytol | -0.16 | -0.18 | 0.06 | -0.34 | 0.20 | 0.22 | -0.06 | -0.20 | -0.12 | -0.02 | -0.03 | 0.04 | 0.12 | 0.10 | 0.41 | -0.05 | -0.02 | -0.14 |
| 20:1 | 0.09 | 0.01 | 0.15 | -0.20 | 0.23 | 0.24 | 0.24 | -0.12 | -0.26 | -0.24 | -0.08 | 0.33 | 0.66** | 0.37 | 0.55* | 0.44 | 0.09 | 0.14 |
| 22:1 | 0.17 | 0.12 | 0.02 | 0.21 | -0.08 | -0.24 | 0.12 | -0.01 | -0.01 | 0.12 | 0.10 | 0.07 | -0.08 | -0.08 | -0.02 | 0.17 | 0.07 | 0.05 |
| 24:1 | -0.03 | 0.01 | -0.26 | 0.18 | -0.04 | -0.17 | -0.14 | -0.05 | -0.10 | -0.03 | -0.08 | -0.06 | -0.07 | -0.14 | -0.00 | -0.34 | 0.19 | -0.01 |

|        | $C_{23}$ | $C_{24}$ | $C_{25}$ | $C_{26}$ | $C_{28}$ | 16:1    | 18:1    | Phytol | 20:1 | 22:1    |
|--------|----------|----------|----------|----------|----------|---------|---------|--------|------|---------|
| $C_{24}$ | 0.28    |          |          |          |          |         |         |        |      |         |
| $C_{25}$ | 0.24    | 0.07     |          |          |          |         |         |        |      |         |
| $C_{26}$ | 0.45    | 0.54*    | 0.39     |          |          |         |         |        |      |         |
| $C_{28}$ | 0.24    | 0.48     | 0.46     | 0.84**   |          |         |         |        |      |         |
| 16:1   | -0.10    | 0.55*    | -0.24    | 0.14     | 0.24     |         |         |        |      |         |
| 18:1   | -0.15    | 0.59**   | -0.16    | 0.35     | 0.46     | 0.79**  |         |        |      |         |
| Phytol | 0.05     | -0.08    | -0.19    | -0.21    | -0.15    | -0.20   | -0.04   |        |      |         |
| 20:1   | -0.03    | -0.09    | -0.26    | -0.28    | -0.24    | 0.40    | -0.01   | 0.35   |      |         |
| 22:1   | -0.18    | 0.15     | -0.17    | 0.10     | 0.20     | 0.44    | 0.61**  | -0.22  | 0.22 |         |
| 24:1   | 0.04     | -0.04    | 0.15     | 0.18     | 0.36     | 0.24    | 0.36    | -0.24  | 0.15 | 0.74**  |

*       $p < 0.01$
**      $p < 0.001$

$C_{22}$ showed a correlation coefficient of 0.75 and 0.77 with $C_{21}$ and $C_{23}$ respectively. Meanwhile $C_{24}$ has strong correlation with $C_{17}$ and $C_{26}$ with $r$ values of 0.75 and 0.54 respectively. $C_{28}$ saturated alcohol has correlation coefficient of 0.84 with $C_{26}$. Interestingly, the biomarker for chlorophyll, phytol, did not correlate strongly with any compounds in this study. Other monounsaturated compounds such as 18:1, correlated strongly with 16:1, 22:1 and $C_{24}$ with $r$ values of 0.79, 0.61 and 0.59 respectively. The 22:1 monounsaturated has $r$ value of 0.74 with 24:1.

Fatty alcohols of all marine wax esters are invariably either saturated short chain alcohols especially $C_{14}$ and $C_{16}$, or monounsaturated alcohols especially 16:1, 18:1, 20:1 and 22:1 (Sargent *et al.*, 1981; Rajendran *et al.*, 1991). Therefore short chain alcohols are often used as marine indicators (Mudge and Norris, 1997; Mudge and Lintern, 1999). Another major source of wax esters is terrestrial higher plants. Fatty alcohols derived from terrestrial plants consist of long saturated carbon chain ($>C_{20}$) and they can be used to indicate terrestrial inputs (Fukushima and Ishiwatari, 1984; Mudge and Norris, 1997). Figure 5.7 shows the mean chain length of fatty alcohols in each sampling station. Only S1, S2, S3, S4 and S10 have relatively shorter mean chain length while the rest of the sampling stations have almost similar mean chain length of fatty alcohols.
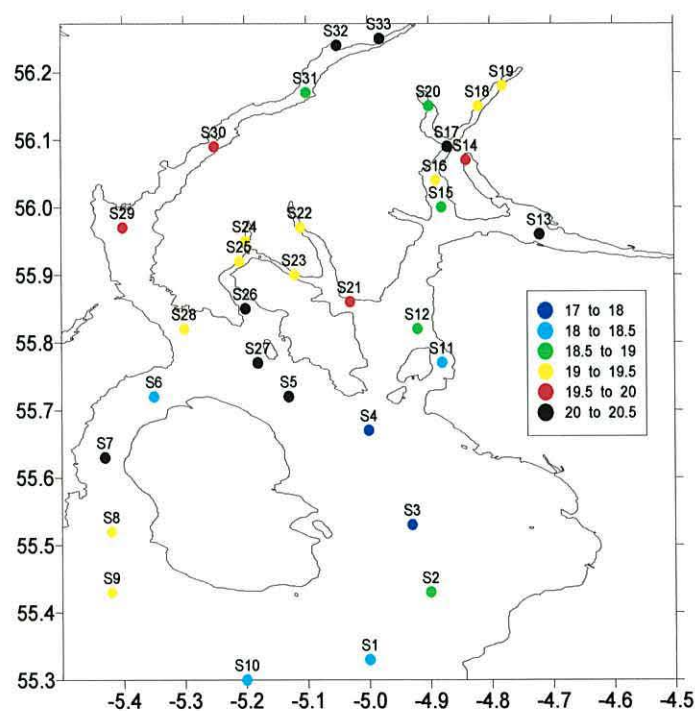


Figure 5.7: Mean chain length of fatty alcohols in the surface sediment of the Clyde Sea

The short/long ratio (similar to L/H ratio by Fukushima and Ishiwatari, 1984) was used by assuming that chain length distribution may reflect input of fatty alcohols from particular sources. The distribution of short/long ratio $(\Sigma C_{11}\text{-}C_{20})/(\Sigma C_{21}\text{-}C_{28})$ are shown in Figure 5.8. The short/long ratios for alcohols have a similar distribution with the same ratios of fatty acids ($r$=0.65, p<0.001). In general, the ratios were greater than 1.0 in most samples indicating that the shorter chain fatty alcohols were more predominant than the longer chain alcohols. This is not unexpected since all samples were collected from marine environments. However the highest value can be seen at S10, which is an open sea sample.
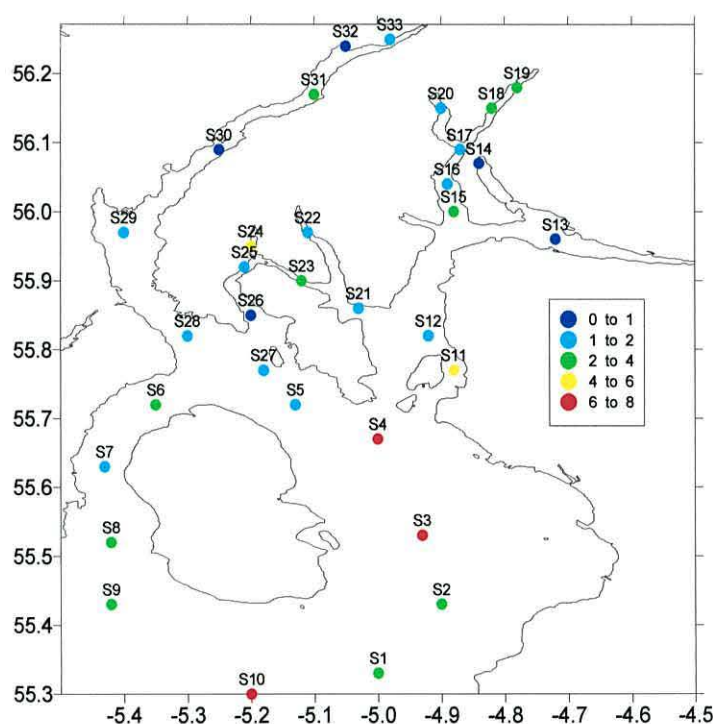


Figure 5.8: Distribution of the short/long ratios of fatty alcohols throughout the surface sediment of the Clyde Sea

The alcohol source index (ASI) can be calculated by dividing the concentration of a terrestrial fatty alcohol with concentration of a marine fatty alcohol (Mudge and Norris, 1997). The ASI was used to estimate the input of terrestrial organic matter to sediments. Figure 5.9 shows the example of ASI distribution using $C_{24}$ and $C_{22}$ as the terrestrial fatty alcohol and $C_{14}$ and $C_{16}$ as marine origin fatty alcohol. The ratios were low in open sea samples and these results coincided with the high short/long ratios in these samples.

158
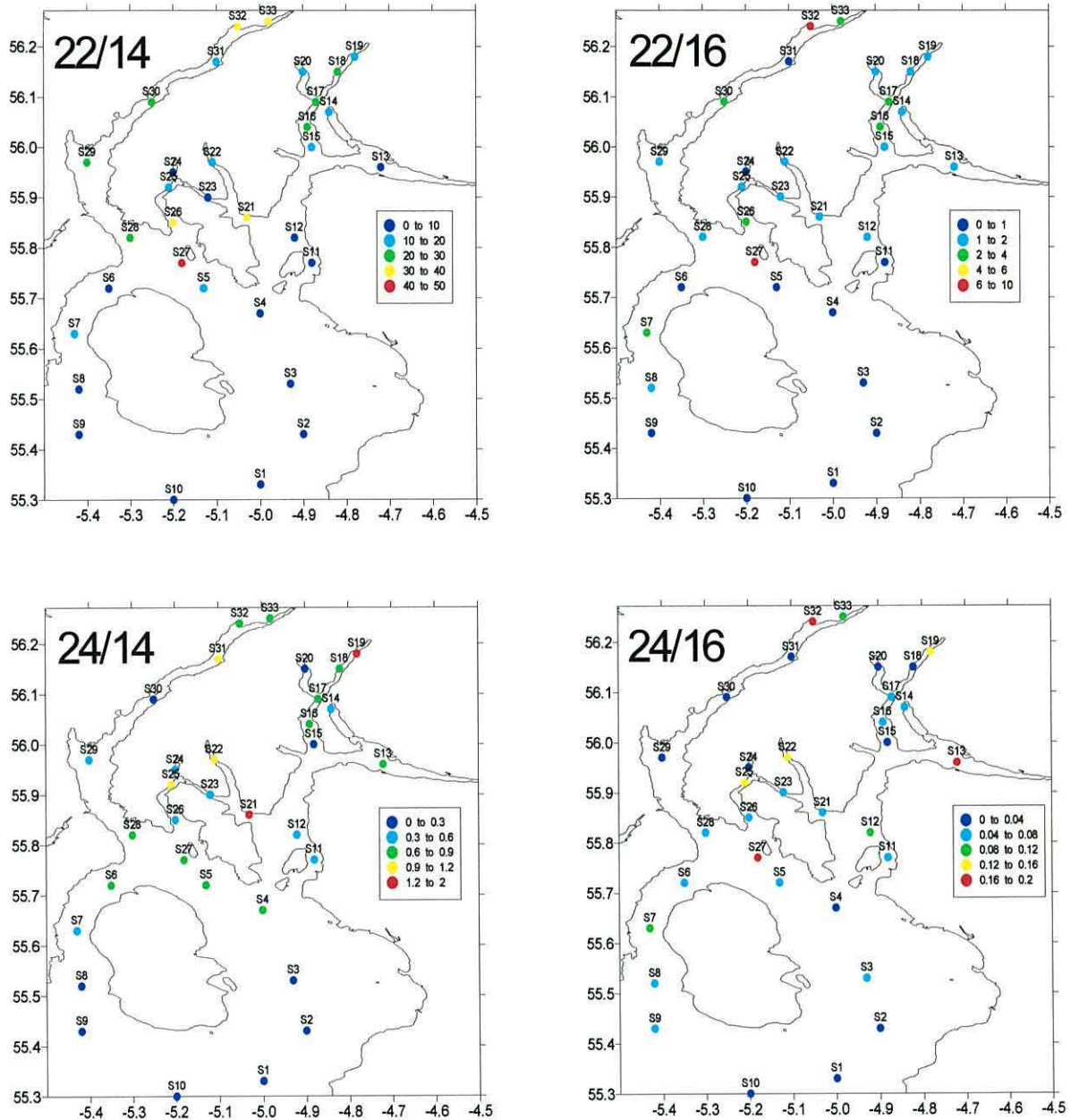
Figure 5.9: Distribution of the ASI ratios throughout the surface sediment of the Clyde Sea

The percentage of branched fatty alcohols (Figure 5.10) has a similar distribution with the percentage of branched fatty acids and coprostanol/cholesterol ratio ($r$=0.51 and 0.64 respectively, p<0.01). Branched fatty acids and fatty alcohols usually result from bacterial metabolism (Parkes, 1987). The strong correlation between branched alcohols with branched fatty acids and coprostanol/cholesterol ratio indicates their link with bacterial biomass. High percentages can be seen at S13, which was collected from the mouth of River Clyde and S21 collected near Rothesay (Isle of Bute). This may suggest that these compounds have direct link to the human influence such as sewage discharges around these areas.
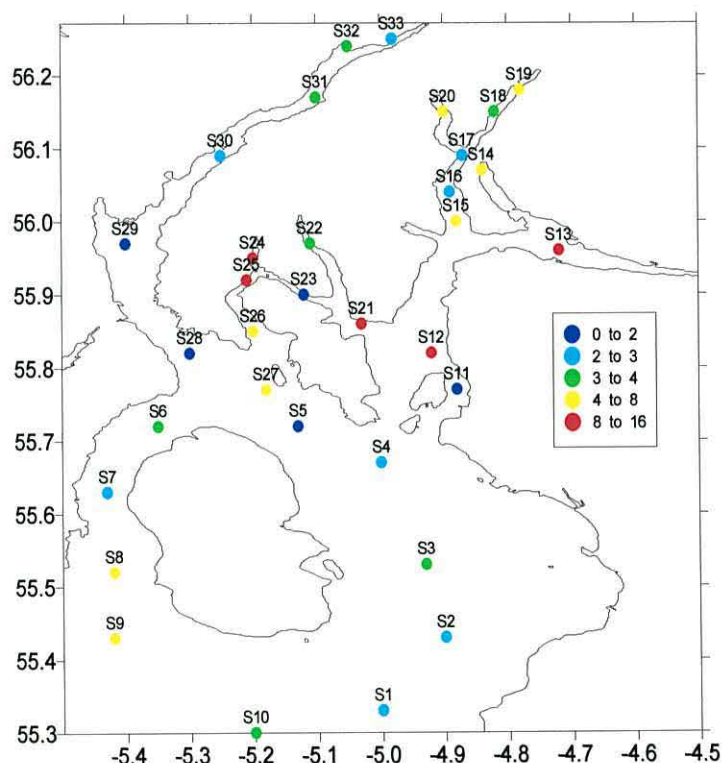


Figure 5.10: Percentage of branched fatty alcohols throughout the surface sediment of the Clyde Sea

### 5.2.3 Sterols

Sterols have been used as tracers of inputs from marine and terrestrial plants and animals (Saliot *et al.*, 1991; Mudge and Norris, 1997). In this study, 20 sterols were identified (Appendix 9, Table 5.4). Cholesterol was the most abundant sterol and was present in all

samples; however, there is no clear trend of cholesterol distribution throughout the sampling sites. Cholesterol originates from variety of planktonic organisms of all trophic levels (Volkman, 1986; Volkman *et al.*, 1987; Hinrichs *et al.*, 1999; Mudge *et al.*, 1999) with zooplankton being the major source.

Within the sterols, correlation was generally low, with 20% of compound pairs showing an *r* value greater than 0.5 (Table 5.3). Cholesterol, which is abundant in many marine organisms, was strongly correlated with 24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22(E)-dien-3β-ol and brassicasterol, with *r* values of 0.65, 0.73 and 0.64 respectively. Open sea samples (S1, S2, S3 and S10) were dominated by brassicasterol, dinosterol and st3 (cholesta-5,22-dien-3β-ol). These sterols are molecular markers of algae and dinoflagellates (Volkman, 1986; Volkman *et al.*, 1998). Brassicasterol is strongly correlated with cholesta-5,22-dien-3β-ol and dinosterol (*r*=0.88 and 0.56 respectively) Brassicasterol is also strongly correlated with 24 norcholesta-5,22-dien-3β-ol), which is often found in the marine environment (Yunker *et al.*, 1995) with an *r* value of 0.82. Correlation between coprostanol and epicoprostanol was weak (*r*=0.39) while cholestanol has positive correlations with both coprostanol and epicoprostanol with *r* values of 0.50 and 0.66 respectively.

β-sitosterol, campesterol and stigmasterol are the most common sterols in vascular plants (Volkman, 1986) although they also have been found in phytoplankton. β-sitosterol had a weak correlation with the other two compounds. On the other hand, campesterol and stigmasterol had a strong correlation (*r*=0.62). The relative distribution of these sterols has been proposed as a source marker for higher plants (Volkman, 1986; Laureillard and Saliot, 1993). While algae contain larger amounts of campesterol and stigmasterol, β-sitosterol dominates in vascular plants. Therefore the ratio between stigmasterol and β-sitosterol could provide an indication of terrestrial contribution in the sediment samples

Table 5.4: Coefficients of correlation between sterols in the Clyde Sea sediment samples

| | st1 | st2 | cop | epi | st3 | st4 | chol | cholest | brass | st5 | st6 | camp | st7 | stig | st8 | st9 | sito | st10 | dino |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| st2 | 0.38 | | | | | | | | | | | | | | | | | | |
| cop | 0.18 | 0.07 | | | | | | | | | | | | | | | | | |
| epi | -0.10 | 0.14 | 0.39 | | | | | | | | | | | | | | | | |
| st3 | 0.80** | 0.31 | 0.06 | -0.07 | | | | | | | | | | | | | | | |
| st4 | 0.52* | 0.89** | 0.09 | 0.05 | 0.39 | | | | | | | | | | | | | | |
| chol | 0.65** | 0.11 | 0.42 | 0.18 | 0.64** | 0.28 | | | | | | | | | | | | | |
| cholest | 0.02 | 0.13 | 0.50* | 0.66** | 0.13 | 0.10 | 0.22 | | | | | | | | | | | | |
| brass | 0.82** | 0.31 | -0.00 | 0.02 | 0.88** | 0.42 | 0.73** | 0.07 | | | | | | | | | | | |
| st5 | 0.73** | 0.35 | 0.10 | 0.16 | 0.58** | 0.54* | 0.62** | 0.26 | 0.66** | | | | | | | | | | |
| st6 | 0.75** | 0.41 | 0.54* | 0.07 | 0.56* | 0.56* | 0.63** | 0.29 | 0.50* | 0.72** | | | | | | | | | |
| camp | 0.47 | -0.01 | 0.35 | 0.30 | 0.34 | 0.13 | 0.56* | 0.28 | 0.28 | 0.66** | 0.66** | | | | | | | | |
| st7 | 0.60** | 0.17 | 0.04 | 0.24 | 0.39 | 0.34 | 0.29 | 0.03 | 0.39 | 0.62** | 0.48 | 0.66** | | | | | | | |
| stig | 0.10 | -0.30 | -0.02 | 0.14 | 0.09 | -0.20 | 0.29 | 0.02 | 0.10 | 0.35 | 0.25 | 0.62** | 0.37 | | | | | | |
| st8 | 0.32 | 0.21 | 0.23 | 0.44 | 0.27 | 0.30 | 0.32 | 0.30 | 0.27 | 0.36 | 0.42 | 0.54* | 0.56* | 0.37 | | | | | |
| st9 | 0.44 | 0.39 | 0.08 | 0.49 | 0.29 | 0.38 | 0.13 | 0.23 | 0.30 | 0.42 | 0.29 | 0.25 | 0.72** | 0.14 | 0.48 | | | | |
| sito | -0.09 | -0.42 | 0.42 | 0.001 | -0.31 | -0.44 | 0.003 | 0.24 | -0.33 | -0.09 | 0.12 | 0.30 | -0.10 | 0.42 | -0.07 | -0.20 | | | |
| st10 | 0.10 | 0.46 | 0.33 | 0.03 | -0.07 | 0.37 | -0.04 | 0.21 | -0.10 | 0.15 | 0.34 | 0.11 | -0.13 | -0.16 | -0.22 | -0.14 | 0.28 | | |
| dino | 0.67** | 0.30 | 0.19 | -0.07 | 0.55* | 0.48 | 0.51* | 0.19 | 0.54* | 0.53* | 0.50* | 0.22 | 0.42 | -0.09 | 0.26 | 0.35 | -0.23 | -0.08 | |
| C30tri | 0.08 | 0.24 | 0.28 | 0.08 | -0.05 | 0.14 | 0.06 | 0.22 | -0.05 | 0.20 | 0.37 | 0.26 | -0.10 | -0.02 | -0.14 | -0.12 | 0.31 | 0.77** | -0.23 |

| * | p < 0.01 |
| ** | p < 0.001 |

(Dachs *et al.*, 1999). The ratios are more than 1 in all sampling sites except for S1, S2 and S10 reflecting their terrigenous inputs.

Table 5.3: Trivial and systematic names of the sterols identified

| Abbreviation | Systematic names |
|---|---|
| st1 | 24 norcholesta-5,22(E)-dien-3β-ol |
| st2 | 24 nor-5α(H)-cholest-22(E)-en-3β-ol |
| cop | 5β-cholestan-3β-ol (coprostanol) |
| epicop | 5β-cholestan-3α-ol (epicoprostanol) |
| st3 | cholesta-5,22(E)-dien-3β-ol |
| st4 | 5α-cholest-22(E)-en-3β-ol |
| chol | cholest-5-en-3β-ol (cholesterol) |
| cholest | 5α-cholestan-3β-ol (cholestanol) |
| brass | 24-methylcholesta-5,22(E)-dien-3β-ol (brassicasterol) |
| st5 | 5α(H)-cholest-7-en-3β-ol |
| st6 | 24-methylenecholest-5-en-3β-ol |
| camp | 24-methylcholest-5-en-3β-ol (campesterol) |
| st7 | 24-methyl-5α(H)- cholestan-3β-ol |
| stig | 24-ethylcholesta-5,22(E)-dien-3β-ol (stigmasterol) |
| st8 | 24-methyl-5α(H)- cholest-7-en-3β-ol |
| st9 | 24-ethyl-5α(H)- cholest-22(E)-en-3β-ol |
| sito | 24-ethylcholest-5-en-3β-ol (sitosterol) |
| st10 | 24-ethyl-5α(H)- cholestan-3β-ol |
| dino | 4α,23,24-trimethyl-5α(H)- cholest-22(E)-en-3β-ol (dinosterol) |
| C30trienol | structure unknown |

The sterol source index (SSI) can be calculated by dividing a terrestrially derived sterol by a marine sterol *e.g.* cholesterol (Mudge and Norris, 1997). Cholesterol was used as the assumed marine sterol (Grimalt and Albaiges, 1990). β-sitosterol, stigmasterol and campesterol were used to calculate the indices. Stigmasterol/cholesterol and campesterol/cholesterol ratios had a similar distribution ($r$=0.71, $p$<0.001) to each other. The ratios for both indices were less than 1.0 throughout the sampling sites indicating that marine sterol was predominant in the study area. Meanwhile, samples collected within the lochs have a higher β-sitosterol/cholesterol ratio as shown in Figure 5.11. β-sitosterol/cholesterol ratios are strongly correlated with stigmasterol/cholesterol ratios ($r$=0.59, $p$<0.01) but not with campesterol/cholesterol ratios. Unfortunately the SSI does

163

not correlate positively with ASI suggesting that SSI alone cannot be used as indicator of terrestrially derived organic matter.
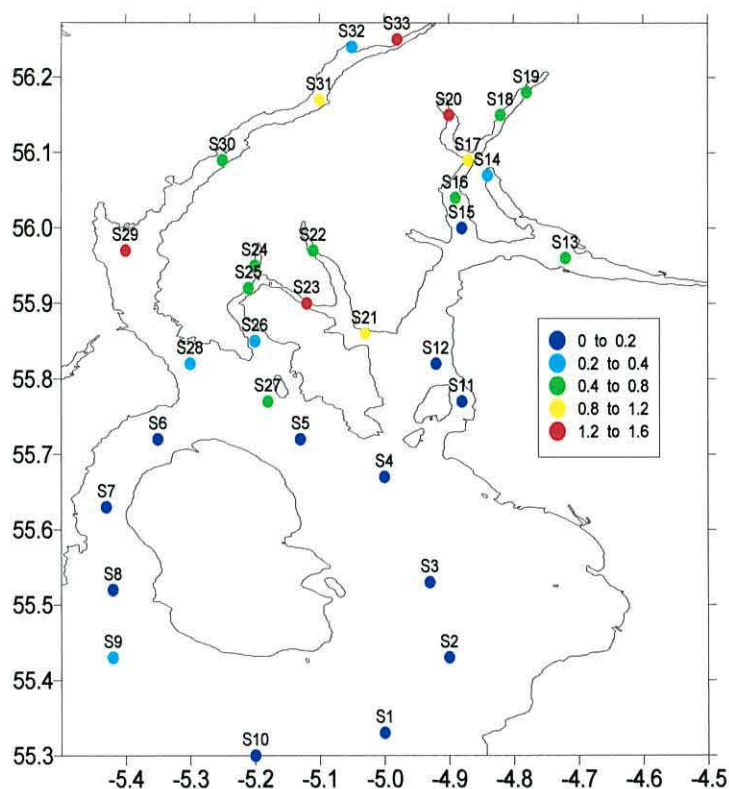


Figure 5.11:Distribution of β-sitosterol/cholesterol ratios throughout the surface sediment of the Clyde Sea

Coprostanol (5β-cholestan-3β-ol) has been used as a sewage tracer in a variety of environments (Venkatesan and Kaplan, 1990; Jeng and Han, 1994; Leeming and Nichols, 1998). Coprostanol is produced in the intestine of mammals by the bacterial transformation of cholesterol, therefore, it present in sewage contaminated waters and sediments. The coprostanol/cholesterol ratio is often used to indicate sewage sources (Grimalt and Albaiges, 1990; Nichols and Espey, 1991; Mudge and Bebianno, 1997; Mudge and Norris, 1997). Figure 5.12 shows the distribution of coprostanol/cholesterol ratios from the sampling sites. These ratios have similar distribution with percentage branched fatty acids ($r$=0.64, p<0.01), suggesting their link to sewage inputs. The highest coprostanol/cholesterol ratio can be seen at S13 reflecting deposition of sewage derived organic matter due to high population around this area. Meanwhile the ratio between
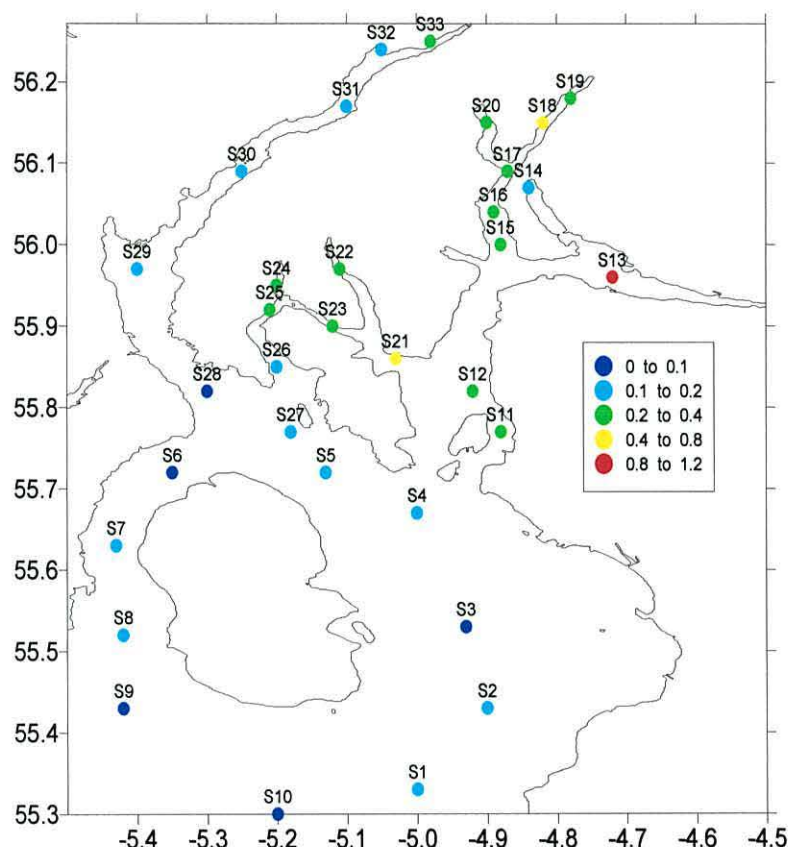
Figure 5.12: Distribution of coprostanol / cholesterol ratios throughout the surface sediment of the Clyde Sea

epicoprostanol and coprostanol can be used to indicate the degree of sewage treatment (Mudge and Lintern, 1999) as epicoprostanol is formed during anaerobic sludge digestion (McCalley *et al.*, 1981). Figure 5.13 shows the scatter plot of the epicoprostanol/coprostanol ratios and coprostanol/cholesterol ratios. Untreated sewage is high in the area such as S21, S18, S17 and S11 with low epicoprostanol/coprostanol ratios. Meanwhile sites as S4 and S27 have a high epicoprostanol/coprostanol ratios indicating the treated sewage in the sediment samples.
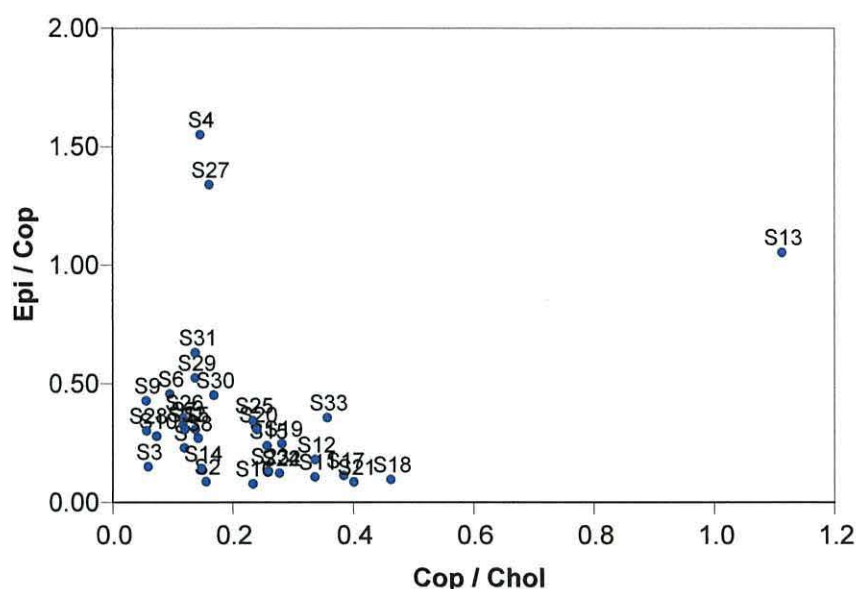
Figure 5.13: Distribution of epicoprostanol/coprostanol and coprostanol / cholesterol ratios throughout the surface sediment of the Clyde Sea

## 5.3 Multivariate statistical analysis

### 5.3.1 Principal component analysis

PCA was carried out in order to get a further insight into the relationships between samples and lipid classes. All the data was transformed into proportion with 0.001 added and then log transformation.

### 5.3.1.1 Fatty acids

Two principal components account for 38.3% of the variance in the data set. The loadings of PC1 (23.7%) and PC2 (14.6%) can be seen in Figure 5.14a. Branched fatty acids originating from bacteria are loaded positively on PC1, while long chain fatty acids indicate terrestrial markers are significantly negatively loaded. Phytoplankton and marine markers such as polyunsaturated fatty acids, short chain acids and 16:1ω7 are positively loaded on PC2. From the score plot (Figure 5.14b) S13 contain most of the bacterial derived fatty acids. Samples that project to the left are enriched with terrestrial markers and samples at the top of the diagram indicating marine inputs.
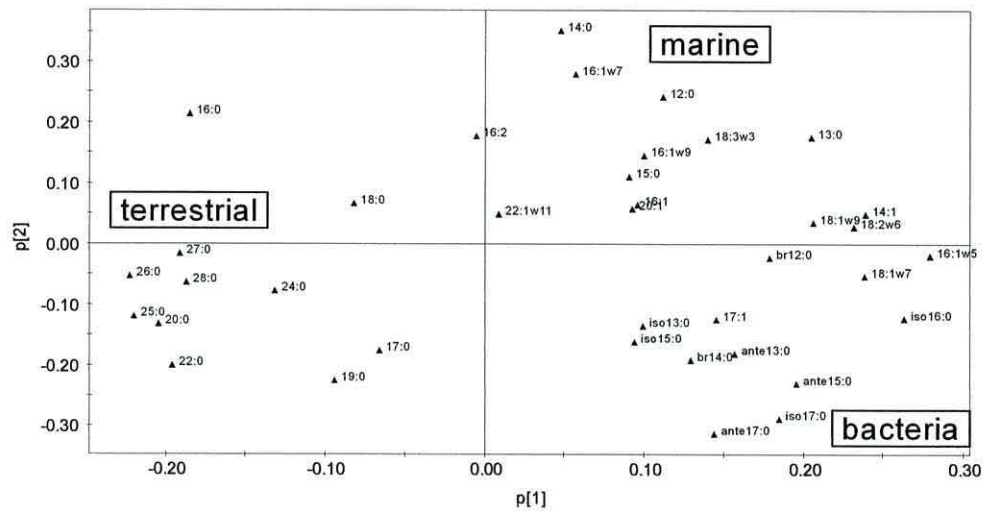
Figure 5.14a: The loadings of each fatty acid on PC1 and PC2 for the Clyde Sea samples
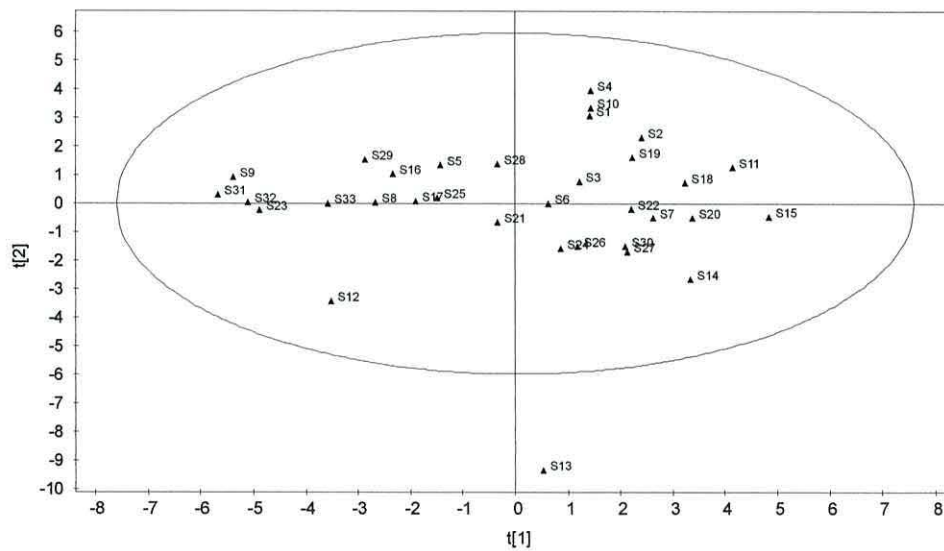


Figure 5.14b: The PCA scores for the first two components in the PCA model of the Clyde Sea samples

The first two principal components are plotted spatially using classed posting map to show those samples that project positively and those that project negatively. Figure 5.15a with PC1 shows samples that were rich in bacterial and marine derived fatty acids (positive

167
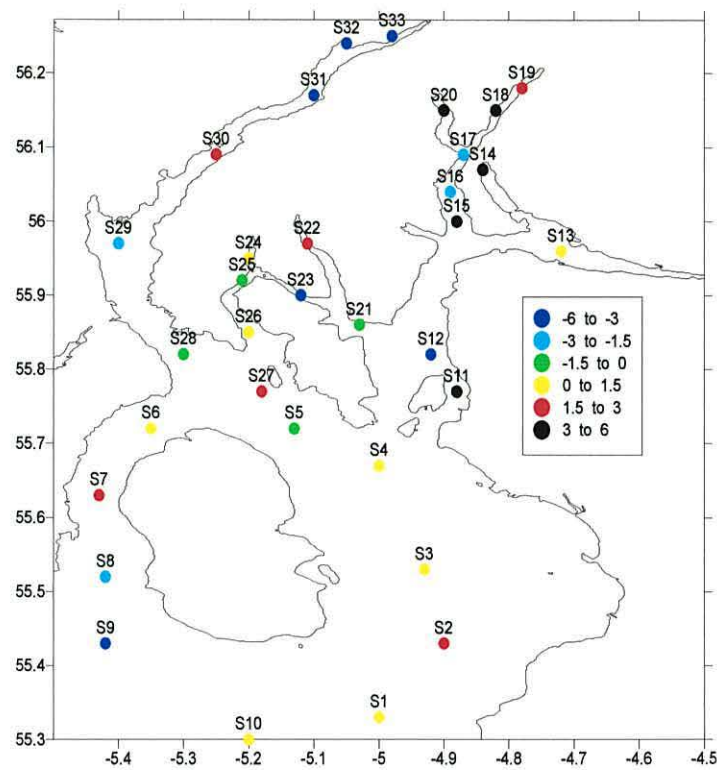
Figure 5.15a: A classed posting of scores on PC1
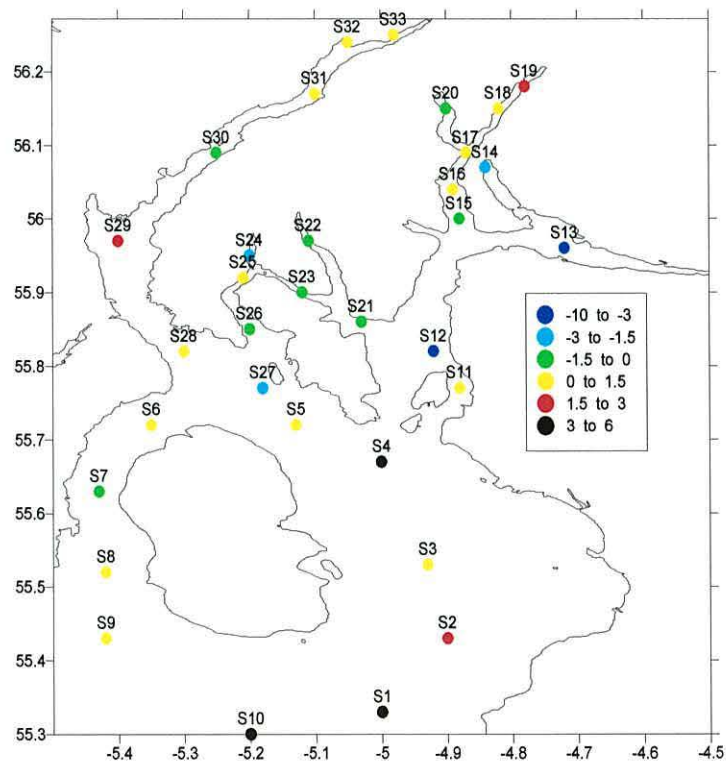


Figure 5.15b: A classed posting of scores on PC2

scores) are distinguished from those that are influenced by terrestrial markers (negative scores). S31, S32 and S33, which are the Loch Fyne samples, have the highest negative scores showing that Loch Fyne is rich with terrestrial inputs. PC2 (Figure 5.15b) distinguished samples with short chain fatty acids (high positive scores) with samples enriched with bacterial derived fatty acids (high negative scores).

### 5.3.1.2 Fatty alcohols

The first two PCs account for 31.6% of the total variance. Geochemical features are not really clear with fatty alcohols. The loadings of each individual compound are shown in Figure 5.16a and the scores are shown in Figure 5.16b. PC1 and PC2 account for 16.2% and 15.4% of the variance in the data. Compounds with the positive loadings on PC1; short chain fatty alcohols and monounsaturated alcohol have a marine source predominate the marine samples as illustrated in Figure 5.16b. Branched fatty alcohols are loaded negatively on PC1, together with long chain fatty alcohols. Phytol, which is a potential marker for chlorophyll has a negative loadings with the terrestrial markers. Therefore it can be assumed that phytol in this sampling area has a terrigenous source. However, phytol did not have strong correlations with these compounds. Again from the score plot (Figure



Figure 5.16a: The loadings of each fatty alcohol on PC1 and PC2 for the Clyde Sea samples

5.16b), S13 contain the greatest amount of bacterial derived organic matter. Samples that are clustered on the bottom side of the diagram are associated with terrestrial markers while marine derived fatty alcohols influence samples on the top right such as S1, S2, S3, S4 and S10.
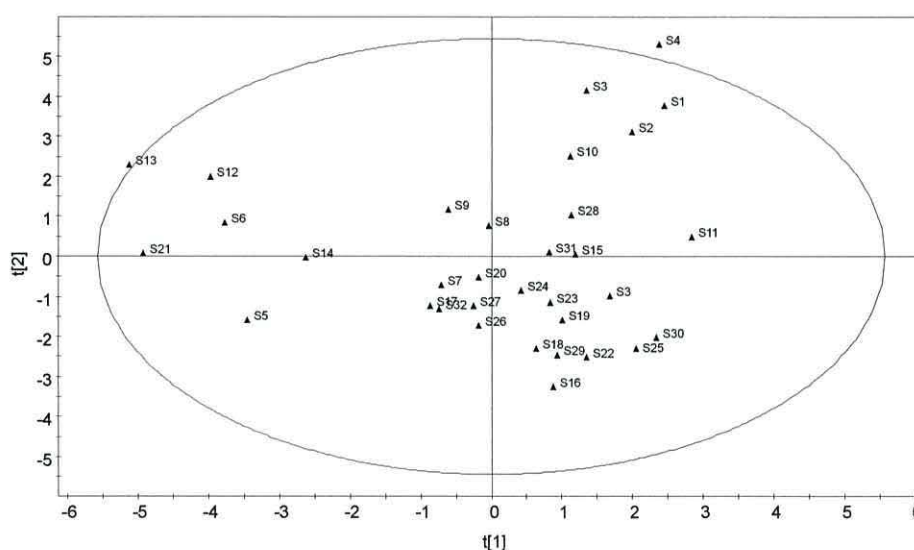


Figure 5.16b: The PCA scores for the first two components in the PCA model of the Clyde Sea samples

### 5.3.1.3 Sterols

PC1 and PC2 account for 27.3% and 16.9% of the variance in the data. Figure 5.17a shows the loadings on the first two components. Algae markers such as brassicasterol, dinosterol and st1 are loaded positively on PC1 while terrestrial derived sterols (sitosterol, stigmasterol and campesterol) are negatively loaded. Meanwhile, sewage markers are positively loaded on PC2. Stigmasterol and campesterol are loaded positively on PC1 together with cholesterol, which can be found in all marine organisms. These sterols have been found in phytoplankton, therefore they are grouped accordingly. Figure 5.17b shows the scores of PC1 and PC2. Samples that are clustered on the top of the diagram such as the Loch Fyne sites contain terrestrial organic matter while marine markers influence samples that are projected on the bottom right. These are all the "offshore" sites. However

S13, which is associated with sewage markers, is clustered together with the inner Clyde sites.
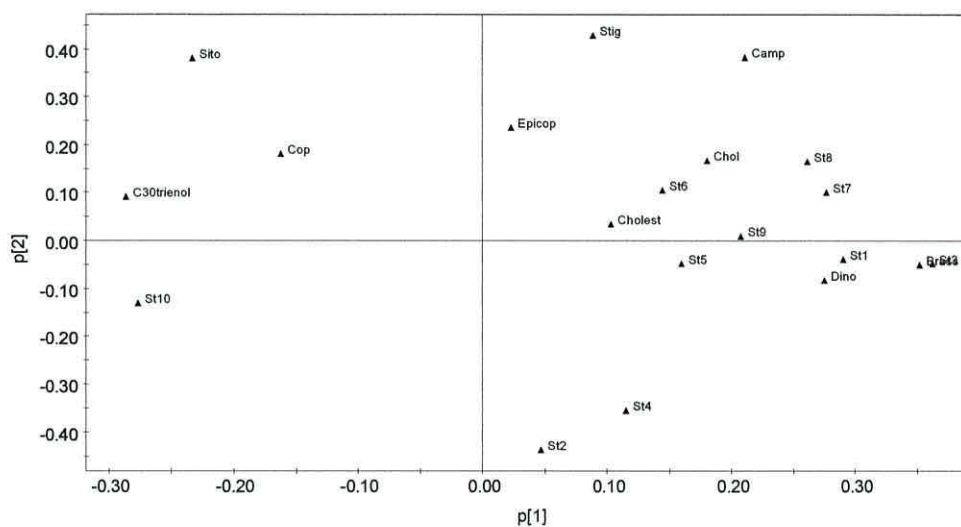


Figure 5.17a: The loadings of each sterol on PC1 and PC2 for the Clyde Sea samples
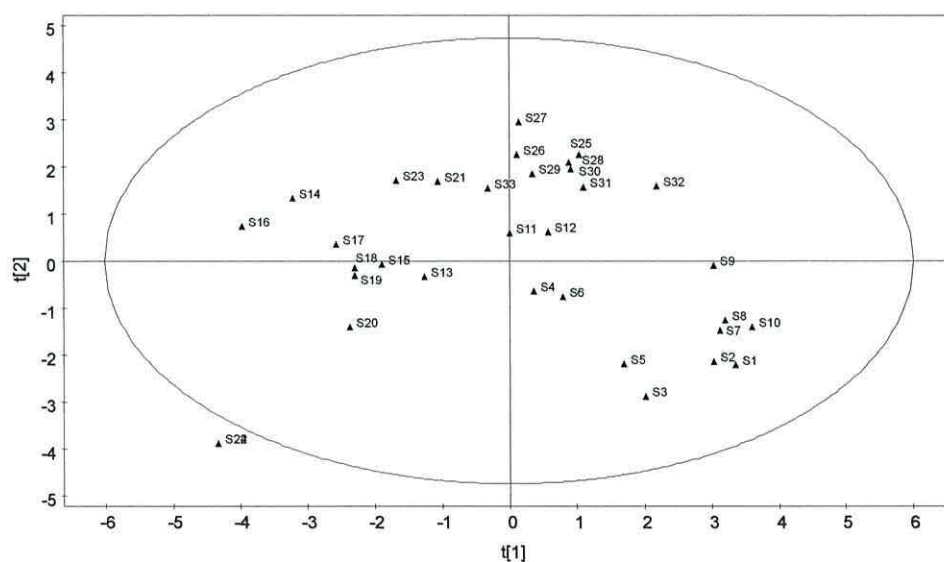


Figure 5.17b: The PCA scores for the first two components in the PCA model of the Clyde Sea samples

**5.3.1.4 Mixed PCA**

All the data was transformed into proportion. PCA was first carried out on the proportion data without the transformation. The proportion data then had 0.001 added and log transformed before second PCA was performed.

a) Proportion data without transformation

Percentage of variance explained in the data for PC1 and PC2 are 14.7% and 11.4% respectively. Figure 5.18a shows the loadings of each compound on PC1 and PC2. Branched fatty alcohols and fatty acids together with coprostanol and epicoprostanol are loaded positively on PC1. Meanwhile marine derived organic matter such as short chain fatty alcohols (C11 and C12), short chain fatty acids (12:0, 13:0 and 14:0) have negative loadings. On PC2, terrestrial derived compounds such as long chain fatty alcohols, long chain fatty acids, $\beta$-sitosterol and stigmasterol are positively loaded.



Figure 5.18a: The loadings of each compound on PC1 and PC2 for the Clyde Sea samples with proportion data (no transformation)

The plot of scores (Figure 5.18b) shows that S13 contain the greatest amount of bacterial and sewage derived organic matter. Samples such as S1, S2, S3, S4 and S10 are associated with marine markers while samples that clustered on the top of the diagram are enriched with terrestrial inputs.

172

Figure 5.18b: The PCA scores for PC1 and PC2 in the PCA model of the Clyde Sea samples
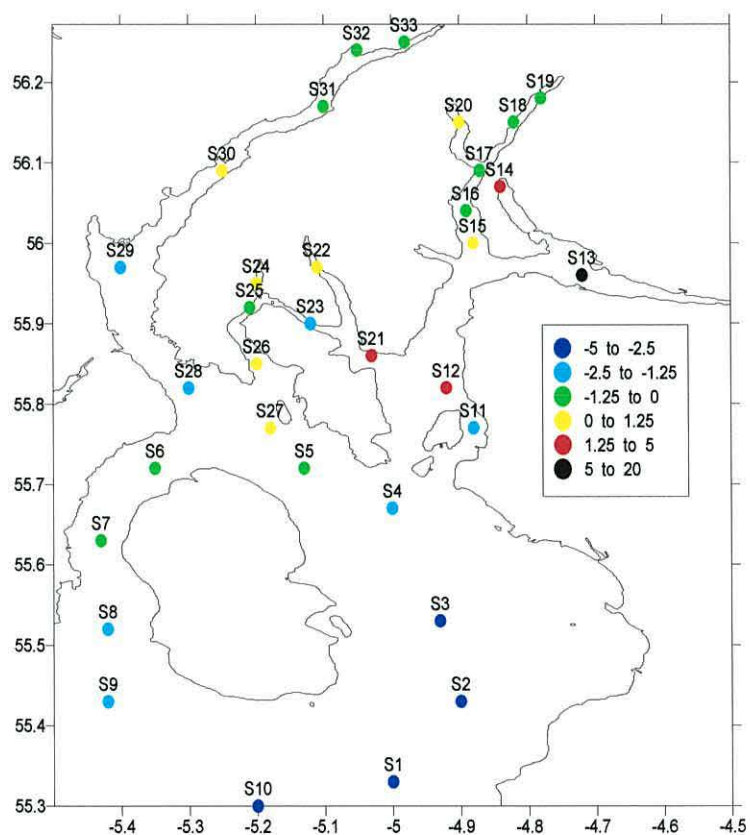


Figure 5.19a: A classed posting of scores on PC1

Figure 5.19a and Figure 5.19b are plotted spatially showing samples projected positive and negatively on each principal component. Figure 5.18a with PC1 shows that S13, which is rich in bacterial and sewage organic matter (black spot) is clearly distinguished from those that are associated with marine derived markers (S1, S2, S3 and S10 with dark blue spots). On the other hand, PC2 distinguishes terrestrial and marine samples (Figure 5.19b).
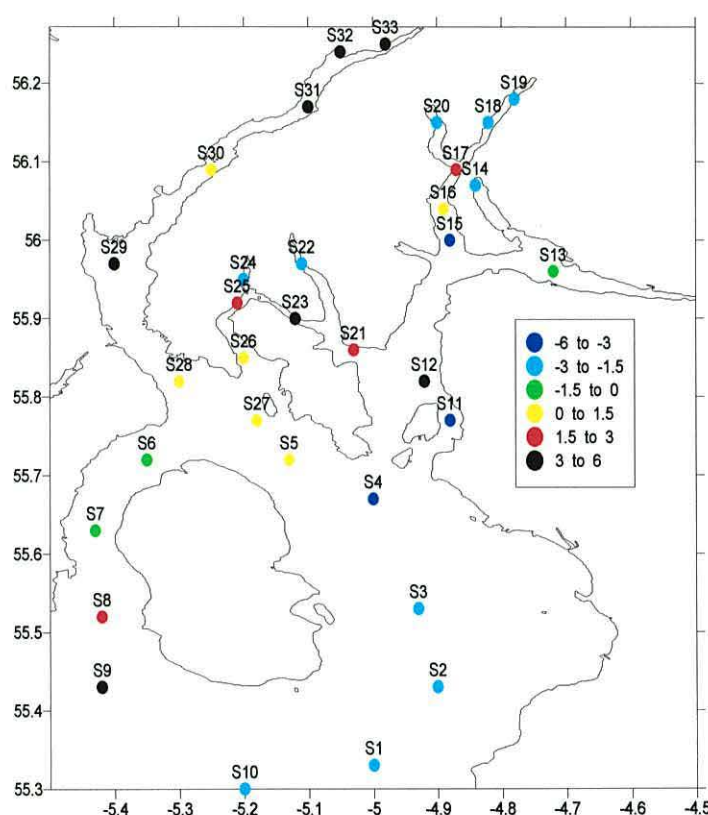


Figure 5.19b: A classed posting of scores on PC2

b) Proportion data, 0.001 added and log transformed

Figure 5.20a shows the loadings on PC1 and PC2, which account for 13.2% and 12.7% of the total variance in the data. The result was similar with the PCA carried out with the proportion data (no transformation) except for PC2. Marine derived compounds such as short chain fatty acids, 16:1$\omega$7 acid, short chain fatty alcohols and brassicasterol are loaded positively on PC2, while terrestrial markers (long chain fatty alcohols, long chain fatty acids and stigmasterol) have negative loadings. The bacterial and sewage markers such as

branched fatty acids, 18:1ω7 acid, branched fatty alcohols and coprostanol are positively loaded on PC1.



Figure 5.20a: The loadings of each compounds on PC1 and PC2 for the Clyde Sea samples with proportion data, added 0.001 (log transformation)



Figure 5.20a: The PCA scores for PC1 and PC2 in the PCA model of the Clyde Sea samples

From the score plot (Figure 5.20b), S1, S2, S3, S4 and S10 contain marine derived organic matter. Samples projecting towards the bottom left are influenced by terrestrial organic matter while samples projecting towards the bottom right especially S13 are associated with bacterial and sewage markers.

Classed posting diagram on PC1 (Figure 5.21a) shows samples that project positively are rich in bacterial or sewage markers, while samples that project negatively are associated with terrestrial derived compounds. Figure 5.21b with PC2 shows that marine marker influence samples with high positive scores, while samples with high negative scores are rich with terrestrial markers.
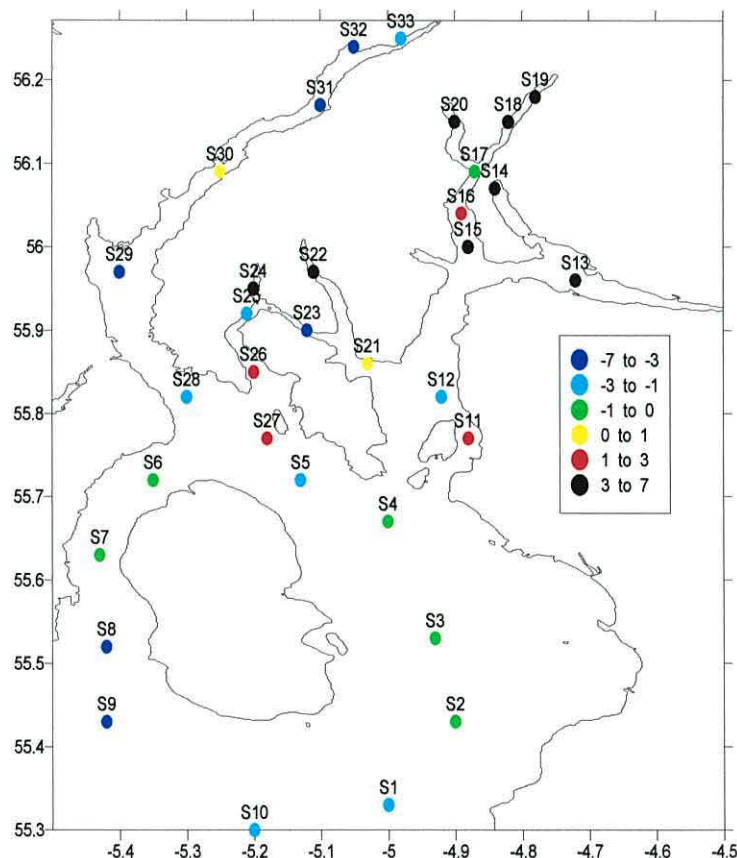


Figure 5.21a: A classed posting of scores on PC1
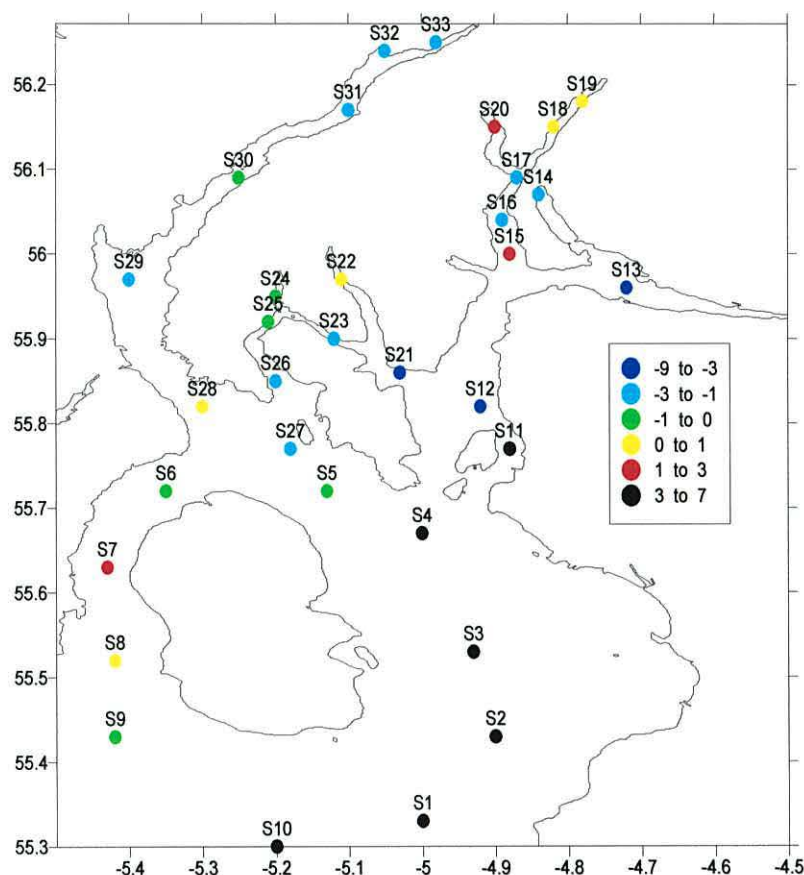
Figure 5.21b: A classed posting of scores on PC2

## 5.3.2 Partial least squares (PLS) path modelling

PLS was carried out on all compounds from the sampling sites to characterise the contribution of biomarkers from the signature block to the other sampling sites. S31, S32 and S33 from Loch Fyne were used as terrestrial signatures (*X*-blocks) and the rest of the sites as *Y*-blocks. S1, S2 and S10 were used as the *X*-block in a PLS model to characterise the marine signatures throughout the Clyde Sea. Meanwhile, to characterise the sewage/bacteria signatures, sites S12, S13 and S21 were used as the *X*-block. The signatures were chosen based on the score plot of the mixed compound PCA. The plot of scores (Figure 5.18b) shows that S12, S13 and S21 contain the greatest amount of bacterial and sewage derived compounds. Samples S1, S2 and S10 are influenced with marine markers while samples that clustered on the top of the PCA plot (S31, S32 and S33) are enriched with terrestrial inputs.

Figure 5.22a shows the comparison of the *X*-block (t1) and *Y*-block (u1) projections for PLS when S31, S32 and S33 were used as terrestrial signatures. The projection was basically linear with sterols such as st7, st8, stigmasterol and β-sitosterol having the largest distant below the line of agreement. These compounds can be found in higher plants. Meanwhile branched fatty acids (bacterial derived compounds) such as *iso*-15:0 and *anteiso*-15:0 have the largest distant above the 1:1 line. From the classed posting map shown in Figure 5.22b, terrestrial signatures are low at sites S3, S4, S5, S13 and S14 with only 0-60% of terrestrial signatures were transported to these sites. These results coincided with the projection plot (Figure 5.22a) where terrestrial derived compounds such as stigmasterol and β-sitosterol behave differently with bacterial derived compounds (branched fatty acids). Terrestrial signatures from S31, S32 and S33 were transported down the Loch Fyne and entered the North Channel following the outflow current that leaves at the southern end of the Kintyre peninsula. These signatures were also transported to the sampling sites around the Isle of Bute. For example 80-90% of the signatures occurred at S25.
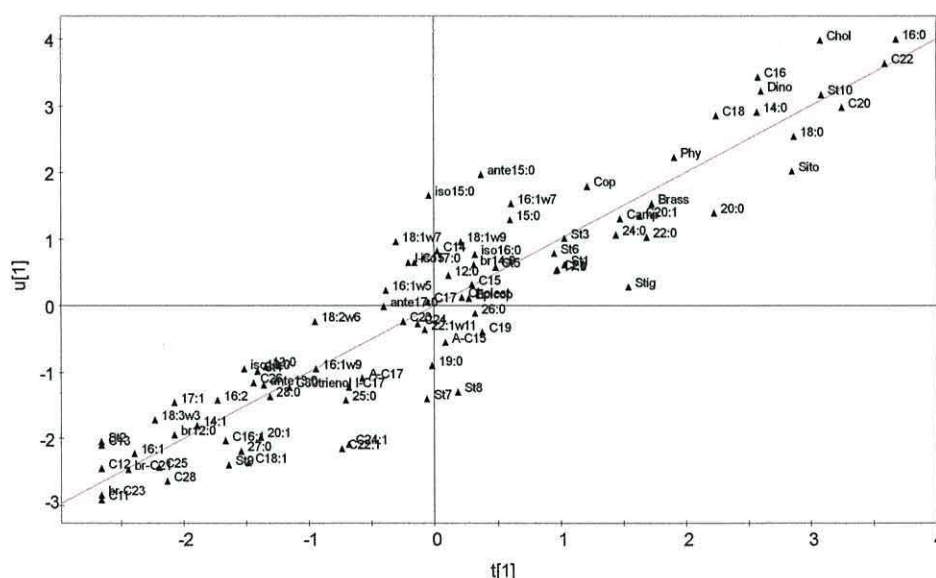


Figure 5.22a: Comparison of t1 and u1 projections for PLS with S31, S32 and S33 as terrestrial signatures
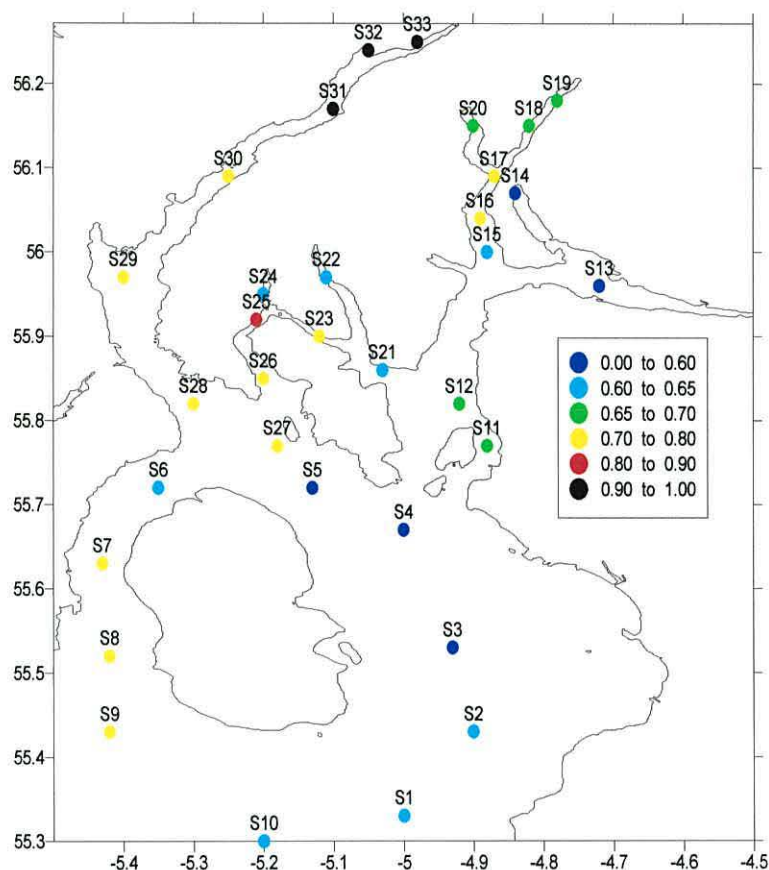
Figure 5.22b: A classed posting map showing the contribution of terrestrial signatures from a PLS model

S1, S2 and S10 were chosen to characterise marine contribution throughout the Clyde Sea samples. The plot of the *X*-block (t1) and *Y*-block (u1) is shown in Figure 5.23a. The plot was essentially linear with marine derived compounds such as polyunsaturated fatty acid 16:2 and C12 fatty alcohol having the highest distant below the 1:1 line. Meanwhile β-sitosterol had the largest distant above the line of agreement. These results show that these compounds behave differently in the two blocks. Percentage of contribution of marine signatures to the other sampling sites is shown in Figure 5.23b. The marine signatures decrease towards the sea lochs except samples S31, S32 (Loch Fyne), S18 (Loch Long) and S20 (Loch Goil), which had 70-80% of the signatures. Marine signatures were transported by current inflow from the North Channel into the Clyde Sea at its southern entrance. Within the Clyde Sea, there is a clockwise circulation around the Island of Arran

with an anticlockwise circulation to the east close to the Scottish coast. Sample S3 illustrates the similarity with the signatures with having 80-90% of signature contribution.
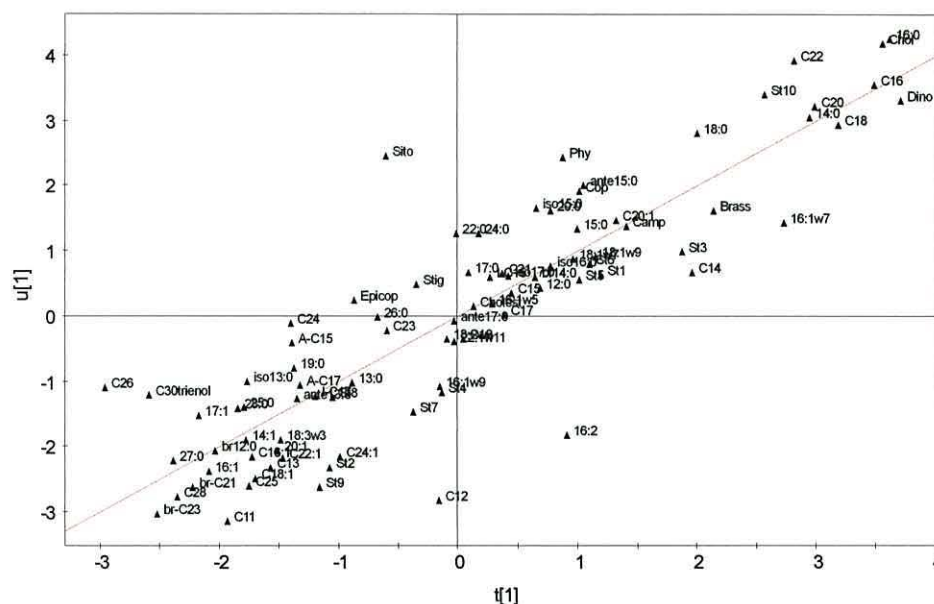


Figure 5.23a: Comparison of t1 and u1 projections for PLS with S1, S2 and S10 as marine signatures
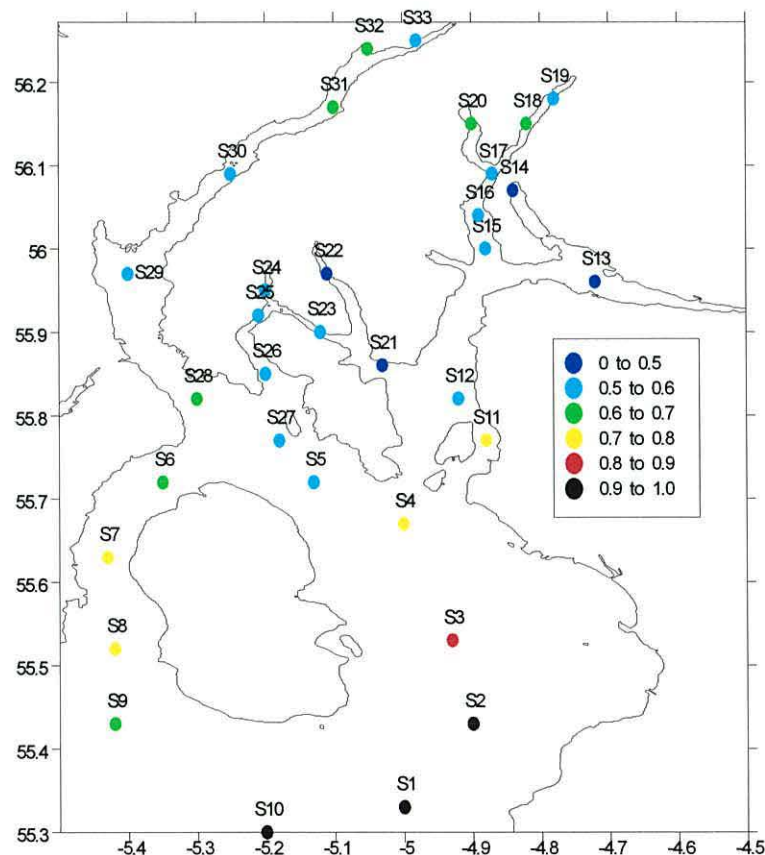


Figure 5.23b: A classed posting map showing the contribution of marine signatures from a PLS model

To characterise sewage/bacteria signatures, S12, S13 and S21 were chosen to be the *X*-block. Figure 5.24a shows the plot of t1 and u1 with the line of agreement showing that C13, *iso*-C17 and *anteiso*-C17 fatty alcohols (bacterial derived compounds) have the largest distant below the 1:1 line. Long chain fatty alcohol, $C_{26}$, was also clustered with this group of compounds. This may be because not only sewage (bacteria) derived compounds, but terrestrial matter also comes in through the sewage route. Meanwhile monounsaturated 20:1 alcohol and phytol (marine derived compounds) have the large distant above the line of agreement.
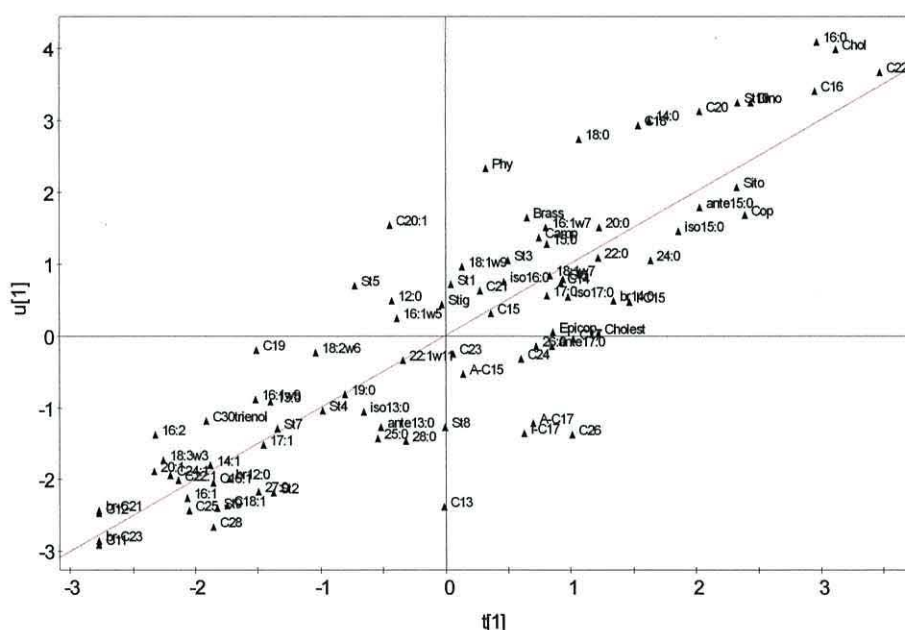


Figure 5.24a: Comparison of t1 and u1 projections for PLS with S12, S13and S21 as sewage signatures

A classed posting map (Figure 5.24b) shows the contribution of sewage (bacteria) signatures throughout the Clyde Sea sampling sites. Sewage signatures are low at the open sea sampling sites (S1, S2, S3, S4 and S10) and this corresponds with the projection plot where bacterial derived compounds behave differently with marine derived compounds such as 20:1 alcohol and phytol. River Clyde and other river sources are responsible for over 60% of the freshwater supply to the Clyde Sea (Poodle, 1986) and drain much of agricultural and industrial western central Scotland, including the large conurbation around

Glasgow. Therefore much of the freshwater entering Clyde Sea is rich with nutrients from agricultural fertilisers and domestic sewage (Haig, 1986). Hence it is possible to conclude that sewage signatures from S12, S13 and S21 will be travelling through the North Channel through the anticlockwise current movements around the Isle of Arran. 60-80% of sewage signatures were transported to Gare Loch, Loch Goil and Loch Long. This may be happening during high tide where sewage materials go up to these sea lochs.
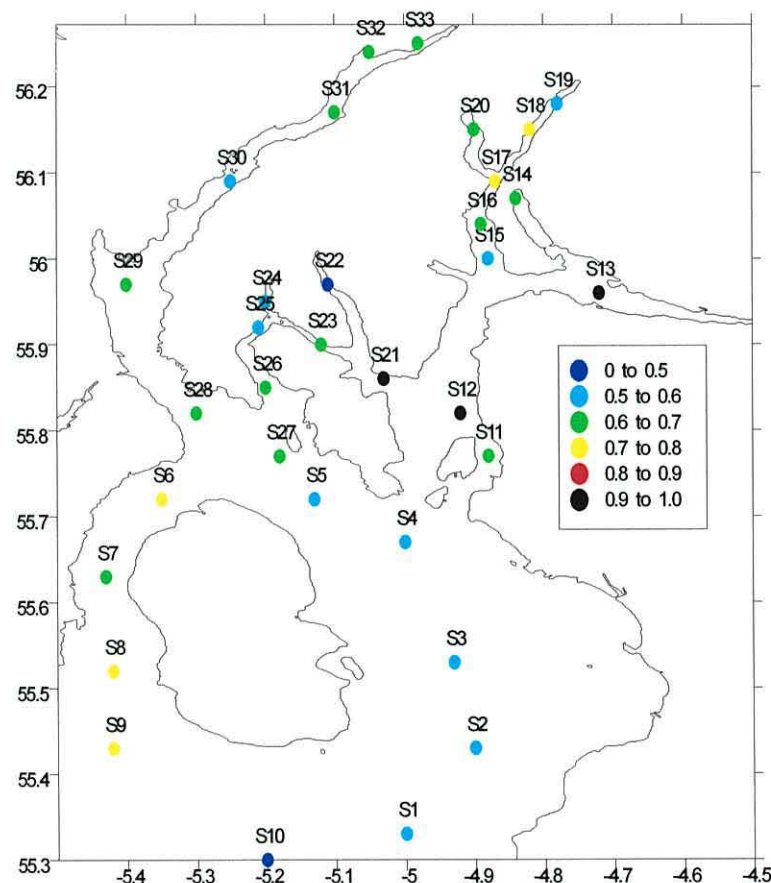


Figure 5.24b: A classed posting map showing the contribution of sewage signatures from a PLS model

There is some overlap between the X-block (signatures) with the Y-block as most molecular markers are common to several types of organisms, both terrestrial and marine. Therefore some markers will have multiple origins. The overlap matrix for PLS model in the Clyde Sea samples is given in Table 5.5. For example, 60% of marine signatures

182

appeared within samples that high in bacterial derived markers and 64% in terrestrial derived markers, respectively, meaning that the marine signature can explain 60% and 64% of the variance in the bacterial and terrestrial signatures because of the similarity of compounds occurred throughout the sampling sites. This fitting is direction dependent. 62% and 50% of bacterial signatures are overlapped in marine and terrestrial samples, reflecting that the bacteria signature describes 62% and 50% of the variance in marine and terrestrial signatures when PLS carried out with bacteria signature. When PLS characterising terrestrial signature, 56% and 41% of the variance in the marine and bacteria signatures were explained by it.

Table 5.5: Overlap matrix for PLS in the Clyde Sea samples

|  | *X*-block | | |
|---|---|---|---|
|  | Marine | Bacteria | Terrestrial |
| Marine | x | 0.62 | 0.56 |
| Bacteria | 0.60 | x | 0.41 |
| Terrestrial | 0.64 | 0.50 | x |

### 5.3.3 Cluster analysis

Cluster analysis of variables and observations were carried out in this study using the Ward's method. Fatty acids, fatty alcohols and sterols were used separately in cluster analysis. Cluster analysis was employed to classify the study area into specific region, each having definite characteristics.

### 5.3.3.1 Fatty acids

The output of the cluster analysis is given as a dendogram (Figure 5.25). There are two major cluster groups divided into 2 smaller subgroups. The first group in cluster group I corresponds to branched fatty acids indicating bacterial markers. The other subgroup consists of marine and algal derived compounds. Meanwhile long chain fatty acids are grouped together within cluster group II associating their terrestrial origin.

Similarity
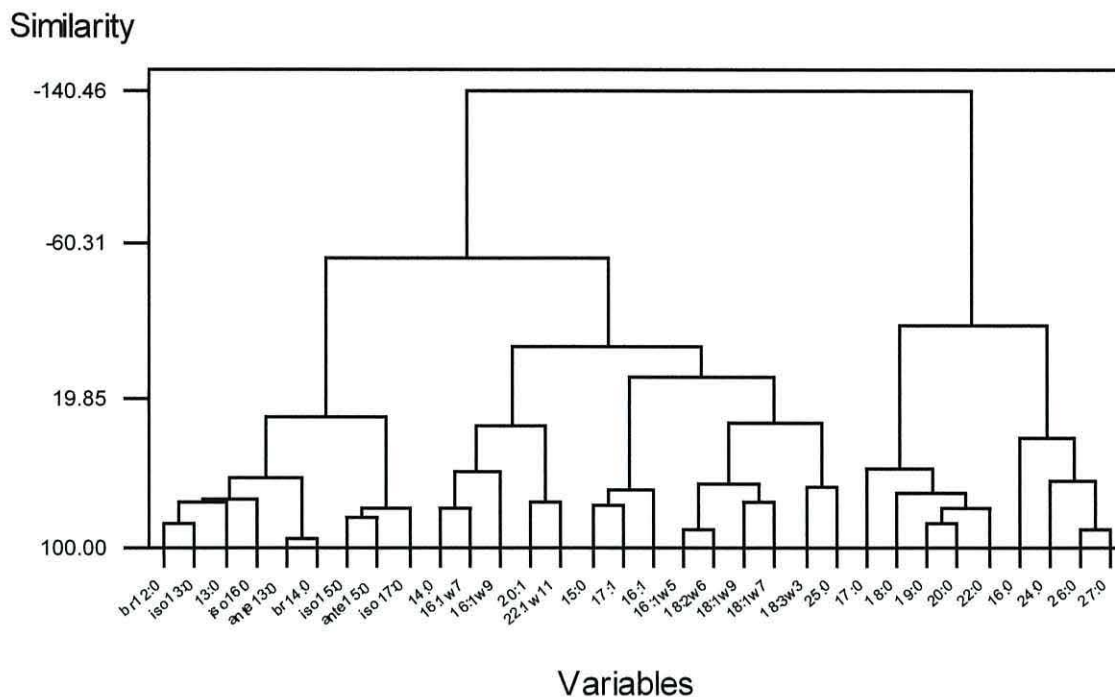


Figure 5.25: Cluster analysis of fatty acids showing the Ward's method for the Clyde Sea samples

## 5.3.3.2 Fatty alcohols

Figure 5.26 shows the dendogram from fatty alcohols cluster analysis. The results are more complex to interpret. There are 3 major cluster groups as shown in Figure 5.25. Cluster group I corresponds to bacterial derived fatty alcohols with branched fatty alcohols clustered together. Meanwhile cluster group II consists of short chain fatty alcohols, therefore this cluster is associated with marine markers. Cluster group III is more complicated as marine, bacterial and terrestrial derived compounds are clustered in one group. Terrestrial derived compounds such as long chain fatty alcohols are also found to be clustered within cluster group I and cluster group II. On the other hand, monounsaturated fatty alcohols are grouped with cluster group I and cluster group III.

Similarity
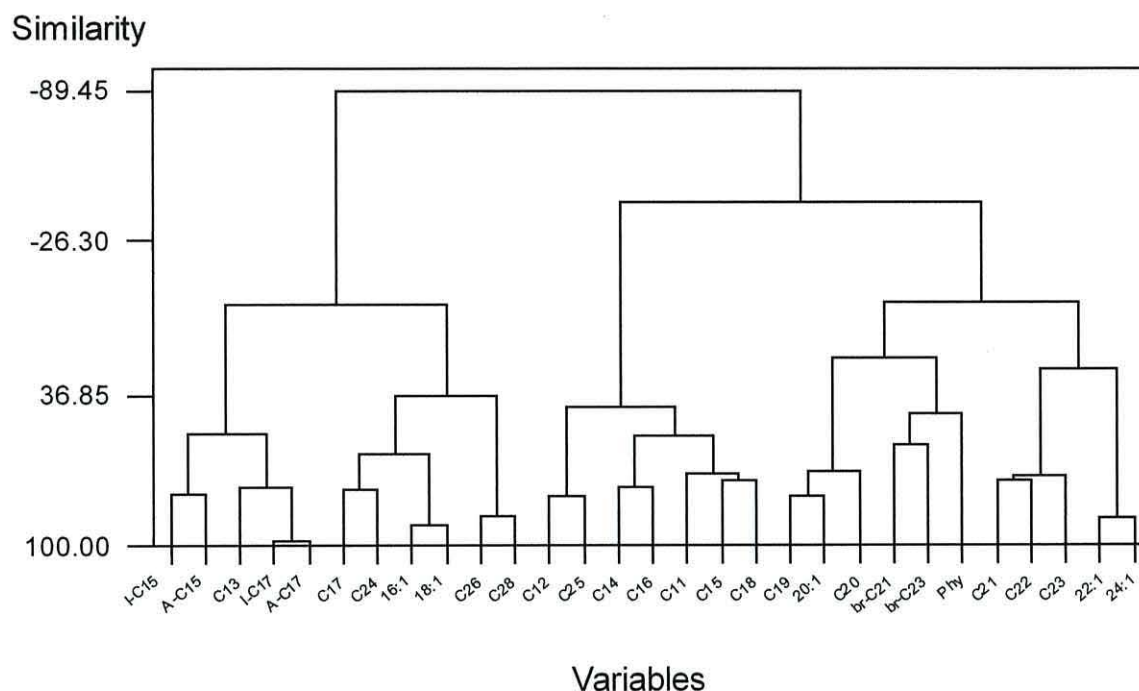


Figure 5.26: Cluster analysis of fatty alcohols showing the Ward's method for the Clyde Sea samples

### 5.3.3.3 Sterols

The cluster analysis results of the sterols are shown in Figure 5.27. Altogether 4 clusters could be explained divided into two bigger cluster groups. Group 1 in the cluster group I contains marine derived sterols such as st1, brassicasterol, cholesterol and dinosterol. Meanwhile the second group include the terrestrial markers (stigmasterol and campesterol). The first group in cluster group II indicates sewage derived markers as coprostanol, epicoprostanol, st9 and cholestanol are clustered together. In the second group of cluster group II, again, the terrestrial derived compounds are clustered together within this group (β-sitosterol, st10 and C30 trienol).
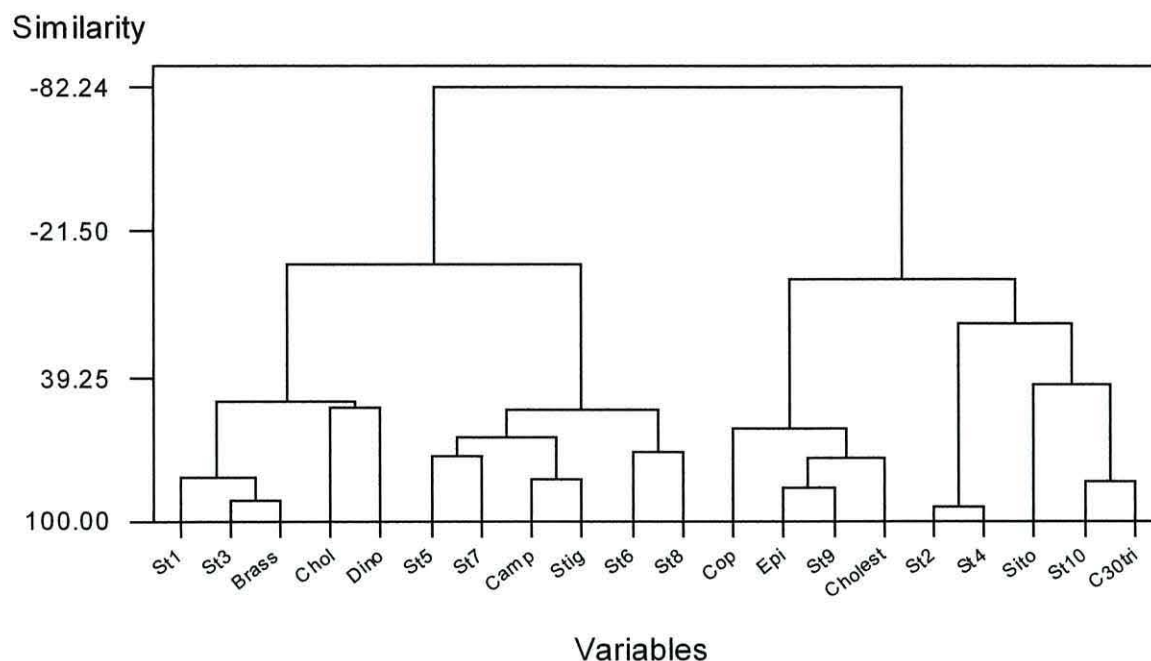
Figure 5.27: Cluster analysis of sterols showing the Ward's method for the Clyde Sea samples

## 5.3.3.4 Mixed compounds

Two main cluster groups were identified from the dendogram (Figure 5.28a). Within the first cluster group, there are two separate clusters. First cluster includes short chain fatty acids and fatty alcohols as well as branched fatty acids. These compounds are abundant in marine organisms and in bacterial biomass. Meanwhile the second cluster consists of marine derived fatty alcohols, fatty acids and sterols. It can be concluded that cluster group I contain a mixture of marine and bacterial derived compounds. The second cluster group includes the terrestrial derived compounds as well as sewage biomarkers such as coprostanol.

Similarity
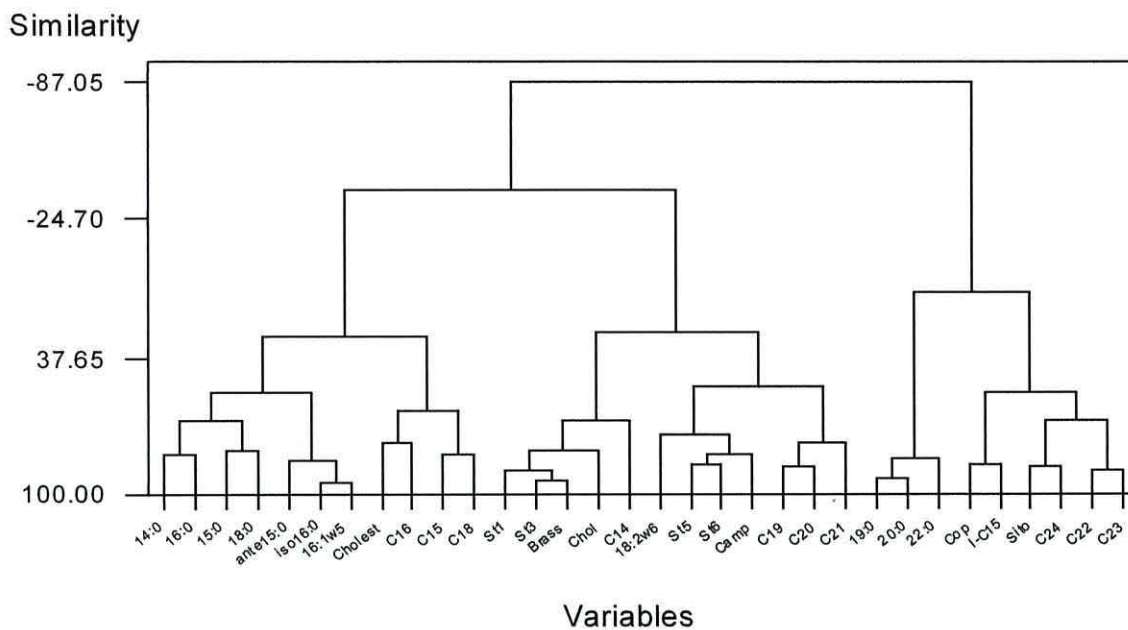


Figure 5.28a: Cluster analysis of mixed compounds showing the Ward's method for the Clyde Sea samples

Similarity
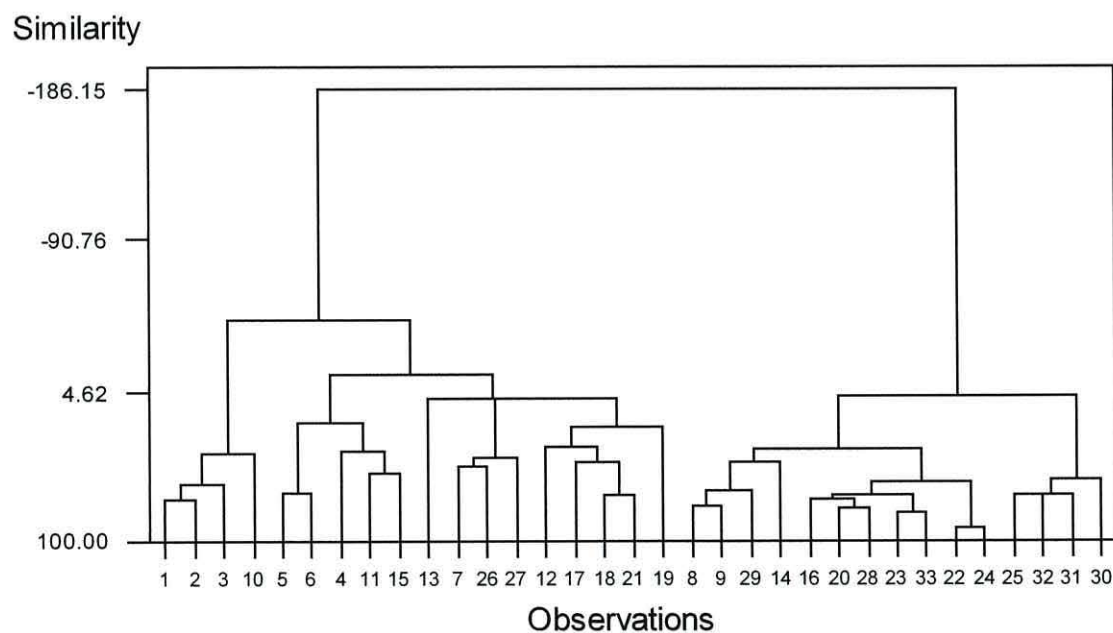


Figure 5.28b: Cluster analysis of sampling sites showing the Ward's method for the Clyde Sea samples

Two main groups were distinguished in the Ward's method dendrograms for the sampling sites (Figure 5.28b). The first cluster group includes samples mainly located in the open seas including samples collected from Loch Long, while the second cluster group consists of samples collected within the sea lochs especially from Loch Fyne. Generally, the CGI corresponds to samples characterised by marine and bacterial derived compounds such as short chain fatty acids and fatty alcohols, saturated $18:2\omega6$, cholesterol, st1, st3 and brassicasterol. The cluster group II consists of samples collected within the lochs. These samples characterised by terrestrial and sewage derived compounds such as long chain fatty acids and fatty alcohols, sitosterol, coprostanol and cholestanol. However, two open sea samples (S8 and S9) were also clustered together with this group not with cluster group I.

## 5.4 Discussion

Although molecular lipid constituents such as fatty acids, fatty alcohols and sterols represent a minor fraction of the total organic carbon, they are known to convey important information on the input of organic matter in the marine environments. A total of 35 fatty acid compounds, 29 fatty alcohols and 20 sterols were identified from the 33 sampling sites around the Clyde Sea.

Fatty acids are major components of most organisms, and hence fatty acids are found commonly recognised in a variety of geochemical samples. Long chain saturated fatty acids are the dominant components in terrestrial higher plant wax, suberin and cutin. Long chain fatty alcohols are also characteristic of land-plant epicuticular waxes (Fukushima and Ishiwatari, 1984; Meyers and Ishiwatari, 1993). Therefore the occurrence of long chain fatty acids and fatty alcohols in marine environments is often interpreted as terrestrial input through riverine or aeolian transport. Fatty acids with chain lengths up to 28:0 were quantified in the sediment samples with saturated 24:0 and 26:0 were the most abundant. Fatty alcohols with similar chain lengths were also identified in the Clyde Sea samples. Some microalgae, on the other hand, have also been found to synthesise such long chain components as well (Volkman *et al.*, 1980, 1998; Venkatesan and Kaplan, 1987).

In general, short chain saturated fatty acids are considered to originate from algae (Carrie *et al.*, 1998; Rohjans *et al.*, 1998; Mudge *et al.*, 1998). The short chain saturates were dominated by 14:0, 16:0 and 18:0 acids. Saturated 14:0 acid is among the major lipid components of phytoplankton, especially the Bacillariophyceae and Prymniophyceae (Reitan *et al.*, 1994). The saturated 16:0 acids were the principal fatty acid measured. This was not surprising as it is the major fatty acid in many organisms such as phytoplankton, higher plants and wastewater discharged from the sewage plants (Reitan *et al.*, 1994; Quemeneur and Marty, 1994; Mudge *et al.*, 1998). In this study, 3 monounsaturated 16-carbon fatty acids (16:1$\omega$5, 16:1$\omega$7, 16:1$\omega$9) were identified. Monounsaturated 16:1 acid is common in algal species (Reitan *et al.*, 1994). 16:1$\omega$7 is the major monounsaturated 16:1 acid, and it is abundant in phytoplankton especially diatom (Volkman *et al.*, 1989; Dunstan *et al.*, 1994; Reitan *et al.*, 1994). Polyunsaturated fatty acids are normally associated with phytoplankton. For example, green algae contain abundant of $C_{16}$ and $C_{18}$ polyunsaturated fatty acids especially with the positional isomers $\omega$3 and $\omega$6 (Volkman *et al.*, 1989; Carrie *et al.*, 1998). Relatively high concentrations of 18:3$\omega$3 and 18:2$\omega$6 polyunsaturated fatty acids were detected in the sampling sediments. A high percentage of monounsaturated fatty acids can be seen at sampling sites S1, S2, S3 and S10, while S1 and S10 also have high value of polyunsaturated fatty acids. Short chain saturated fatty acids and unsaturated fatty acids were high in concentration at S1, S2, S3 and S10, which are situated at the open seas. The same observation can be seen with the 16:1$\omega$7/16:0 ratios that have been used as indicator for diatoms (Skerratt *et al.*, 1995; Mudge *et al.*, 1998). These ratios are high at the open sea sampling sites.

Short chain fatty alcohols are often used as marine indicators (Grimalt and Albaiges, 1990; Mudge and Norris, 1997). Short chain fatty alcohols were more prominent than the longer chain compounds in the Clyde Sea sediment samples. These observations were shown by the short/long ratios for fatty alcohols where the ratios are generally > 1.0, indicating that the shorter chain homologues were more predominant than the longer chain compounds. The short/long ratios for fatty acids have a similar distribution with the same ratios of fatty alcohols. The ratios are > 1.0 in most samples. This is not unexpected since all samples were collected from marine environments although it is interesting to observe gradients even within areas that may be considered "marine". S10, which is an open sea sample, has

the highest value of short/long ratios of fatty acids and fatty alcohols. Another indices, the ASIs, were used to estimate the input of terrestrial organic matter to sediments. These ratios were low in the open sea samples suggesting that marine organic matter dominated these samples. These results were also coincided with the high short/long ratios of fatty acids and fatty alcohols. Within sterols, the SSIs were used to describe the degree of influence of terrestrial organic matter within marine sediments. Nearly all of the values were < 1.0 indicating the dominance of marine sterols in these areas.

Cholesterol was present in all samples and was the principal sterol in more than half of the sampling sites. Cholesterol is present in many marine organisms from algae (Volkman, 1986) to marine animals (Borchjensen and Mollerup, 1996) as well as domestic sewage discharges. In this study, cholesterol was strongly correlated with 24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22(E)-dien-3β-ol, brassicasterol and dinosterol. 24 norcholesta-5,22(E)-dien-3β-ol is not unique to marine organisms but are often found in the marine environment (Volkman, 1986; Yunker *et al.*, 1995). In the Clyde Sea sediment samples, 24 norcholesta-5,22(E)-dien-3β-ol was relatively high in concentrations in samples collected around the Isle of Arran and samples collected from Loch Fyne. Another sterol, brassicasterol, is the major sterol in the Prymnesiophyceae and also in several diatoms and dinoflagellates. S7 and S10 have the highest concentration of this sterol, suggesting high phytoplankton biomass in these areas. Dinosterol, which is a characteristic of dinoflagellates, occurred in high concentration in samples collected around the Isle of Arran with the highest concentration at S10. Therefore, these results suggesting that open sea samples were dominated by marine derived sterols.

Three sterols are often found in large amounts in terrestrial higher plants: β-sitosterol, stigmasterol and campesterol (Laureillard and Saliot, 1993). Thus, these sterols have been commonly used as tracers of continentally derived organic matter inputs into marine systems (Huang and Meinschein, 1976; Lajat *et al.*, 1990; Laureillard and Saliot, 1993; Yunker *et al.*, 1995; Mudge and Norris, 1997), but these compounds also have planktonic sources (Volkman, 1986; Volkman *et al.*, 1998). These sterols were found in all Clyde Sea sediment samples. Campesterol and stigmasterol have strong correlation with each other but weak correlation with β-sitosterol. β-sitosterol/stigmasterol ratios were used to indicate

terrestrial contribution in the sediment samples (Dachs *et al.*, 1999). All the ratios > 1.0 in all sampling sites except for S1, S2 and S10 reflects their terrigenous inputs.

In general, odd chain length fatty acids are derived from bacterial sources (Rajendran *et al.*, 1991). Branched fatty acids have been used together with branched fatty alcohols as biomarkers of bacterial lipid contribution. The spatial distribution of branched fatty acids and branched fatty alcohols from the Clyde Sea sediment samples was similar with the highest value at the mouth of the River Clyde. The odd/even ratios of fatty acids have a similar distribution with the percentage of branched fatty acids demonstrating their link with bacterial biomass. Within the sterol series, 5β-coprostanol is one of the major sterols in human and higher animal faeces and it has been used as an indicator of sewage pollution, and also for a quantitative evaluation of faecal contamination in coastal waters and sediments (Venkatesan and Kaplan, 1990; Nichols and Espey, 1991; Laureillard and Saliot, 1993; Mudge and Bebianno, 1997). The spatial distribution of 5β-coprostanol/cholesterol ratios within the Clyde Sea sediment samples were similar with percentage branched of fatty acids, suggesting their link to sewage inputs. The highest ratio of 5β-coprostanol/cholesterol was at the mouth of the River Clyde reflecting deposition of sewage derived organic matter due to high population around this area.

PCA was also carried out on the Clyde Sea sediment samples in order to get further insight into the relationships between samples and lipid classes. PCA has been conducted on fatty acids, fatty alcohols and sterols individually. Only fatty acid PCA shows that PCA clearly separates the biomarkers according to their geochemical sources. Polyunsaturated fatty acids, short chain acids and 16:1ω7 acid principally have a marine and algal source in the sediment samples. Saturated 14:0 acid and 16:1ω7 monounsaturated acid are the strongest indicators of marine inputs. PCA also make a clear separation between the terrestrial derived fatty acids and bacterial derived fatty acids. Long chain fatty acids project to the left on the PCA plot with saturated 26:0 acids as the strongest indicator of terrestrial input. Branched fatty acids, indicator of bacterial input project on the bottom right on the PCA plot. Geochemical features are not really clear with fatty alcohols PCA. Branched chain fatty alcohols were clustered together with the long chain fatty alcohols, suggesting the mixed sources of these compounds. On the other hand, short chain and unsaturated

alcohols have positive loadings with saturated $C_{12}$ as the strongest indicator of marine inputs. Within the sterol PCA, cholesta-5,22(E)-dien-3β-ol and brassicasterol became the strongest indicator of marine inputs. Stigmasterol and campesterol were clustered together with all marine derived sterols. These sterols were grouped accordingly as these sterols have also been found in phytoplankton (Volkman, 1986).

PCA with the proportion data (with and without transformation) have been carried out on the mixed compound of fatty acids, fatty alcohols and sterols. Both PCAs only explained a small percentage of variance in the data. PC1 and PC2 explain 26.1% and 25.9% of the variance for PCA with proportion data without transformation and proportion data, added 0.001 (log transformed) respectively. *Anteiso*-17:0 acid is the strongest indicator for bacterial derived compounds as branched fatty acids, branched fatty alcohols, coprostanol and epicoprostanol project to the right of the PCA plot. Saturated 14:0 acid, on the other hand, became the strongest indicator of marine inputs. Marine derived compounds such as short chain fatty acids, short chain fatty alcohols, brassicasterol, 24 norcholesta-5,22(E)-dien-3β-ol and cholesta-5,22(E)-dien-3β-ol project to the left of the PCA plot with negative loadings. Long chain fatty alcohols and fatty acids, with β-sitosterol and stigmasterol project to the top of the PCA plot reflecting their terrigenous source. From the score plot, S1, S2, S3 and S10 contain marine derived organic matter. Similar to the other PCA, the results show that S13 contain the greatest amount of bacterial and sewage derived organic matter.

The contribution of biomarkers from the signature blocks to the other sampling sites can be seen by PLS analysis. In the Clyde Sea sediment samples, S31, S32 and S33 were used as the terrestrial signatures; S1, S2 and S10 were used to characterise marine signatures; S12, S13 and S21 as sewage signatures. Current movements are important for biomarker transportation around the Clyde Sea before the deposition in the sediments. Terrestrial signatures from S31, S32 and S33 were transported down the Loch Fyne and entered the North Channel following the outflow current. 80-90% of the signatures occurred at S25 showing that these signatures were also transported around the Isle of Bute. The marine signatures decrease towards the sea lochs, and they were transported by current inflow from the North Channel into the Clyde Sea at its southern entrance. Sample S3, which was

located near S2, showing the similarity with the signatures with having 80-90% of variance explained. The River Clyde is responsible for draining much of agricultural and industrial waste to the western Scotland. Therefore much of the freshwater entering the Clyde Sea is rich with nutrient and domestic sewage. Hence, the sewage signatures will be transported through the North Channel through the anticlockwise movements around the Isle of Arran. Sewage signatures are low at the open sea sampling sites. PLS is very useful in showing the movements of biomarker compounds and to determine their contribution in the marine environments.

Cluster analysis was another multivariate analysis that was carried out on the Clyde Sea sediment samples to classify the study area into specific region, each having definite characteristics. Two cluster groups were identified in cluster analysis of fatty acids and sterols individually. The first group represents a mixture of marine and bacterial derived compounds, while the second group contains the bacterial derived fatty acids. Saturated 16:0 and 18:0 acids were also clustered together with this group. These two components are the most abundant fatty acids in this dataset and are associated to aquatic source organisms (Carrie *et al.*, 1998; Mudge *et al.*, 1998). These results, therefore, suggest that marine derived compounds also dominated this cluster group. Marine and algal derived sterols are clustered separately from the sewage and terrestrial derived sterols. Brassicasterol and cholesta-5,22(E)-dien-3β-ol are the strongest indicator of marine sterols. They were clustered together with the greatest similarity. There are 3 cluster groups identified in the cluster analysis of fatty alcohols. Cluster analysis for fatty alcohols on the Clyde Sea sediment samples is more complicated with marine, terrestrial and bacterial derived compounds clustered together with each other. Only the first smaller cluster in the group I corresponds to bacterial derived fatty alcohols with branched fatty alcohols clustered together. Greatest similarity can be seen at *iso*-$C_{17}$ and *anteiso*-$C_{17}$, suggesting that these compounds were the strongest indicators of bacterial inputs. Two cluster groups were also observed in analysis of the mixed compounds. Marine inputs was represented by the first cluster group with short chain fatty alcohols and fatty acids, 24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22(E)-dien-3β-ol, brassicasterol and cholesterol clustered together. The second cluster group, on the other hand, represents the terrestrial inputs with long chain fatty alcohols and fatty acids and β-sitosterol grouped together. Bacterial

derived compounds can be seen in both clusters, showing that bacterial inputs occurred together with marine and terrestrial input especially for diagenesis processes. Samples mainly located in the open seas together with samples collected from Loch Long are characterised by marine and bacterial derived organic matter. While, samples collected within the lochs are characterised by terrestrial and sewage derived compounds.

## 5.5 Conclusions

In general, all sampling sites are characterised by marine derived organic matter. This can be seen on the results from ASIs, SSIs and short/long ratios. The terrestrial markers such as long chain fatty alcohols and fatty acids can be found in high concentration in samples collected within the lochs. S13, which is collected at the mouth of River Clyde, is different from other samples as it contains the greatest amount of sewage and bacterial markers. PCA on individual group and mixed compounds confirmed these observations. PLS analysis showed there was a transportation of organic matter in the Clyde Sea sediments via current movements. Meanwhile cluster analysis differentiated the study area into two main regions. The first cluster group defined mainly the open sea samples, which are characterised by marine derived compounds, while the second cluster group represented the loch samples with terrigenous and sewage inputs.

## CHAPTER 6: CORE SAMPLES FROM LOCH RIDDON, SCOTLAND

### 6.1 Introduction

Organic matter in marine sediments is derived from living organisms. The evaluation of lipid biomarkers in sediments provides specific information on the sources of organic matter, as well as on the particular conditions of deposition and burial (Volkman *et al.*, 1987; Colombo *et al.*, 1997). The composition and depth distribution of lipids in sediments is determined by the different sources (marine, terrestrial, anthropogenic) and the chemical and microbial transformations.

In this chapter, the distribution and composition of fatty acids, fatty alcohols and sterols were studied in sediment vertical profiles to follow the fate of lipids from various sources. The multivariate statistical analysis will be applied on these multiple lipid markers to contribute a better understanding of the limited specificity of some lipid markers.

A 150cm core was taken from the Loch Riddon, Scotland ($55^o$ 57'N, $5^o$ 11'W) (Figure 6.1) in May, 1998 with *RV Prince Madog*. Loch Riddon sometimes called Loch Ruel extends north for 3 miles, but the head of the loch dries out for 1 1/2 miles. Loch Riddon has hills on both sides, which affects the local weather. The core was sub-sampled by cutting the first 5cm, and then the core was sectioned at 3cm intervals from 5-35cm and 5cm intervals from 35cm down to 150 cm. All samples were refrigerated until analysis.

### 6.2 Results

### 6.2.1 Fatty acids

Fatty acids are often the most abundant lipid in sediment as they are abundant in most organisms. Sources of fatty acids include bacteria, micro algae, higher plants and marine fauna. Fatty acids such as 14:0, 16:0, 16:1ω7, 20:5, 22:5 and 22:6 are abundant in phytoplankton while 16:0, 18:1ω9, 18:0, 20:5 and 22:6 are often dominant in zooplankton (Wakeham and Lee, 1989; Hama, 1991). Saturated straight chain fatty acids 20:0-30:0 in sediments are probably derived from the surface waxes of higher plants (Colombo *et al.*, 1997), however they can also be found in micro algae and bacteria (Volkman *et al.*, 1998).
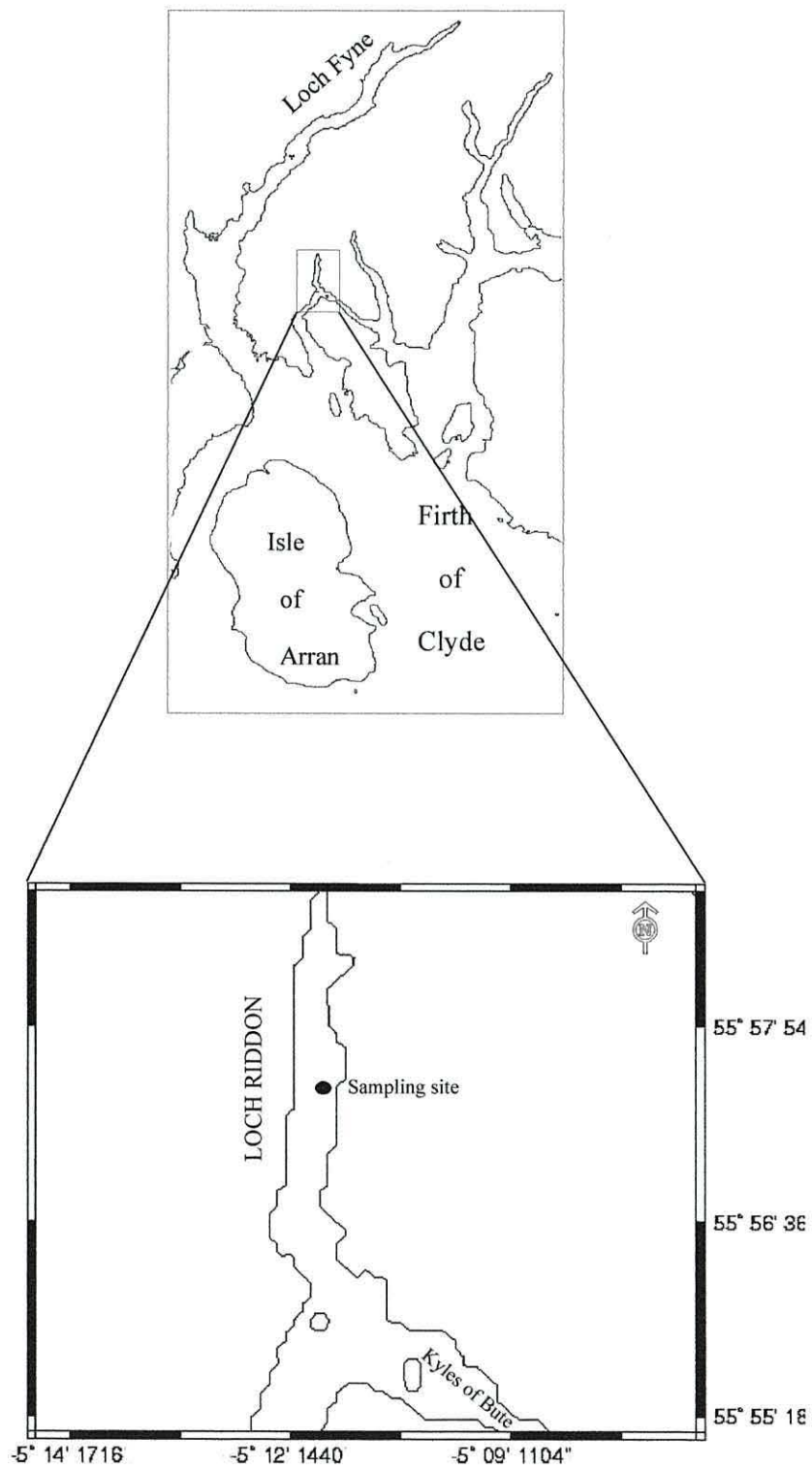
Figure 6.1: Location of sampling site
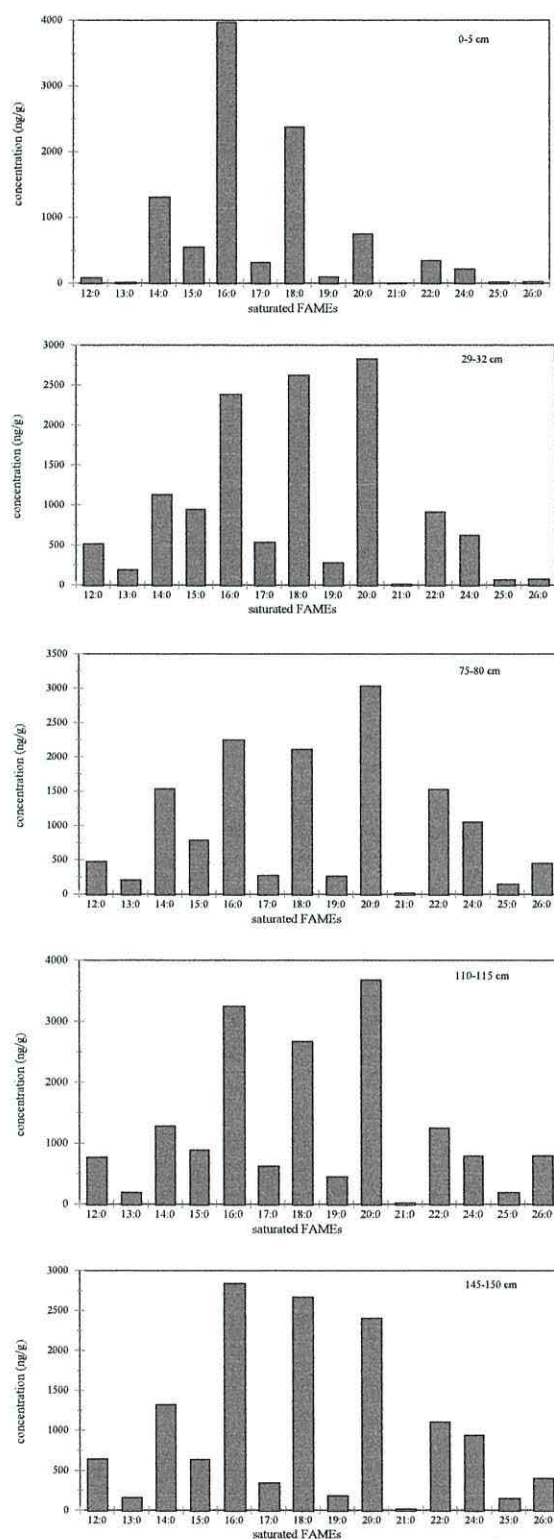
Figure 6.2: Distribution of saturated fatty acids in Loch Riddon sediment

A total of 33 fatty acids were found throughout the core (Appendix 10) with normal saturated fatty acids ranging from 12:0 to 26:0. Within the fatty acid compounds, correlation was very low, with only 21% of compound pairs showing an *r* value greater than 0.5 (Table 6.1). Short chain fatty acids were not strongly correlated to each other except for the saturated 12:0 acid, which was correlated strongly with 13:0 saturated acid, with a correlation coefficient of 0.71. The monounsaturated 16:1ω7 acids was correlated with 16:0 and 16:1ω5, with *r* values of 0.58 and 0.70 respectively. The polyunsaturated acid 18:2ω6, was strongly correlated with branched fatty acids such as *anteiso*-15:0, *iso*-17:0 and *anteiso*-17:0, with *r* values of 0.75, 0.65 and 0.78 respectively. With 18:1ω7, 18:2ω6 has correlation coefficient value of 0.66. The branched fatty acid, *anteiso*-15:0, was strongly correlated with *iso*-17:0 and *anteiso*-17:0, with *r* values of 0.73 and 0.68 respectively. There were good correlations between the long chain compounds. For example, 24:0 saturated acid was positively correlated with 25:0 and 26:0 saturated acids with *r* values of 0.73 and 0.62 respectively. The saturated 25:0 acid has correlation coefficient of 0.92 with 26:0 saturated acids.

Characteristically, short chain fatty acids are major components in the surface sediment samples but usually decrease in deep samples. However, longer chain fatty acids are minor in surface sediment samples, and increase in deep samples as shown in Figure 6.2. Vertical profiles of fatty acids show a decrease in monounsaturated (Figure 6.3) and polyunsaturated (Figure 6.4) fatty acids with increasing depth. The decrease of short chain fatty acids and increase of longer chain down the core can be seen in Figure 6.2. This reflects the preferential utilisation of marine organic matter as unsaturated and shorter chain fatty acids are primary substrates for marine organisms (Nishimura and Baker, 1987; Colombo *et al.*, 1997). Short chain fatty acids are less stable and degrade more rapidly than the longer chain compounds. Therefore, the general decrease of short chain fatty acids is probably caused by microbial and chemical degradation during early diagenesis. The increase of long chain fatty acids may be due to greater preservation of these compounds (Kawamura and Ishiwatari, 1984; Nishimura and Baker, 1987; Colombo *et al.*, 1997). Another possible reason for the increase is the production of long chain fatty acids by chemical reactions of other organic compounds such as oxidation of fatty alcohols (Kawamura and Ishiwatari, 1984) since the presence of phytanic acid in sediments is a

Table 6.1: Coefficients of correlation between fatty acids in the Loch Riddon core

| | br12:0 | 12:0 | iso13:0 | ante13:0 | 13:0 | br14:0 | 14:0 | 14:1 | iso15:0 | ante15:0 | 15:0 | iso16:0 | 16:0 | 16:1ω9 | 16:1ω7 | iso17:0 | 16:1ω5 | ante17:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12:0 | 0.71** | | | | | | | | | | | | | | | | | |
| iso13:0 | 0.62** | 0.66** | | | | | | | | | | | | | | | | |
| ante13:0 | 0.62** | 0.61** | 0.87** | | | | | | | | | | | | | | | |
| 13:0 | 0.56** | 0.71** | 0.81** | 0.90** | | | | | | | | | | | | | | |
| br14:0 | 0.58** | 0.83** | 0.66** | 0.71** | 0.78** | | | | | | | | | | | | | |
| 14:0 | 0.20 | 0.44 | 0.33 | 0.33 | 0.40 | 0.44 | | | | | | | | | | | | |
| 14:1 | 0.39 | 0.26 | 0.46 | 0.39 | 0.30 | 0.34 | 0.15 | | | | | | | | | | | |
| iso15:0 | 0.10 | 0.36 | 0.18 | 0.26 | 0.38 | 0.54* | 0.51* | 0.30 | | | | | | | | | | |
| ante15:0 | 0.27 | 0.25 | 0.38 | 0.18 | 0.15 | 0.16 | 0.40 | 0.45 | 0.26 | | | | | | | | | |
| 15:0 | 0.25 | 0.50* | 0.50* | 0.52* | 0.60** | 0.69** | 0.40 | 0.26 | 0.50* | 0.51* | | | | | | | | |
| iso16:0 | 0.41 | 0.42 | 0.65** | 0.74** | 0.69** | 0.61** | 0.40 | 0.37 | 0.39 | 0.34 | 0.71** | | | | | | | |
| 16:0 | 0.10 | 0.17 | 0.07 | -0.11 | 0.08 | 0.16 | 0.30 | 0.03 | 0.39 | 0.48 | 0.28 | 0.15 | | | | | | |
| 16:1ω9 | 0.31 | 0.24 | 0.56* | 0.44 | 0.40 | 0.20 | 0.16 | 0.66** | 0.32 | 0.67* | 0.36 | 0.50* | 0.28 | | | | | |
| 16:1ω7 | -0.15 | -0.29 | -0.19 | -0.37 | -0.32 | -0.48 | 0.04 | -0.001 | -0.02 | 0.44 | -0.14 | -0.11 | 0.58** | 0.35 | | | | |
| iso17:0 | 0.39 | 0.43 | 0.34 | 0.22 | 0.23 | 0.33 | 0.48 | 0.28 | 0.44 | 0.73** | 0.44 | 0.37 | 0.56* | 0.53* | 0.44 | | | |
| 16:1ω5 | 0.12 | 0.13 | 0.12 | 0.03 | 0.10 | 0.06 | 0.55* | 0.29 | 0.47 | 0.62** | 0.26 | 0.27 | 0.57** | 0.48 | 0.70** | 0.69** | | |
| ante17:0 | 0.42 | 0.36 | 0.72** | 0.63** | 0.57** | 0.53* | 0.40 | 0.36 | 0.29 | 0.68** | 0.71** | 0.76** | 0.42 | 0.63** | 0.14 | 0.58** | 0.39 | |
| 17:0 | 0.12 | 0.30 | 0.22 | 0.40 | 0.51* | 0.62** | 0.25 | -0.16 | 0.52* | 0.03 | 0.70** | 0.60** | 0.35 | 0.00 | -0.21 | 0.22 | 0.08 | 0.50* |
| 16:2 | -0.002 | -0.18 | 0.07 | 0.002 | -0.10 | -0.07 | 0.12 | 0.26 | 0.06 | 0.27 | 0.07 | 0.22 | 0.19 | 0.19 | 0.28 | 0.20 | 0.28 | 0.33 |
| 17:1 | 0.35 | 0.44 | 0.30 | 0.34 | 0.43 | 0.49 | 0.24 | 0.06 | 0.39 | 0.55* | 0.70** | 0.48 | 0.41 | 0.41 | 0.14 | 0.62** | 0.34 | 0.61** |
| 18:0 | -0.19 | -0.07 | -0.20 | -0.21 | -0.01 | 0.04 | -0.21 | -0.23 | -0.15 | -0.21 | 0.04 | -0.20 | 0.13 | -0.19 | -0.16 | -0.15 | -0.32 | -0.12 |
| 18:1ω9 | 0.18 | 0.16 | 0.22 | 0.24 | 0.32 | 0.19 | 0.06 | -0.05 | 0.11 | 0.35 | 0.33 | 0.34 | 0.41 | 0.43 | 0.21 | 0.41 | 0.23 | 0.50* |
| 18:1ω7 | 0.37 | 0.38 | 0.56* | 0.43 | 0.42 | 0.32 | 0.17 | 0.46 | 0.32 | 0.63** | 0.47 | 0.43 | 0.30 | 0.75** | 0.31 | 0.68** | 0.43 | 0.60** |
| 18:2ω6 | 0.23 | 0.12 | 0.36 | 0.33 | 0.23 | 0.24 | 0.24 | 0.37 | 0.24 | 0.75** | 0.59** | 0.58** | 0.42 | 0.66** | 0.38 | 0.65** | 0.50* | 0.78** |
| 19:0 | 0.09 | 0.26 | 0.14 | 0.29 | 0.42 | 0.47 | 0.10 | -0.44 | 0.16 | -0.34 | 0.39 | 0.34 | 0.05 | -0.37 | -0.40 | -0.15 | -0.21 | 0.13 |
| 20:0 | 0.14 | 0.30 | 0.25 | 0.44 | 0.51* | 0.54* | 0.07 | -0.33 | 0.10 | -0.38 | 0.34 | 0.35 | -0.11 | -0.32 | -0.60** | -0.25 | -0.37 | 0.13 |
| 20:1 | 0.20 | 0.15 | 0.04 | 0.12 | 0.17 | 0.18 | 0.06 | 0.01 | 0.003 | -0.22 | -0.10 | 0.07 | 0.07 | -0.20 | -0.30 | -0.11 | -0.13 | -0.03 |
| 21:0 | 0.38 | 0.50* | 0.35 | 0.43 | 0.54* | 0.48 | 0.14 | -0.05 | 0.03 | -0.13 | 0.21 | 0.25 | 0.06 | -0.01 | -0.42 | -0.14 | -0.20 | 0.14 |
| 22:0 | 0.03 | 0.16 | 0.23 | 0.35 | 0.47 | 0.41 | 0.08 | -0.18 | 0.12 | -0.33 | 0.28 | 0.33 | -0.01 | -0.16 | -0.49 | -0.31 | -0.28 | 0.13 |
| 24:0 | 0.01 | 0.11 | -0.004 | 0.16 | 0.36 | 0.36 | -0.01 | -0.26 | 0.10 | -0.31 | 0.25 | 0.18 | -0.01 | -0.26 | -0.53* | -0.30 | 0.33 | -0.02 |
| 25:0 | -0.21 | 0.04 | -0.10 | 0.10 | 0.16 | 0.26 | -0.10 | -0.46 | 0.04 | -0.60** | 0.16 | 0.03 | -0.10 | -0.56* | -0.55* | -0.48 | -0.48 | -0.22 |
| 26:0 | -0.28 | 0.10 | -0.14 | -0.10 | 0.12 | 0.20 | -0.18 | -0.54* | -0.01 | -0.56** | 0.13 | -0.11 | -0.08 | -0.57** | -0.50* | -0.48 | -0.53 | -0.31 |

| | 17:0 | 16:2 | 17:1 | 18:0 | 18:1ω9 | 18:1ω7 | 18:2ω6 | 19:0 | 20:0 | 20:1 | 21:0 | 22:0 | 24:0 | 25:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16:2 | -0.001 | | | | | | | | | | | | | |
| 17:1 | 0.61** | -0.12 | | | | | | | | | | | | |
| 18:0 | 0.20 | -0.07 | 0.17 | | | | | | | | | | | |
| 18:1ω9 | 0.47 | -0.16 | 0.70** | 0.30 | | | | | | | | | | |
| 18:1ω7 | 0.11 | 0.02 | 0.54* | -0.24 | 0.40 | | | | | | | | | |
| 18:2ω6 | 0.34 | 0.32 | 0.58** | -0.12 | 0.52 | 0.66** | | | | | | | | |
| 19:0 | 0.75** | -0.13 | 0.23 | 0.28 | 0.20 | -0.27 | -0.11 | | | | | | | |
| 20:0 | 0.64** | -0.24 | 0.13 | 0.23 | 0.15 | -0.29 | -0.16 | 0.91** | | | | | | |
| 20:1 | 0.10 | -0.06 | -0.17 | 0.06 | 0.03 | -0.15 | -0.15 | 0.08 | 0.13 | | | | | |
| 21:0 | 0.28 | -0.04 | 0.04 | 0.04 | -0.04 | -0.20 | -0.15 | 0.49 | 0.62** | 0.11 | | | | |
| 22:0 | 0.49 | -0.08 | -0.10 | 0.17 | -0.03 | -0.25 | -0.13 | 0.71** | 0.80** | 0.12 | 0.73** | | | |
| 24:0 | 0.47 | -0.29 | 0.10 | 0.42 | 0.16 | -0.29 | -0.21 | 0.69** | 0.75** | 0.17 | 0.58** | 0.82** | | |
| 25:0 | 0.48 | -0.23 | -0.16 | 0.34 | -0.13 | -0.47 | -0.45 | 0.80** | 0.79** | 0.15 | 0.45 | 0.78** | 0.73** | |
| 26:0 | 0.42 | -0.42 | -0.06 | 0.37 | -0.10 | -0.43 | -0.52* | 0.69** | 0.68** | 0.07 | 0.41 | 0.62** | 0.62** | 0.92** |

\*    $p < 0.01$

\*\*   $p < 0.001$

product of phytol oxidation (Ishiwatari *et al.*, 1980). Longer chain fatty alcohols that were found in these samples are relatively minor components, compared to longer chain fatty
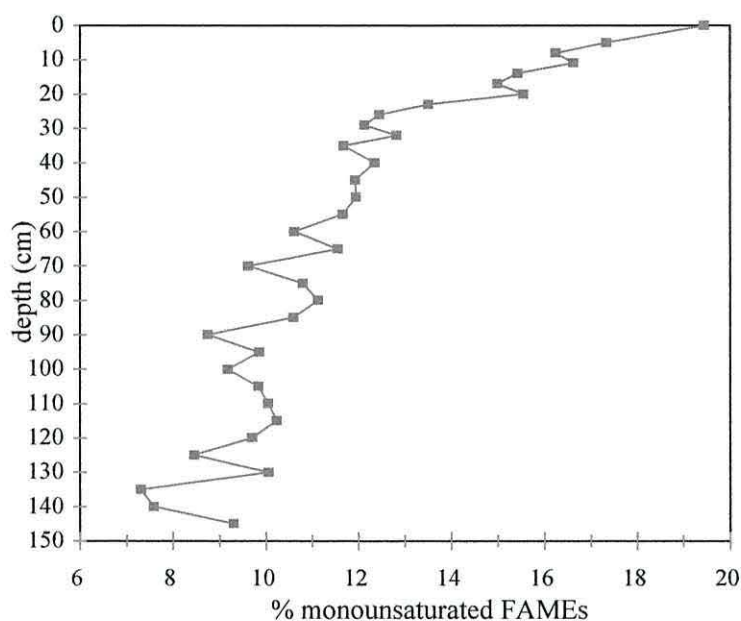


Figure 6.3: Vertical distribution of percentage monounsaturated fatty acids in sediment core of Loch Riddon
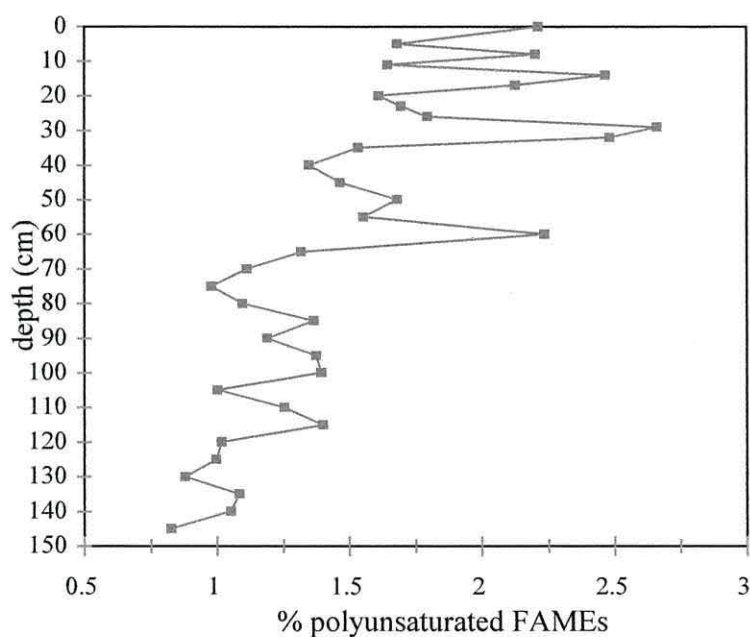


Figure 6.4: Vertical distribution of percentage polyunsaturated fatty acids in sediment core of Loch Riddon

acids. Thus the distribution of fatty alcohols is not parallel with fatty acids. Therefore, the increase of longer chain fatty acids indicates an increased contribution of terrestrial organic matter in Loch Riddon.

Figure 6.5 shows a vertical profile of the short/long ratio (similar to L/H ratio by Fukushima and Ishiwatari, 1984). The ratio $(\Sigma12:0-20:0)/(\Sigma21:0-26:0)$ decrease with increasing depth but it is above 1.0 throughout the core showing great concentrations of short chain fatty acids. These observations indicate that the marine derived fatty acids were more dominant than the terrestrial fatty acids in the sediment core.
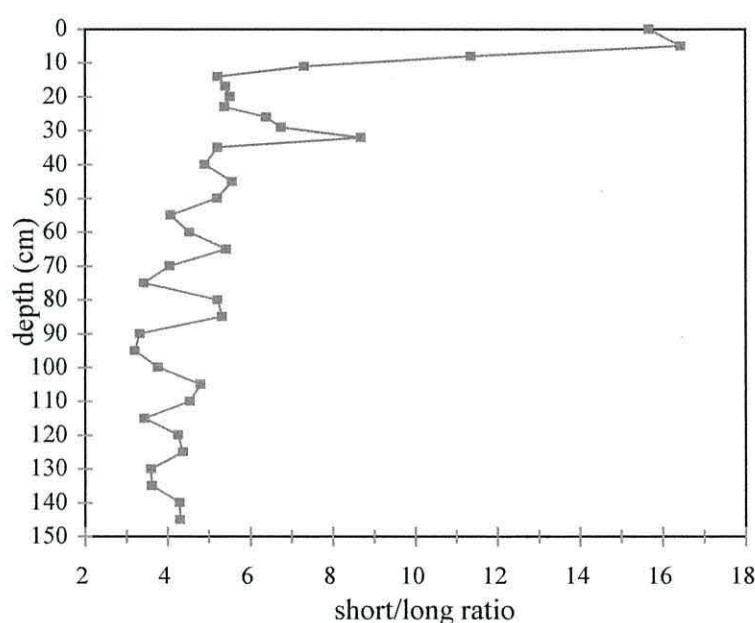


Figure 6.5: Vertical distribution of short/long ratio of fatty acids in sediment core of Loch Riddon

Bacteria are the major source of branched fatty acids principally *iso* and *anteiso* odd chain length fatty acids and also 18:1ω7 acid (Wakeham and Ertel, 1988; Volkman *et al.*, 1998). The peak at 32-35 cm (Figure 6.6) may be associated with an increase of bacteria degrading organic matter in the sub oxic layer. Figure 6.7 shows the profile of 18:1ω7/18:0 ratios with depth. The vertical profile of 18:1ω7/18:0 ratios are similar with percentage of branched fatty acid (correlation coefficient, $r=0.76$, $p<0.01$) supporting its link to bacterial activity.
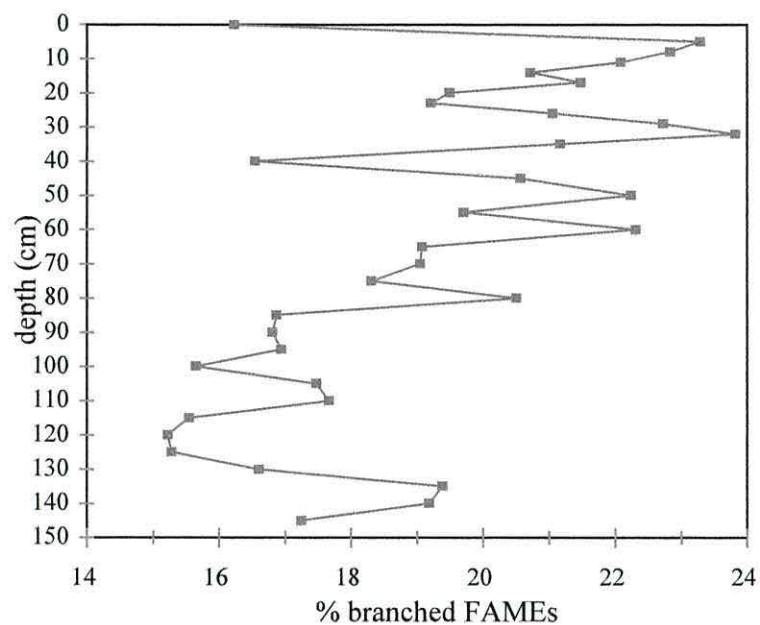
Figure 6.6: Vertical distribution of percentage branched fatty acids in sediment core of Loch Riddon
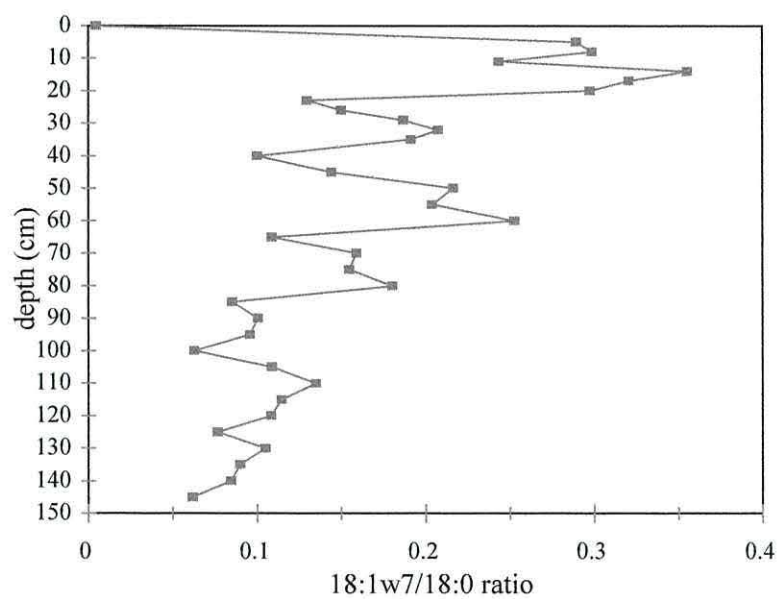


Figure 6.7: Vertical distribution of 18:1ω7/18:0 ratios in sediment core of Loch Riddon

## 6.2.2 Fatty alcohols

In total, 18 compounds with saturated alcohols from $C_{12}$ to $C_{24}$ were found in the core samples showing a maximum at $C_{16}$ and a secondary maximum at $C_{20}$ (Appendix 11). The short chain alcohols ($<C_{20}$) are commonly associated with zooplankton and marine invertebrates either as direct inputs or derived from the diet of these organisms. Therefore they are often used as marine indicators. On the other hand, terrestrial higher plants are the major source of long chain fatty alcohols that have used as terrestrial markers (Grimalt and Albaiges, 1990; Yunker *et al.*, 1995; Mudge and Norris, 1997).

Correlation was generally low within the fatty alcohol compounds. Forty six percent (46%) of the compound pairs showing an *r* value greater than 0.5 (Table 6.2). The saturated $C_{14}$, which appeared to be prominent in the top samples, correlated most strongly with $C_{12}$, $C_{16}$ and $C_{18}$, with *r* values of 0.65, 0.68 and 0.82 respectively. The branched fatty alcohol, *iso*-$C_{17}$ was strongly correlated with other branched compounds such as *anteiso*-$C_{17}$, *iso*-$C_{15}$ and *anteiso*-$C_{15}$, with *r* values of 0.99, 0.76 and 0.59 respectively. The chlorophyll biomarker, phytol, was correlated most strongly with short chain fatty alcohols and the zooplankton monounsaturated 20:1 alcohol, showing *r* values of 0.83, 0.77, 0.88 and 0.98 with $C_{12}$, $C_{14}$, $C_{18}$ and 20:1 respectively. Meanwhile, the monounsaturated 20:1 also has strong positive correlations with $C_{12}$, $C_{14}$ and $C_{18}$ with *r* values of 0.78, 0.72 and 0.82 respectively. Long chain fatty alcohols have high concentrations in the deeper section of the core and have good correlation with each other. For example, correlation coefficients for $C_{24}$ with $C_{20}$, $C_{21}$, $C_{22}$ and $C_{23}$ are 0.61, 0.54, 0.56 and 0.80 respectively.

The distribution of individual saturated fatty alcohols in the sediment core from Loch Riddon is shown in Figure 6.8. The concentration of short chain fatty alcohols decreases towards the deeper sections while the concentration of the long chain compounds increases. The decreasing concentrations of short chain fatty alcohols was accompanied by disappearance of unsaturated 20:1 suggesting that preferential degradation seemed to occur in the surface sediments. Figure 6.9 shows the increase with depth in the mean chain length of fatty alcohols down the core. The terrestrial input into Loch Riddon is thought to be large since non-coniferous forests surround the area. Therefore the increase of long chain fatty alcohols may be due to contribution of terrestrial organic matter.

Table 6.2: Coefficients of correlation between fatty alcohols in the Loch Riddon core

| | $C_{12}$ | $C_{14}$ | $isoC_{15}$ | $anteC_{15}$ | $C_{15}$ | $C_{16}$ | $isoC_{17}$ | $anteC_{17}$ | $C_{17}$ | $C_{18}$ | $C_{19}$ | $C_{20}$ | $C_{21}$ | $C_{22}$ | $C_{23}$ | C24 | Phytol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_{14}$ | 0.65** | | | | | | | | | | | | | | | | |
| $isoC_{15}$ | 0.65** | 0.50* | | | | | | | | | | | | | | | |
| $anteC_{15}$ | 0.50* | 0.47 | 0.29 | | | | | | | | | | | | | | |
| $C_{15}$ | 0.59** | 0.64** | 0.88** | 0.27 | | | | | | | | | | | | | |
| $C_{16}$ | 0.60** | 0.68** | 0.87** | 0.19 | 0.90** | | | | | | | | | | | | |
| $isoC_{17}$ | 0.88** | 0.72** | 0.76** | 0.59** | 0.70** | 0.65** | | | | | | | | | | | |
| $anteC_{17}$ | 0.85** | 0.72** | 0.76** | 0.59** | 0.69** | 0.65** | 1.00** | | | | | | | | | | |
| $C_{17}$ | 0.86** | 0.73** | 0.87** | 0.43 | 0.81** | 0.80** | 0.93** | 0.93** | | | | | | | | | |
| $C_{18}$ | 0.74** | 0.82** | 0.47 | 0.46 | 0.50* | 0.63** | 0.73** | 0.73** | 0.73** | | | | | | | | |
| $C_{19}$ | 0.08 | 0.05 | 0.33 | -0.09 | 0.06 | 0.27 | 0.10 | 0.08 | 0.20 | 0.17 | | | | | | | |
| $C_{20}$ | -0.12 | -0.23 | 0.12 | -0.27 | -0.13 | -0.05 | -0.08 | -0.10 | -0.004 | -0.33 | 0.45 | | | | | | |
| $C_{21}$ | -0.03 | -0.32 | -0.21 | -0.21 | -0.41 | -0.24 | -0.37 | -0.38 | -0.31 | -0.21 | 0.61** | 0.47 | | | | | |
| $C_{22}$ | -0.33 | -0.45 | -0.26 | -0.15 | -0.47 | -0.38 | -0.36 | -0.36 | -0.33 | -0.23 | 0.50* | 0.44 | 0.86** | | | | |
| $C_{23}$ | -0.19 | -0.48 | 0.16 | -0.26 | -0.10 | 0.01 | -0.20 | -0.20 | -0.09 | -0.40 | 0.66** | 0.61** | 0.47 | 0.36 | | | |
| $C_{24}$ | -0.30 | -0.60** | -0.06 | -0.24 | -0.28 | -0.25 | -0.27 | -0.28 | -0.20 | -0.51* | 0.50* | 0.61** | 0.54* | 0.56* | 0.80** | | |
| Phytol | 0.83** | 0.77** | 0.61** | 0.63** | 0.57** | 0.59** | 0.91** | 0.92** | 0.84** | 0.88** | 0.12 | -0.29 | -0.34 | -0.31 | -0.35 | -0.42 | |
| 20:1 | 0.78** | 0.72** | 0.62** | 0.64** | 0.55* | 0.55* | 0.90** | 0.92** | 0.82** | 0.82** | 0.12 | -0.22 | -0.28 | -0.24 | -0.30 | -0.35 | 0.98** |

| * | $p < 0.01$ |
|---|---|
| ** | $p < 0.001$ |
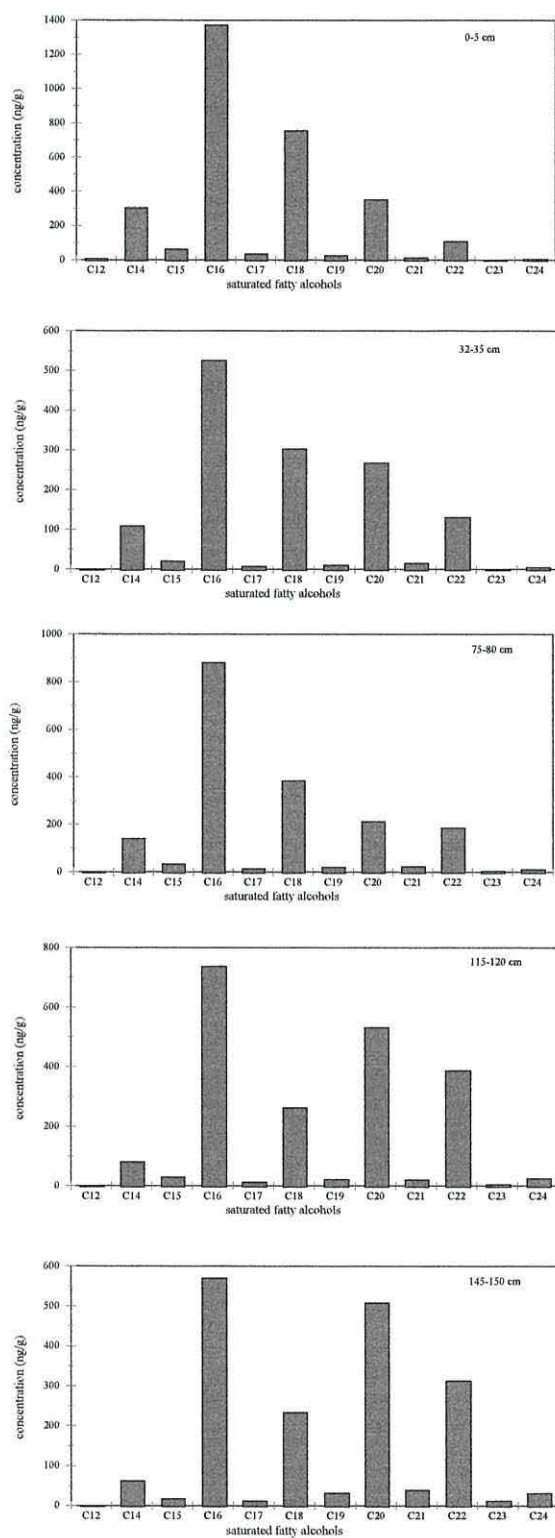
Figure 6.8: Distribution of saturated fatty alcohols in Loch Riddon sediment
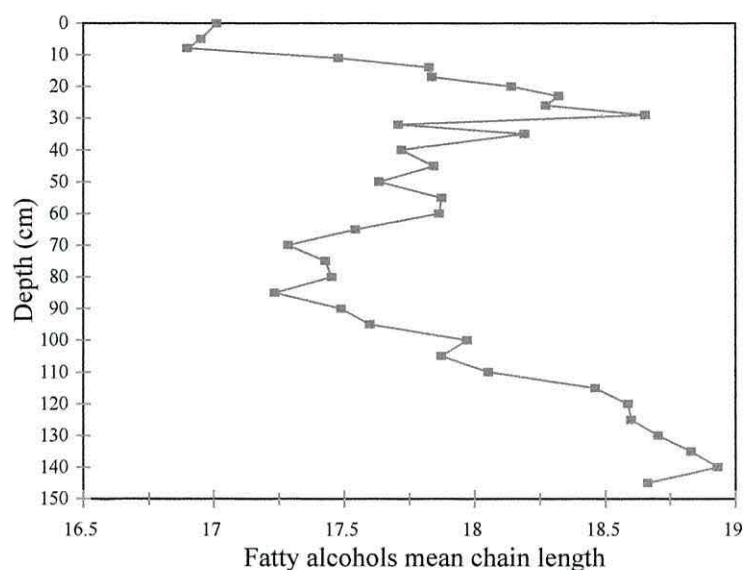
Figure 6.9: Profile of mean chain length of fatty alcohols in the Loch Riddon core

The percentage of branched fatty alcohols was small and decreased towards the deeper section of the core with maximum at the surface and at 20-23 cm interval (Figure 6.10). Branched fatty acids and fatty alcohols usually result from bacterial metabolism (Parkes,
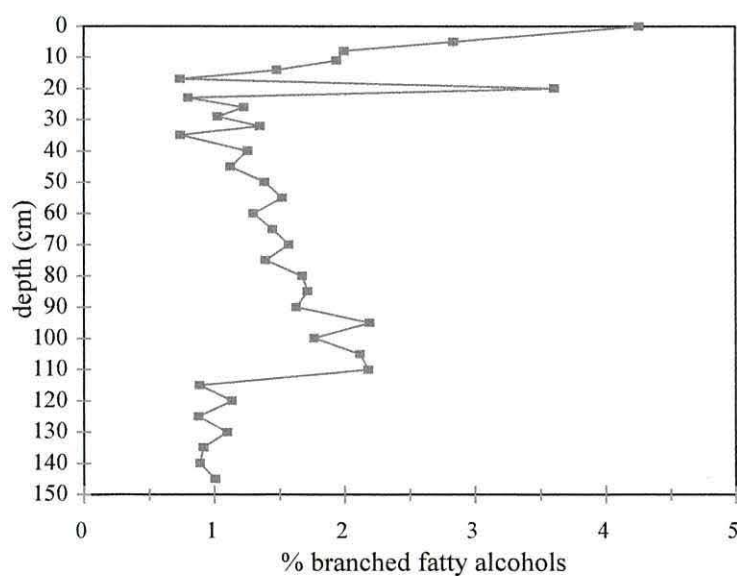


Figure 6.10: Vertical distribution of percentage branched fatty alcohols in sediment core of Loch Riddon

1987). These peaks are probably associated with an increase of bacterial activity due to a greater food source.

The short/long $(\Sigma C_{12}\text{-}C_{20})/(\Sigma C_{21}\text{-}C_{24})$ ratio decreased with depth (Figure 6.11). This may be due to degradation of short chain fatty alcohols. Nearly all the ratios were above 1.0 except for the 3 bottom samples. This observation indicates that the short chain compounds were dominant in these core sediments. The distribution of short/long ratio of fatty alcohols is similar to the same ratios of fatty acids ($r=0.69$, $p<0.001$).
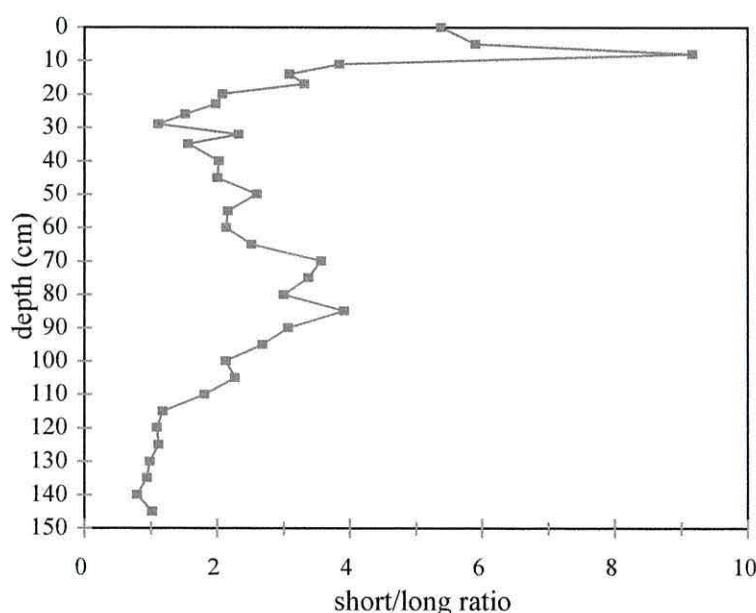


Figure 6.11: Vertical profile of short/long ratio of fatty alcohols in sediment core of Loch Riddon

The Alcohol Source Index (ASI) can be used to estimate the input of terrestrial derived material to sediments (Mudge and Norris, 1997). The ASI is calculated by dividing the concentration of terrestrial fatty alcohols by the concentration of marine fatty alcohols. It may be hypothesised that the ratio would increase with depth. In this study $C_{14}$ and $C_{16}$ were chosen as marine fatty alcohols while $C_{22}$ and $C_{24}$ were assumed to be of terrestrial origin. The ratios increase with depth, and indicate the continual input of higher plant materials in the past. $C_{24}$ appears to be the strongest terrestrial marker. For example, $C_{24}/C_{16}$ ratios increase by a factor of 16, while $C_{22}/C_{16}$ increase by a factor of 7. $C_{14}$ appears to be the strongest marine marker. The $C_{24}/C_{14}$ ratio increase by a factor of 33,

208

whilst $C_{22}/C_{14}$ ratio increases by a factor of 14. Figure 6.12 shows the vertical profiles of $C_{24}/C_{14}$ ratio throughout the core. The $C_{24}/C_{16}$, $C_{22}/C_{16}$ and $C_{22}/C_{14}$ ratios are not shown here. The proportions of $C_{24}$ never exceed the $C_{14}$ suggesting the dominance of marine fatty alcohols. This observation is similar to the short/long data where the ratios are above 1.0 showing greater concentrations of short chain fatty alcohols in the core sediments.
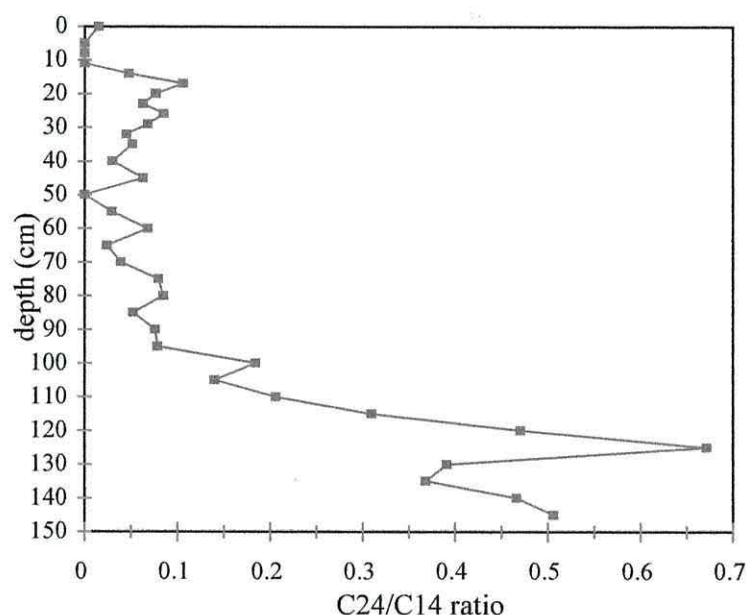


Figure 6.12: Vertical profile of $C_{24}/C_{14}$ ratio in sediment core of Loch Riddon

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), which is a potential marker for chlorophyll (Killops and Killops, 1993) was also found in the samples. Figure 6.13 shows the phytol concentration down the core, which is similar with the distribution of short/long ratios ($r=0.70$, $p<0.001$). Phytol also strongly correlated with dinosterol ($r=0.74$, $p<0.001$), brassicasterol ($r=0.88$, $p<0.001$) and cholesta-5,22-dien-3$\beta$-ol ($r=0.92$, $p<0.001$), which are marine sterols. These results show the inverse distribution of phytol with $\beta$-sitosterol ($r=-0.40$, $p<0.05$), which originates from higher plants suggesting that the phytol observed here was mainly of marine origin.
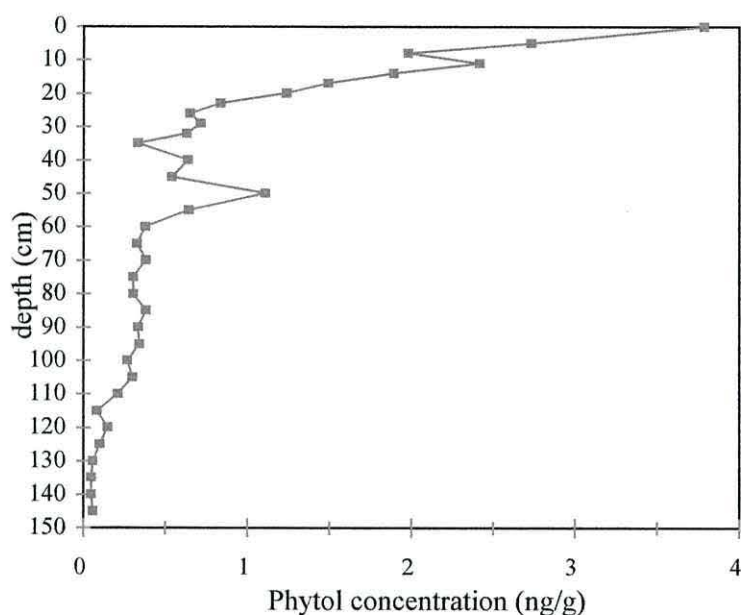
Figure 6.13: Concentration of phytol in sediment core of Loch Riddon

### 6.2.3 Sterols

As well as fatty acids and fatty alcohols, the sterols were also quantified in each sample from Loch Riddon. Sterols have been successfully used as tracers of inputs from various species of marine and terrestrial plants and animals. Sterols are stable and better preserved in sedimentary environment (Volkman, 1986; Saliot *et al.*, 1991). Seventeen sterols were found and quantified (Table 6.4, Appendix 12) from the core. The major sterols found were cholesterol, brassicasterol, β-sitosterol, campesterol and dinosterol.

Figure 6.14 shows the cholestanol/cholesterol ratio throughout the Loch Riddon core. This ratio indicates the anaerobic reduction in the core. Cholestanol generally present in sewage sludge, therefore, can be used as sewage biomarker (McCalley *et al.*, 1981). When the sewage increases, the sediment becomes anaerobic when the oxygen used by the bacteria was greater than the diffusion rates. Therefore, it shows that the sediment core might be lacking in oxygen.

Table 6.4: Coefficients of correlation between sterols in the Loch Riddon core

| | st1 | cop | epi | st2 | st3 | chol | cholest | brass | st4 | st5 | camp | st6 | stig | sito | st7 | dino |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| cop | 0.97** | | | | | | | | | | | | | | | |
| epi | 0.93** | 0.93** | | | | | | | | | | | | | | |
| st2 | 0.98** | 0.97** | 0.95** | | | | | | | | | | | | | |
| st3 | 0.46 | 0.40 | 0.62** | 0.50* | | | | | | | | | | | | |
| chol | 0.95** | 0.96** | 0.88** | 0.93** | 0.30 | | | | | | | | | | | |
| cholest | 0.92** | 0.93** | 0.95** | 0.94** | 0.37 | 0.91** | | | | | | | | | | |
| brass | 0.95** | 0.95** | 0.92** | 0.95** | 0.41 | 0.95** | 0.94** | | | | | | | | | |
| st4 | 0.95** | 0.95** | 0.93** | 0.95** | 0.39 | 0.94** | 0.96** | 0.96** | | | | | | | | |
| st5 | 0.93** | 0.96** | 0.84** | 0.90** | 0.30 | 0.94** | 0.87** | 0.90** | 0.92** | | | | | | | |
| camp | 0.83** | 0.84** | 0.79** | 0.81** | 0.34 | 0.82** | 0.80** | 0.90** | 0.82** | 0.79** | | | | | | |
| st6 | 0.34 | 0.27 | 0.25 | 0.28 | -0.05 | 0.42 | 0.33 | 0.33 | 0.40 | 0.41 | 0.18 | | | | | |
| stig | 0.28 | 0.24 | 0.34 | 0.32 | 0.19 | 0.27 | 0.39 | 0.42 | 0.36 | 0.25 | 0.42 | 0.20 | | | | |
| sito | -0.36 | -0.43 | -0.35 | -0.36 | -0.09 | -0.38 | -0.36 | -0.23 | -0.39 | -0.43 | -0.02 | -0.10 | 0.25 | | | |
| st7 | 0.31 | 0.37 | 0.25 | 0.28 | -0.05 | 0.46 | 0.29 | 0.32 | 0.34 | 0.48 | 0.27 | 0.38 | 0.27 | -0.14 | | |
| dino | 0.84** | 0.90** | 0.78** | 0.83** | 0.16 | 0.92** | 0.83** | 0.83** | 0.83** | 0.91** | 0.75** | 0.38 | 0.22 | -0.41 | 0.63** | |
| C30tri | -0.20 | -0.21 | -0.17 | -0.18 | -0.10 | -0.17 | -0.14 | -0.05 | -0.17 | -0.21 | -0.01 | -0.10 | 0.23 | 0.28 | -0.30 | -0.24 |

| | |
|---|---|
| * | $p < 0.01$ |
| ** | $p < 0.001$ |

211
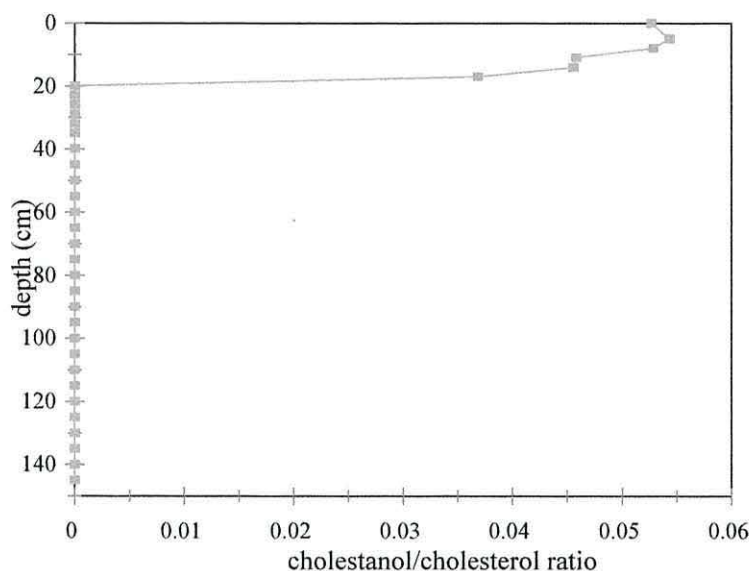
Figure 6.14: Vertical profile of cholestanol/cholesterol ratio in sediment core of Loch Riddon

Similar to fatty alcohols, the correlation was generally low within the sterol compounds, with 43% of the compound pairs showing an $r$ value greater than 0.5 (Table 6.3). Marine and sewage derived compounds were strongly correlated to each other. Brassicasterol was strongly correlated with 24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22(E)-dien-3β-ol, cholesterol, coprostanol, epicoprostanol and cholestanol, with $r$ values of 0.95, 0.95, 0.95, 0.95, 0.92 and 0.94 respectively. Meanwhile coprostanol was correlated most strongly with 24 norcholesta-5,22(E)-dien-3β-ol, epicoprostanol, cholesta-5,22(E)-dien-3β-ol, cholesterol and cholestanol, with $r$ values of 0.97, 0.93, 0.97, 0.96 and 0.93 respectively. Interestingly, higher plant sterols such as β-sitosterol, stigmasterol and campesterol did not correlate well with each other. Campesterol, on the other hand, was strongly correlated with 24 norcholesta-5,22(E)-dien-3β-ol, brassicasterol, cholesterol and coprostanol, with $r$ values of 0.83, 0.90, 0.82 and 0.84 respectively.

Table 6.3: Trivial and systematic names of the sterols identified

| Abbreviation | Systematic names |
| --- | --- |
| st1 | 24 norcholesta-5,22(E)-dien-3β-ol |
| cop | 5β-cholestan-3β-ol (coprostanol) |
| epicop | 5β-cholestan-3α-ol (epicoprostanol) |
| st2 | cholesta-5,22(E)-dien-3β-ol |
| st3 | 5α-cholest-22(E)-en-3β-ol |
| chol | cholest-5-en-3β-ol (cholesterol) |
| cholest | 5α-cholestan-3β-ol (cholestanol) |
| brass | 24-methylcholesta-5,22(E)-dien-3β-ol (brassicasterol) |
| st4 | 5α(H)-cholest-7-en-3β-ol |
| st5 | 24-methylenecholest-5-en-3β-ol |
| camp | 24-methylcholest-5-en-3β-ol (campesterol) |
| st6 | 24-methyl-5α(H)- cholestan-3β-ol |
| stig | 24-ethylcholesta-5,22(E)-dien-3β-ol (stigmasterol) |
| sito | 24-ethylcholest-5-en-3β-ol (sitosterol) |
| st7 | 24-ethyl-5α(H)- cholestan-3β-ol |
| dino | 4α,23,24-trimethyl-5α(H)- cholest-22(E)-en-3β-ol (dinosterol) |
| C30trienol | structure unknown |

Marine sources account for most of the sterols in surface sediments. Cholesterol is present in many marine organisms mainly zooplankton (Volkman *et al.*, 1987). Brassicasterol has been found in several algal groups (Volkman *et al.*, 1998). The $C_{26}$ sterol, 24 norcholesta-5,22(E)-dien-3β-ol (st1) is also found. This sterol is not unique to marine organisms but is often found in the marine environment (Volkman, 1986, Yunker *et al.*, 1995; Mudge *et al.*, 1999). Another sterol, cholesta-5,22-dien-3β-ol (st2) has been reported as a good biomarker for diatoms (Nichols *et al.*, 1993). β-sitosterol, campesterol and stigmasterol, on the other hand are the most common sterols in vascular plants (Volkman, 1986) even though they also have been found in phytoplankton in selected environments.
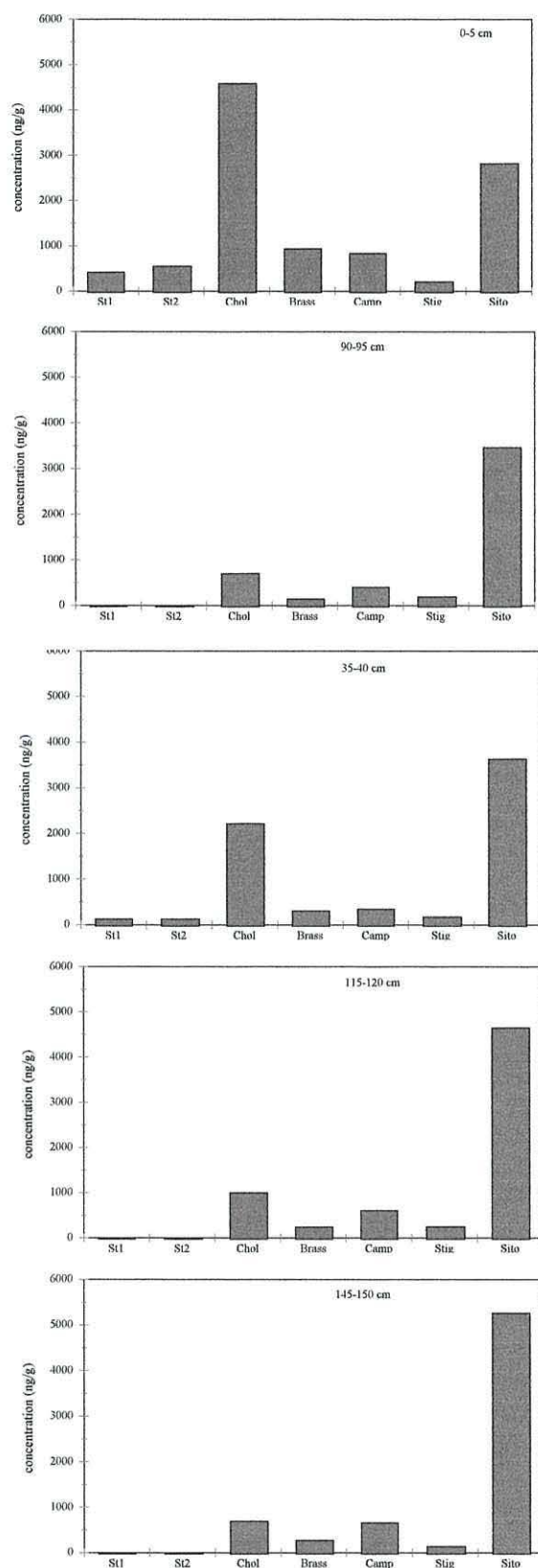
Figure 6.15: Distribution of sterols in Loch Riddon sediment

Figure 6.15 is a histogram of concentration of seven sterols from different depth sections in the core. Marine sterols, which include algal and zooplankton markers (st1, st2, cholesterol and brassicasterol), decrease in concentration while terrigenous $C_{29}$ sterols increase with depth. It is apparent that marine sterols have been subjected to degradation with depth. Volkman *et al.* (1987) established that organic matter of marine origin was remineralized at or near the sediment/water interface in marine sediment off Peru. The increase of $C_{29}$ sterols might be due to change in source. β-sitosterol, campesterol and stigmasterol could be originated from marine algae (Volkman, 1986), but it is not always. In this study, β-sitosterol, campesterol and stigmasterol were assumed to be originating from terrigenous sources considering the high concentration of other biomarkers indicative of plant vascular materials such as long chain fatty acids and fatty alcohols analysed in this study. Therefore, degradation and changing in source were both take place in the sediment core.

β-sitosterol and campesterol were used to calculate the Sterol Source Index (SSI). The SSI was calculated by dividing terrestrially derived sterol with cholesterol (Mudge and Norris, 1997). Cholesterol was assumed to be a marine sterol (Grimalt and Albaiges, 1990). β-sitosterol/cholesterol appeared to be the strongest index, increasing by a factor of 12 between the top and bottom of the core. The campesterol/cholesterol ratio increased by a
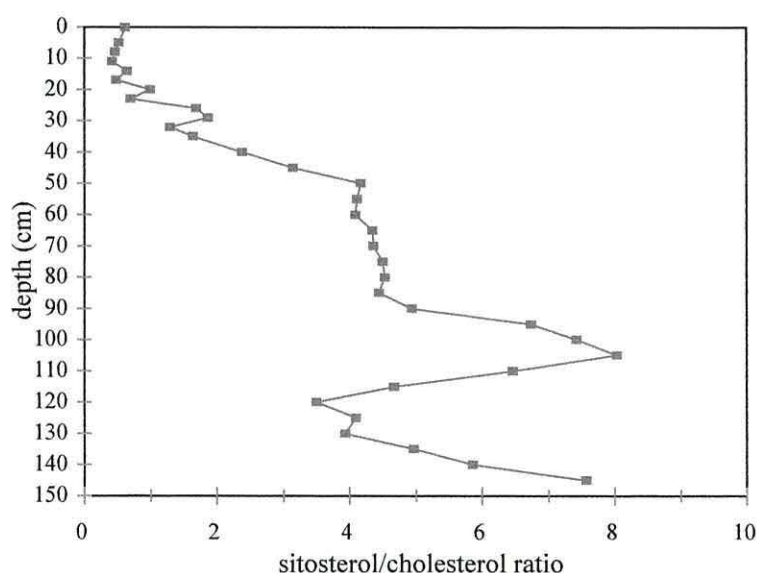


Figure 6.16: Vertical profile of β-sitosterol/cholesterol ratio in sediment core of Loch Riddon

factor of 5. Figure 6.16 shows the vertical distribution of β-sitosterol/cholesterol ratio down the core. Unfortunately, the SSI does not correlate significantly with the ASI. For example, correlation coefficient ($r$) for β-sitosterol/cholesterol and $C_{24}/C_{14}$ was only 0.44 and 0.39 for β-sitosterol/cholesterol and $C_{24}/C_{16}$ ratios.

Figure 6.17 shows that the bioturbation was not seen in the core as the profiles of the core were preserved. Bioturbation is the physical activity by the marine fauna by turning over the sediments, which cause the sediments to become mixed.
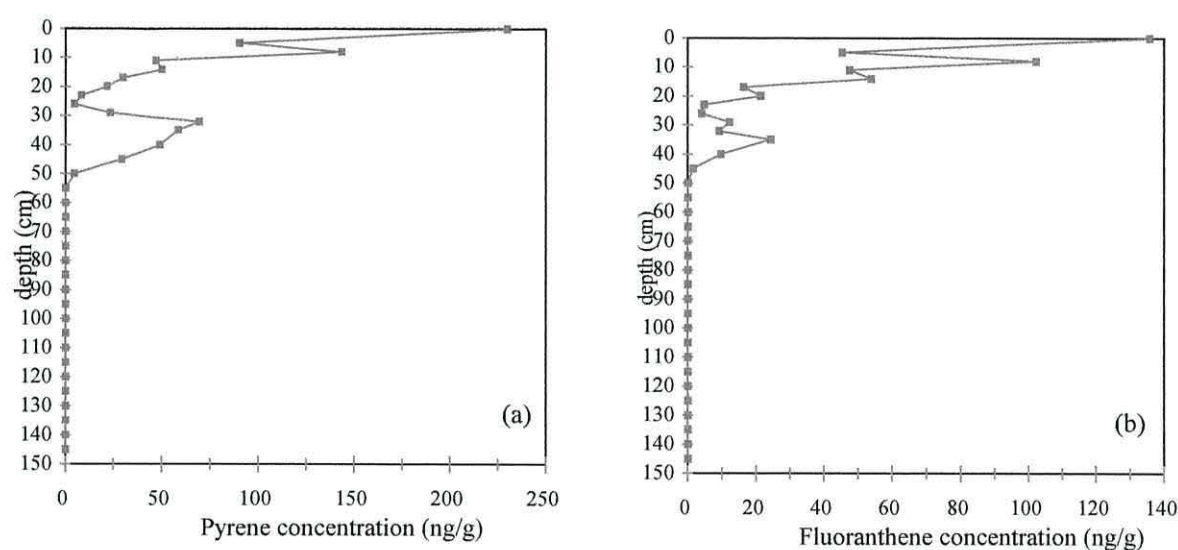


Figure 6.17: Concentration of pyrene and flouranthene in Loch Riddon sediment

The presence of polycyclic aromatic hydrocarbons (PAHs) in sediments is generally considered to reflect inputs from the combustion of organic matter, such as wood and fossil fuels (Killops and Killops, 1993, Pereira *et al.*, 1999). Profiles of pyrene and fluoranthene concentration with depth are shown in Figure 6.17a and 6.17b. Concentrations of these PAHs started to increase around the beginning of the Industrial Revolution (mid-1700's) in response to increasing urbanisation and industrialisation around the area. The decline in the concentrations of the PAHs (between 35-25cm) may be due to a change from coal to oil and gas as home heating fuels (Gustafson *et al.*, 1997). Urban runoff containing PAHs

derived from abrasion of street asphalt and automobile tyres, vehicular emission and chemical products, which evolved from chemical industry, may be the reasons for the increasing PAHs from the past to the sampling time. The concentration of pyrene and fluoranthene in Loch Riddon are generally low compared to PAHs found in Richardson Bay, California (Pereira *et al.*, 1999). It is possible that sediments in Richardson Bay have greater sources of PAHs than in Loch Riddon as some oil refineries are located in the Richardson Bay. It is possible to determine the age of the sections in the core through a variety of methods. The traditional dating technique using $^{210}$Pb is not suitable in this study as the half-life of $^{210}$Pb is 22.4 years. The age of Loch Riddon core is estimated to be 250 years at the 50 cm depth and the sediments were collected to 150 cm, hence, the age of the core might be more than 800 years. Therefore, a longer lived element is needed such as $^{14}$C, with the half-life of 5730 years. However, the facilities for doing such a technique were not available. Polycyclic aromatic hydrocarbons (PAHs) can be used as an alternative way to know the age of sections of the core.

Sterols have been widely used as indicators of sewage and other organic matter discharges. Coprostanol (5β-cholestan-3β-ol) has been shown to be a reliable marker of sewage pollution (Sherwin *et al.*, 1993; Jeng and Han, 1994). Coprostanol has also been used to show sewage pollution history from a sediment core (Venkatesan and Mirsadeghi, 1992; Jeng and Han, 1994; Jeng and Han, 1996). Coprostanol/cholesterol ratios have been used by several researchers to indicate the relative abundance of sewage in sediment (Jeng and Han, 1996; Mudge and Bebianno, 1997). The concentration of coprostanol in the Loch Riddon core has similar distribution with concentration of pyrene and fluoranthene ($r$=0.73 and $r$=0.82 respectively, p<0.001). This may suggest a link between these compounds to the extent of human influence around this area. Figure 6.18a shows the profile of coprostanol/cholesterol ratio throughout the core. The top of the core has the highest ratio, which decreases with depth. The depth that the ratios increase supposedly suggests an increase in population around this region due to the rapid growth of the industrialisation. Epicoprostanol is generally present in treated sewage sludge (Mc Calley *et al.*, 1981; Mudge *et al.*, 1999). Epicoprostanol/coprostanol ratios can be used to indicate the degree of treatment. Epicoprostanol can only be observed from the 17-20cm depths until the top of the core. Figure 6.18b shows the epicoprostanol/coprostanol profiles throughout the

core. The depth 17-20 cm indicates the time when sewage treatment plants become operational.
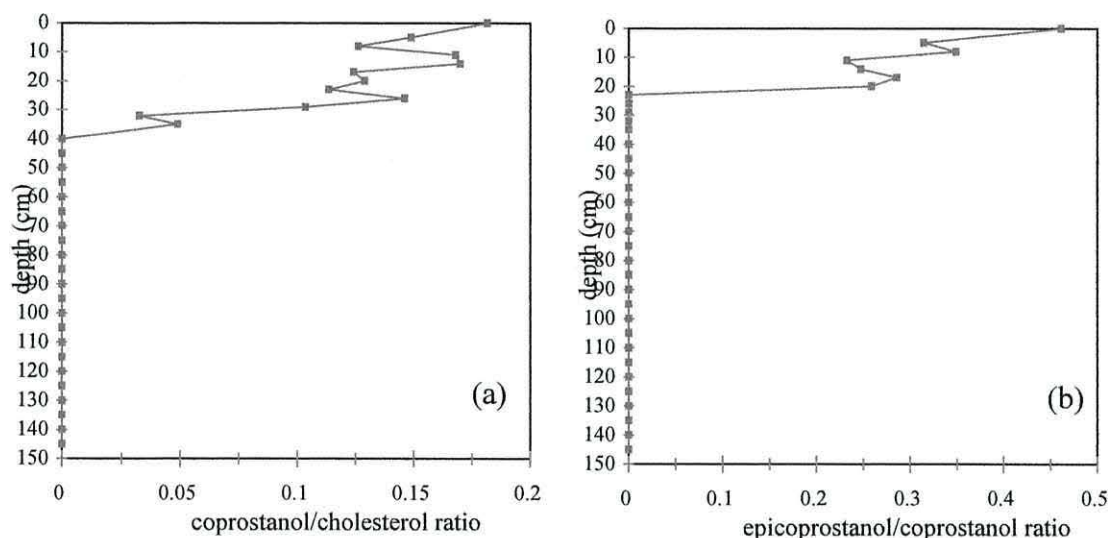


Figure 6.18: Vertical profiles of epicoprostanol/coprostanol ratios and coprostanol/cholesterol ratio in sediment core of Loch Riddon

## 6.3 Multivariate statistical analysis

### 6.3.1 Principal component analysis

Principal Component Analysis (PCA) was carried out on individual chemical group as well as on combination of fatty acids, fatty alcohols and sterols. PCA performed in this chapter was using proportion data, added 0.001 with log transformation.

#### 6.3.1.1 Fatty acids

The loadings for each compound on Principal Component 1 (PC1) and Principal Component 2 (PC2) are shown in Figure 6.19. PC1 and PC2 account for 34.1% and 22.1% of the variance in the data. The data shows that the bacterial and marine/algal derived fatty acids ($18:1\omega7$, $16:1\omega7$ and short chain fatty acids) clustered on the left side with negative loadings on PC1, while terrestrial input fatty acids (long chain moieties) clustered at the right side with positive loadings. Bacterial derived compounds were divided into 2 groups. Both groups have negative loadings on PC2. The first group was negatively loaded on PC1. Bacteria 1 and 2 were not well separated but distinct. Another group of bacterial

218

derived fatty acids was loaded positively on PC1. From the diagram C19:0 loads with terrestrial compounds. It may not be degraded or C19:0 is having terrigenous source.
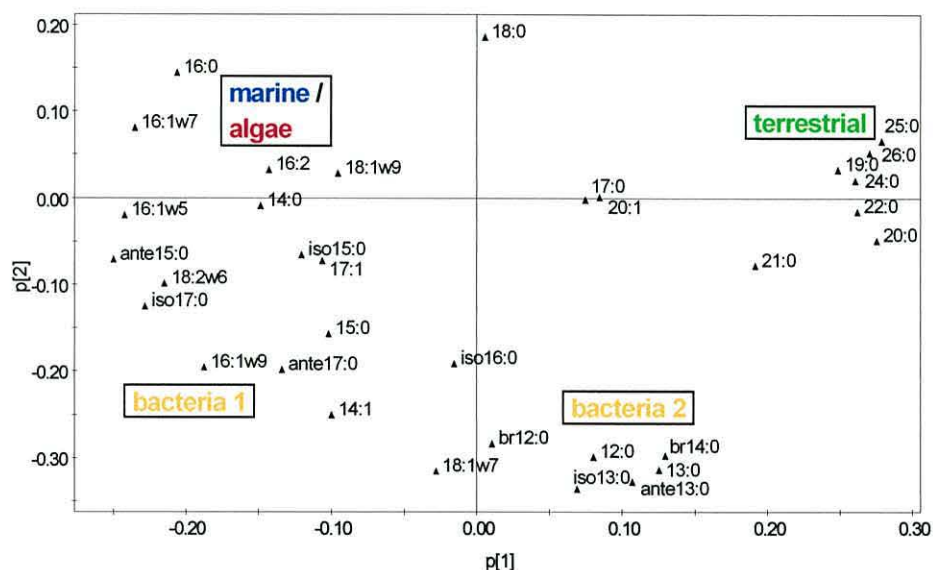


Figure 6.19: The loadings for each fatty acid on PC1 and PC2 in the PCA model for Loch Riddon

### 6.3.1.2 Fatty alcohols

Figure 6.20 illustrates the loadings of fatty alcohols in PC1 and PC2, which account for 45.6% and 18.1% respectively, of the variance in the data. PC1 appears to correspond directly to source input with strong positive loadings representing marine/algae and bacterial derived fatty alcohols (phytol, short and branched chain alcohols). The bacterial group could be aerobic as they occurred in the surface sample, which is rich with oxygen. Monounsaturated 20:1 alcohol, which is produced by zooplankton, is also positively loaded on PC1. Strong negative loadings on PC1 representing terrigenous input compounds such as long chain fatty alcohols. $C_{19}$ - $C_{21}$ alcohols are loaded with the longer chain alcohols. They may have been from terrestrial input or they were not readily degraded in the sediment. Another group of bacterial fatty alcohols was loaded negatively on PC2.
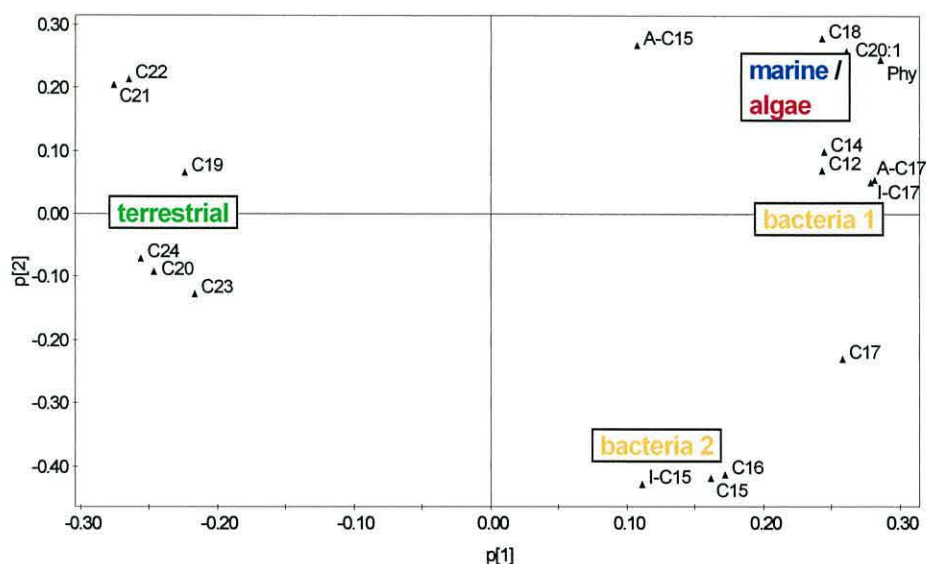
Figure 6.20: The loadings for each fatty alcohol on PC1 and PC2 in the PCA model for Loch Riddon

### 6.3.1.3 Sterols

The loadings on PC1 (57.8%) and PC2 (13.5%) can be seen in Figure 6.21. Compounds that are algal and sewage (bacterial) markers such as cholesterol, brassicasterol, coprostanol and epicoprostanol are loaded positively on PC1. Meanwhile, the terrestrial markers ($\beta$-sitosterol, campesterol and stigmasterol) are negatively loaded together with C30 trienol. These observations correspond to PCA applied to fatty acids and fatty alcohols individually. Sterol st7 (sitostanol) is a compound produced in an *in situ* reduction of $\beta$-sitosterol and loads negatively on PC2. Therefore this may be due to anaerobic bacteria domination.
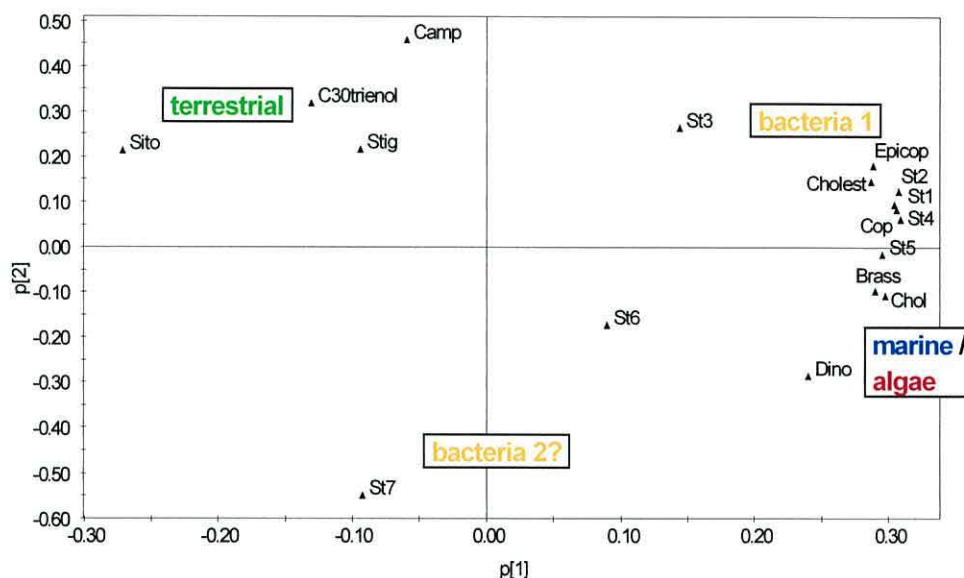
Figure 6.21: The loadings for each sterol on PC1 and PC2 in the PCA model for Loch Riddon

### 6.3.1.4 Mixed PCA

PCA on the mixture of compounds was performed to compare and confirm individual class observations of the distribution of biomarkers throughout the core. The loadings for each compound are shown in Figure 6.22a and the scores in Figure 6.22b. PC1 and PC2 account for 34.9% and 20.5% of the variance in the data. PC1 has strong positive loadings for marine and bacterial compounds as well as algal derived organic matter and strong negative loadings for mixed group of terrestrial derived compounds including C30 trienol, β-sitosterol and long chain fatty acids and fatty alcohols. There are also some mixed non-attributable or undiagnostic compounds that do not fit in with other groups (St3, $C_{15}$, $C_{16}$ and $C_{17}$ saturated alcohols, saturated 17:0 and 18:0 fatty acids). Samples in PC1 (Figure 6.22b) therefore, are separated according the relative importance that terrigenous, bacterial or marine inputs have for each sample from the Loch Riddon core. The plot of scores clearly distinguishes the core with marine and bacterial derived organic matter dominates the upper part of the core while terrestrial organic matter dominates the deeper sediments. Group 1 bacterial dominated the surface sample together with marine markers. They may

be aerobic bacteria. These results may be due to the degradation of marine lipids and the preservation of the terrestrial derived organic matter.
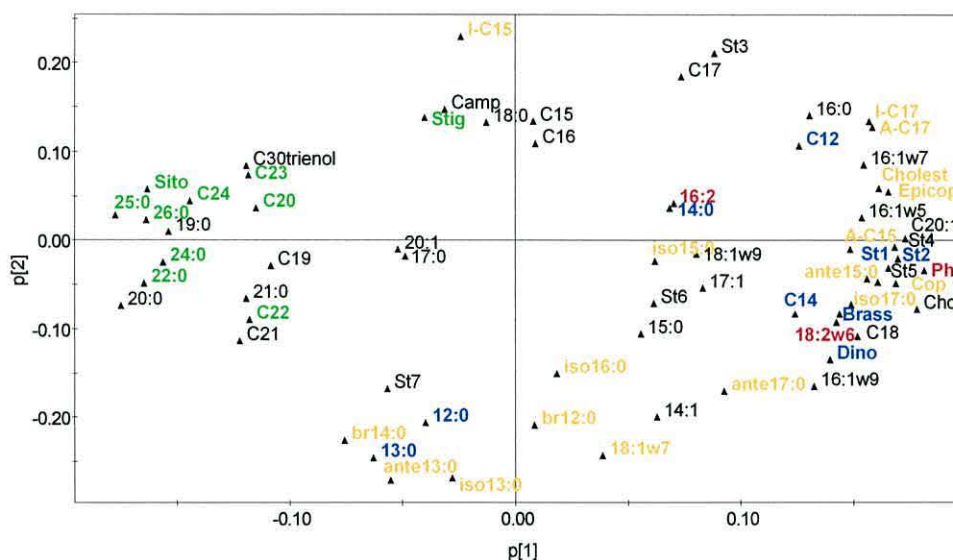


Figure 6.22a: The loadings for each compound on PC1 and PC2 in the PCA model for Loch Riddon
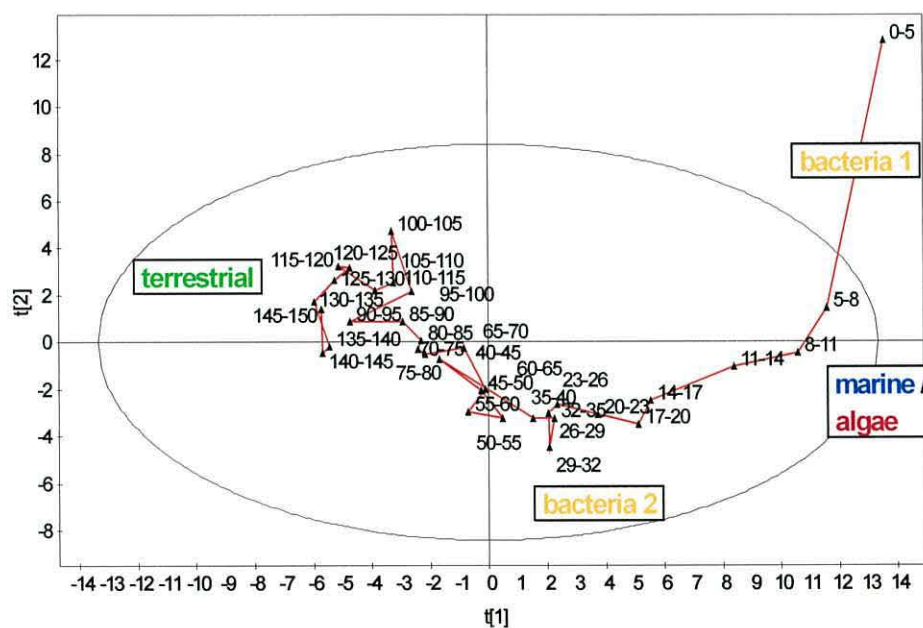


Figure 6.22b: The PCA scores for the first two components in the Loch Riddon sediment

## 6.3.2 Partial least squares (PLS) path modelling

In performing the PLS, the top samples of the core were used to characterised the biomarker signatures (*X*-block). The PLS program then uses the multivariate relationships between variables to quantify the amount of top sample materials in the rest of the core (*Y*-block). From the fit diagram (Figure 6.23) there was a big change from the surface to 5cm depth. Almost 23% of the signature decreased and this may due to rapid diagenesis. The slower rate of signature change can be seen from 5cm to 50cm. Change in signature may be due to change of sources or degradation of selected compounds. The diagram shows that in 40cm the change of signatures is 50%. The remaining core indicates a much slower rate showing that they are same material/compounds with constant source input or there is nothing left to degrade. And these are called refractile materials.
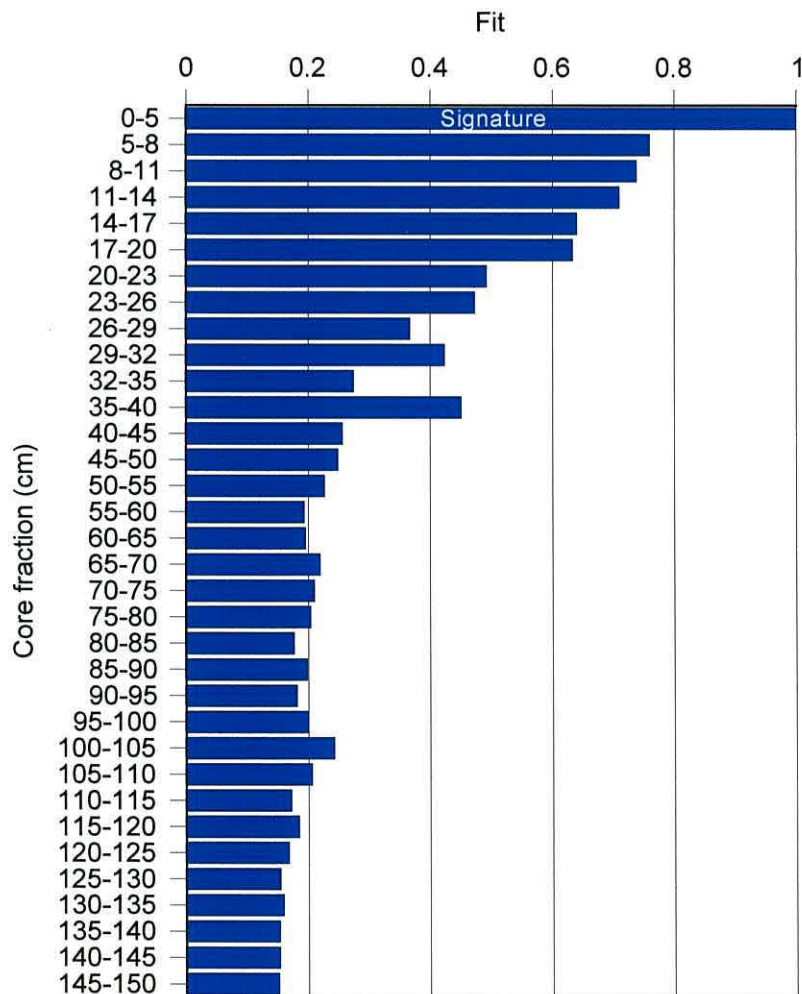
Figure 6.23: The fit diagram

PLS was then carried out with three different sets of signatures. The signatures used were the surface sediment samples from the Clyde Sea. S1, S2 and S10 were used as the *X*-block to characterise the marine markers. To characterise the sewage (bacterial) signatures, S12, S13 and S21 were chosen to be the *X*-block, while S31, S32 and S33 were used to characterise the terrestrial signatures. The fit diagram (Figure 6.24) shows that the marine signatures are greatest at the surface and decrease with depth. This is likely to be due to degradation of more labile compounds such as short chain fatty acids and fatty alcohols as well as polyunsaturated and monounsaturated compounds. Meanwhile no change of
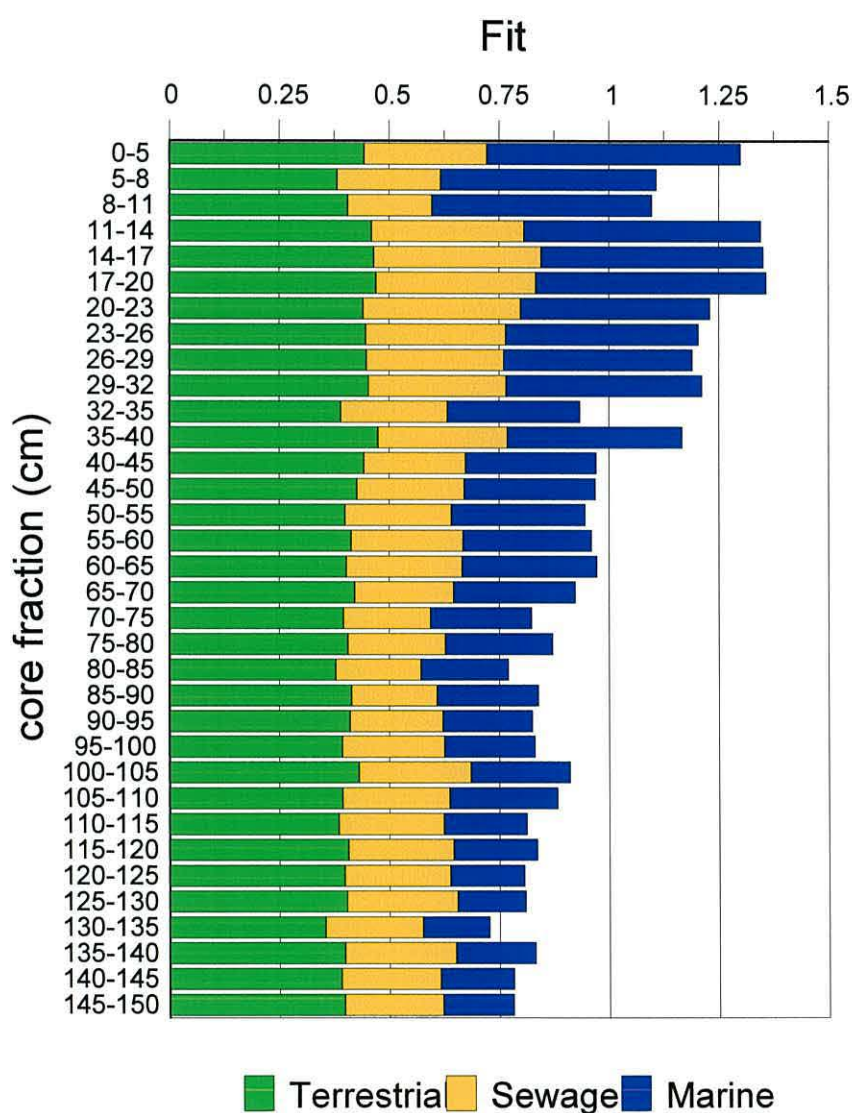


Figure 6.24: The fit diagram in the PLS model with the Clyde Sea surface sediment samples as signatures

terrestrial signatures can be seen down the core. Although the surface signature diagram (Figure 6.23) shows changes in the signatures, terrestrial materials were constant down the core. There is slight elevation of sewage signatures at the surface that could be due to anaerobic bacteria. From 32cm, the sewage signatures were constant and it may be associated with resident bacteria population.

Figure 6.25 shows the proportion of fit diagram of each signature. Care is needed for interpretation when using proportion. Proportion of marine signatures shows real change down the core. It may be due to degradation of short chain compounds. Meanwhile, terrestrial signatures increase down the core, which may be an artefact of the decreasing of marine signatures.
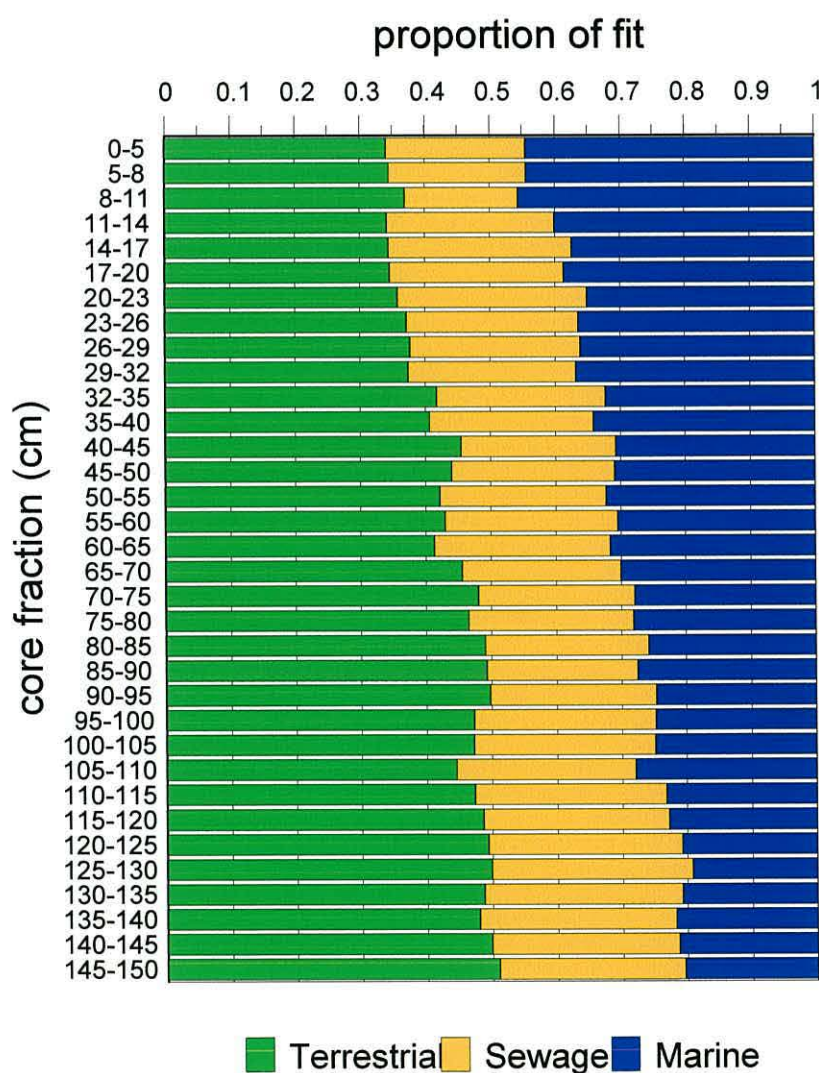


Figure 6.25: The proportion of fit diagram

Plot of the *X*-block (t1) and *Y*-block (u1) when characterising marine markers is shown in Figure 6.26. The projection was essentially linear with 16:2 polyunsaturated fatty acid, which is an algae marker, having the largest distant below the line of agreement. Meanwhile, β-sitosterol has the largest distant above the line. β-sitosterol is always used as a terrestrial marker. The diagram also shows that this *X*-block signature is appropriate for the *Y*-block data with a reasonable relationship between u1 and t1.
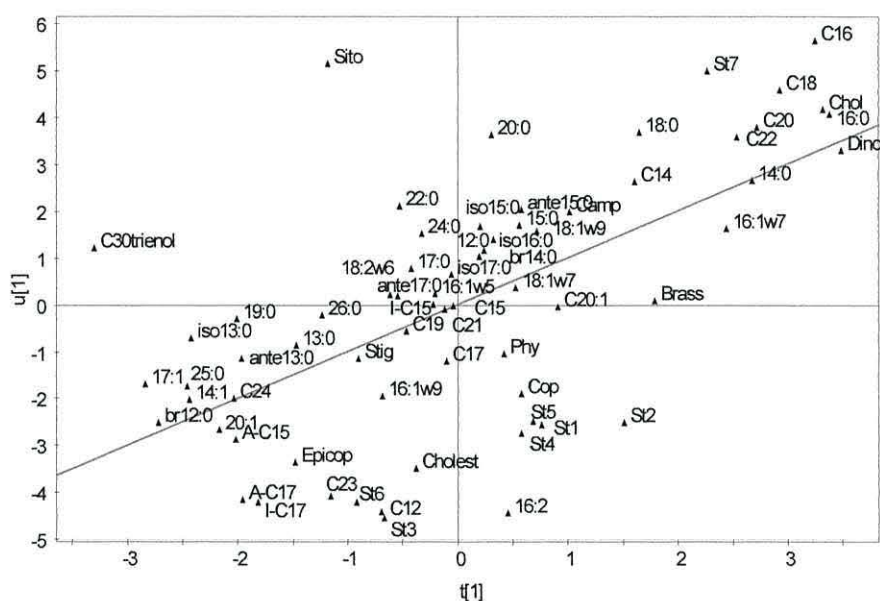


Figure 6.26: Comparison of t1 and u1 projections for PLS with S1, S2 and S10 as marine signatures

Figure 6.27 shows the comparison of *X*-block (t1) and *Y*-block (u1) when S12, S13 and S21 were used as sewage signatures. The trend line was drawn within the diagram. The trend is fine but there is different relationship between compounds in these *X*-blocks to the *Y*-block. Similar shape of trend line with same compounds can be seen when using S31, S32 and S33 as terrestrial signatures. It is shown in Figure 6.28. These results indicate that these *X*-block signatures may not be correct to use with the *Y*-block (Loch Riddon core samples).
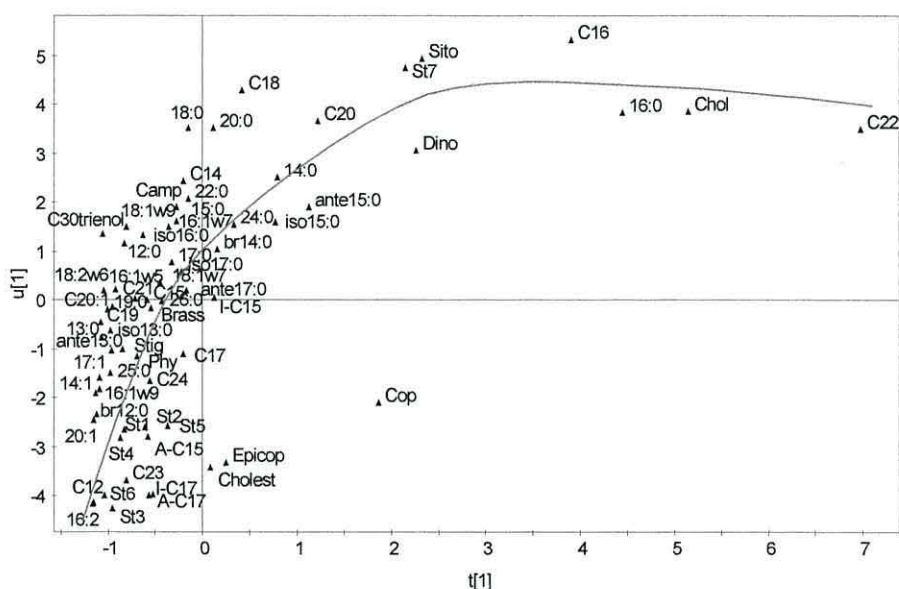
Figure 6.27: Comparison of t1 and u1 projections for PLS with S12, S13 and S21 as sewage signatures
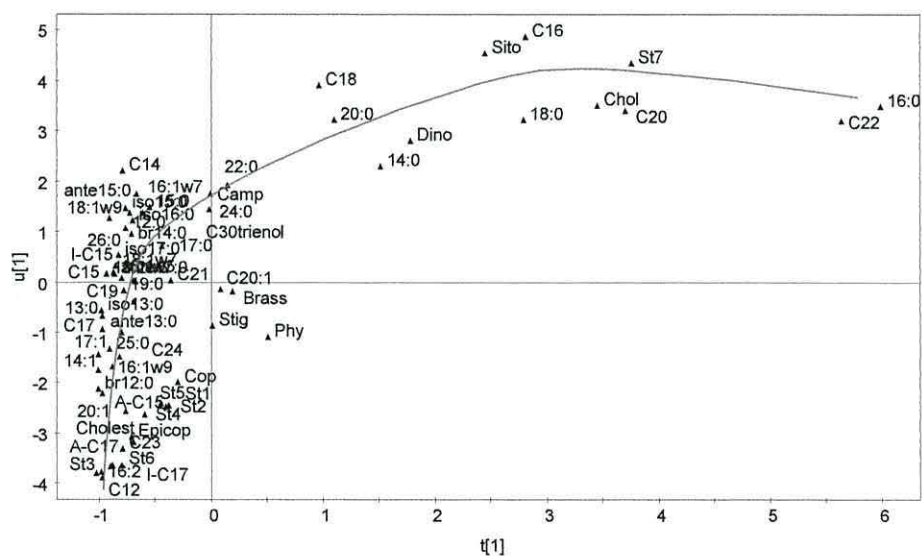


Figure 6.28: Comparison of t1 and u1 projections for PLS with S31, S32 and S33 as terrestrial signatures

## 6.3.3 Cluster analysis

Cluster analysis was used in this study to classify the core section with the geochemical data. Cluster analysis was carried out separately on fatty acids, fatty alcohols and sterols. The Ward's method with correlation coefficient distance was used throughout the analysis.

### 6.3.3.1 Fatty acids

The cluster analysis results (hierarchical clustering, Ward's method) on fatty acids are shown in Figure 6.29. Altogether 2 clusters could be interpreted, cluster group I is divided into 2 smaller subgroups. The first and second clusters in cluster group I contain short chain fatty acids as well as the branched compounds. Short chain fatty acids are the marine markers while branched fatty acids are associated with bacterial communities. These observations are supported by the PCA showing 2 groups of bacterial derived compounds occurred in the sediment samples. The second cluster group indicates the terrestrial derived material with all the long chain fatty acids are clustered together.
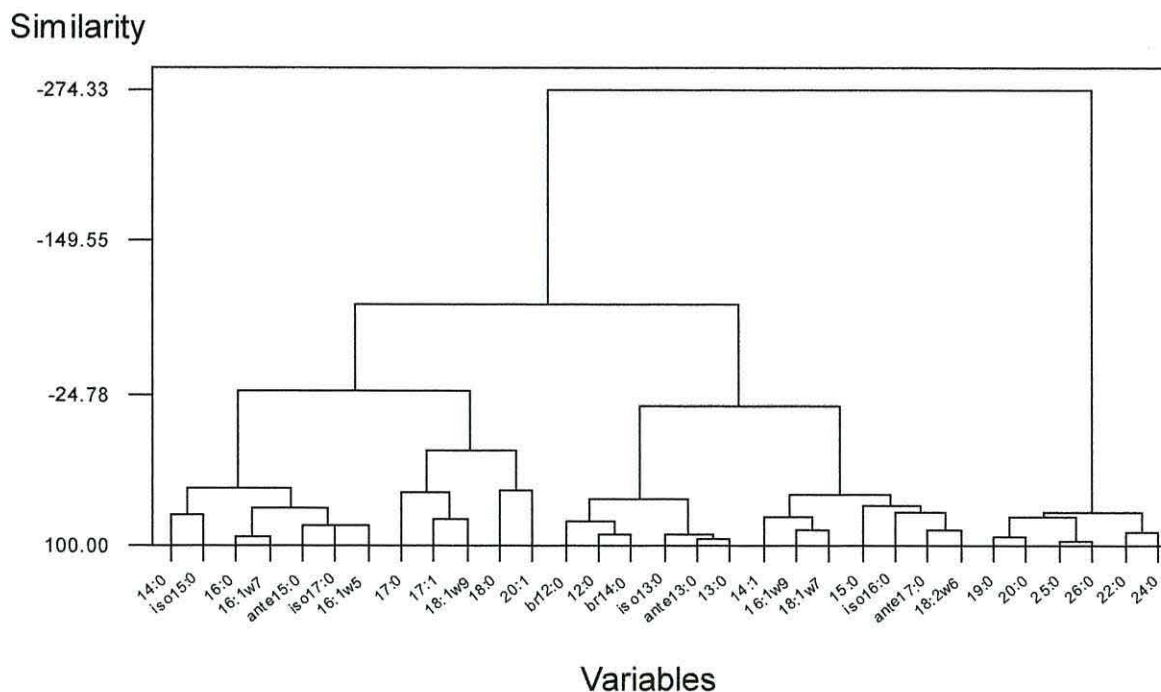


Figure 6.29: Cluster analysis of fatty acids showing the Ward's method for the Loch Riddon core

### 6.3.3.2 Fatty alcohols

Cluster analysis that was performed on fatty alcohols shows similar results with fatty acids (Figure 6.30). Two cluster groups could be seen with the first divided into 2 smaller groups. Cluster group I contain marine/algal derived organic matter (short chain fatty alcohols as well as phytol and monounsaturated 20:1) and bacterial compounds (branched chain fatty alcohols). Meanwhile cluster group II is influenced by long chain fatty alcohols associated with terrestrial markers.
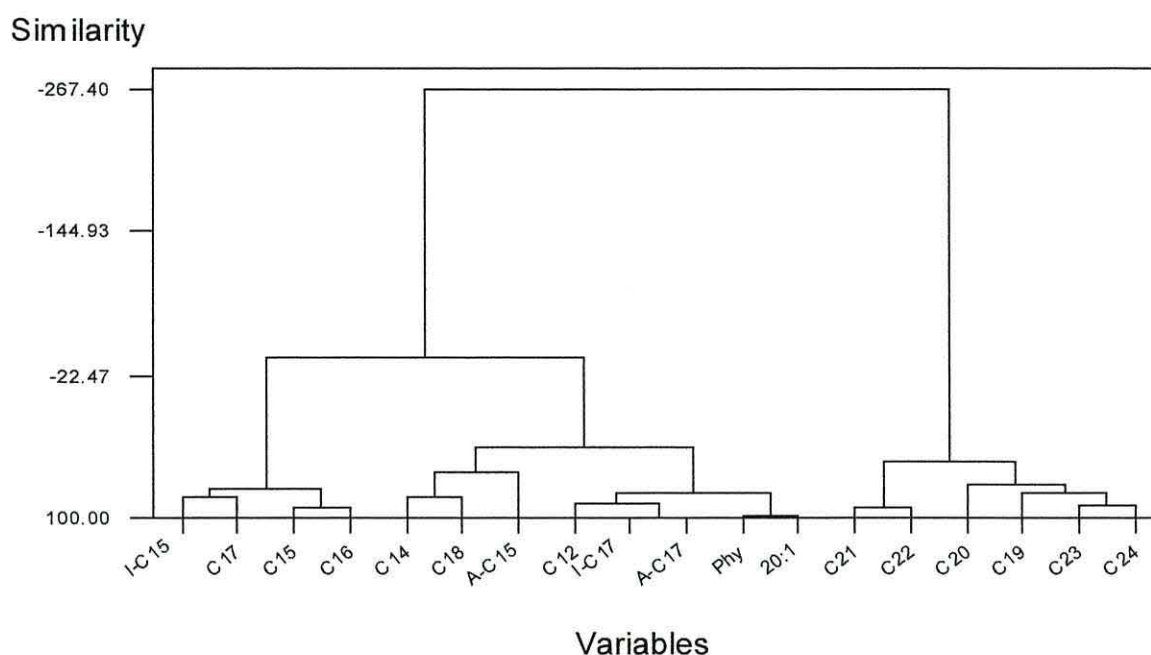


Figure 6.30: Cluster analysis of fatty alcohols showing the Ward's method for the Loch Riddon core

### 6.3.3.3 Sterols

The dendogram from cluster analysis of sterols is shown in Figure 6.31. The results enabled the identification of two main cluster groups. Cluster group I consists of marine (st1, st2, brasscisterol and cholesterol) and sewage derived compounds (coprostanol, epicoprostanol and cholestanol). Dinosterol, which is always found in dinoflagellates, is also clustered within this group, thus supporting a marine origin for these compounds. Meanwhile cluster group II includes the terrestrial inputs such as β-sitosterol, stigmasterol and C30 trienol.

Similarity
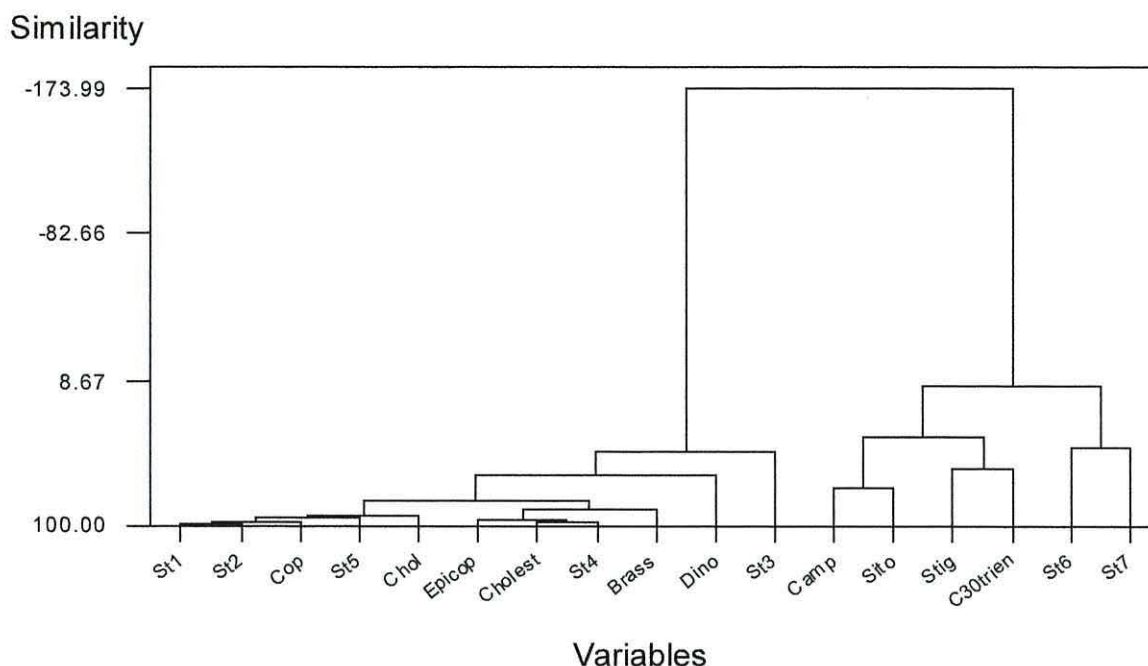


Figure 6.31: Cluster analysis of sterols showing the Ward's method for the Loch Riddon core

### 6.3.3.4 Mixed compounds

The dendogram based on mixed compounds (Figure 6.32a) shows 4 clusters divided into 2 bigger cluster groups. Cluster group I include long chain fatty alcohols together with stigmasterol, β-sitosterol and C30 trienol indicating their terrestrial origin. Short and branched chain fatty acids dominated the other group in the cluster group I. These are marine and bacterial derived compounds. Meanwhile cluster group II is associated with marine and sewage markers. Compounds such as short chain fatty alcohols together with st1, cholesterol and dinosterol are known as marine markers. Coprostanol, which is a sewage derived compound, is also clustered with these compounds.
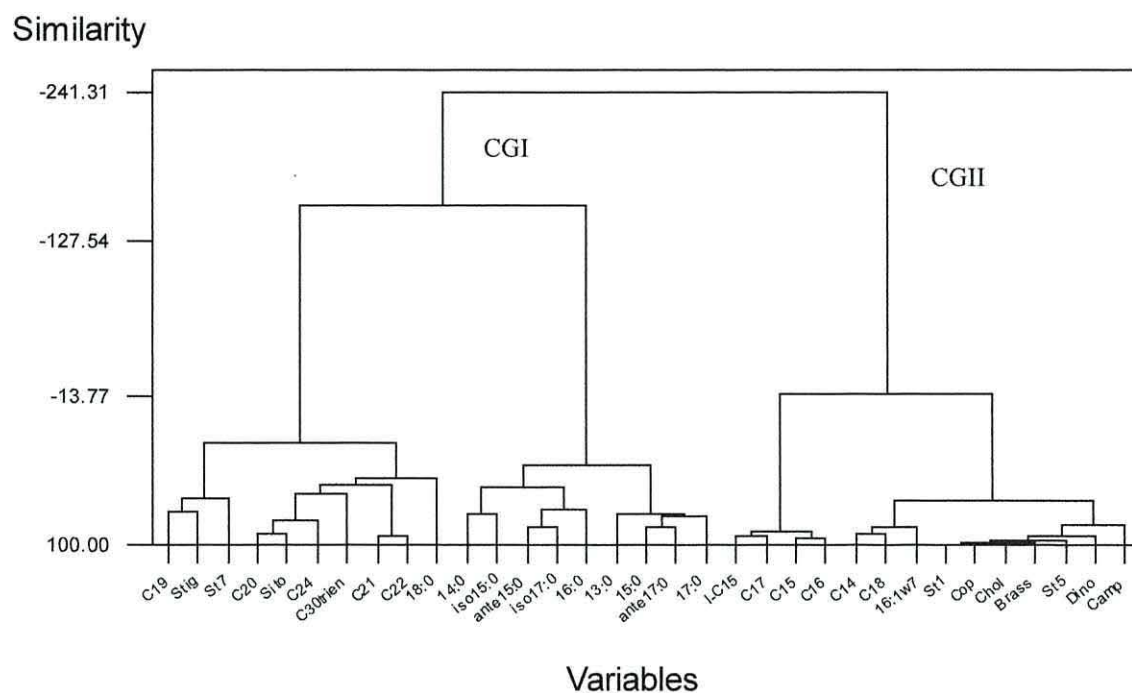
Figure 6.32a: Cluster analysis of mixed compounds showing the Ward's method for the Loch Riddon core
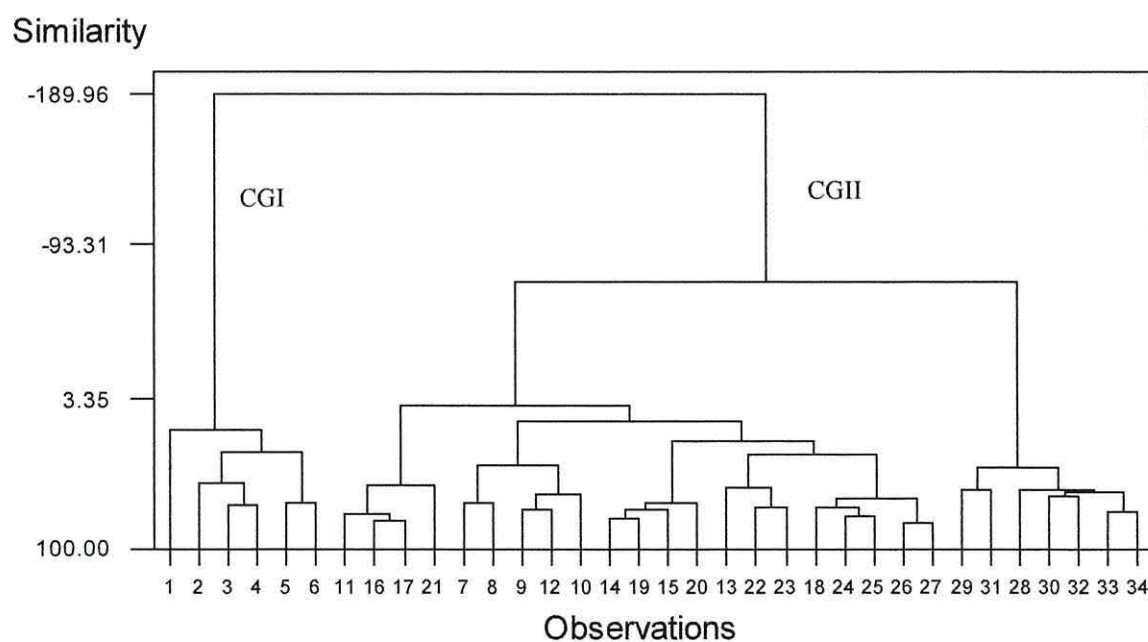


Figure 6.32b: Cluster analysis of samples (depth) showing the Ward's method for the Loch Riddon core

Figure 6.32b shows the cluster analysis results of the core depths as objects. In general CGI (the smaller group) corresponds to samples characterised by marine and bacterial/sewage derived compounds. The CGII consists of the deeper section of the core and characterise by terrestrial derived organic matter.

### 6.3.4 Factor analysis

The same variables were used in this analysis as in PCA, PLS path modelling and cluster analysis. All the data used for factor analysis was obtained by subtracting the mean from each observation and dividing by standard deviation. Factor analysis that was carried out only on fatty alcohols was using raw data, as extraction cannot be done with the autoscored, proportion or log transformation data. Initial extraction was carried out first to decide the final number of factors to extract. In this study, the Kaiser-Gutmann rule was used, where the number of factors to be extracted should be equal to the number of factors having an eigenvalue (variance) greater than 1.

### 6.3.4.1 Fatty acids

The factor analysis of fatty acids in the Loch Riddon gave eight factors describing 85.32% of the data variability. The rotated factor matrix, eigenvalues, percentage of variance and cumulative of variance of 8 factors are given in Table 6.5.

The first factor, which accounts for 30.50% of the total variance and this, constitutes the main factor. It is characterised by high loadings of straight chain fatty acids (17:0, 18:0, 19:0, 20:0, 21:0, 22:0, 24:0, 25:0 and 26:0 acids). Therefore this factor represents terrestrial inputs, as long chain fatty acids are found in great abundant in higher plants. The second factor (which accounts for 24.81% of the total variance) is mainly associated with high loadings of short chain (12:0 and 14:0 acids), odd chain length (13:0, 15:0 and 21:0 acids) and branched fatty acids (*br*-12:0, *iso*-13:0, *anteiso*-13:0, *br*-14:0, *iso*-16:0 and *anteiso*-17:0). Monounsaturated 18:1ω7 also has high loading in this factor. Branched and odd chain length fatty acids and 18:1ω7 are associated with bacterial biomass. Therefore this factor corresponds to bacterial inputs. The third factor (which accounts for 9.44% of the total variance) implies alga and bacterial inputs. It is characterised by high loadings of branched fatty acids together with monounsaturated (16:1ω9, 17:1, 18:1ω9 and 18:1ω7)

and polyunsaturated 18:2ω6 fatty acids. Factors 4-8 are characterised by the mixture of sources of compounds and together these factors account for 20.57% of the total variance.

Table 6.5: Rotated factor matrix for fatty acids

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 | Factor 8 |
|---|---|---|---|---|---|---|---|---|
| *br*-12:0 | -0.101 | 0.756 | 0.144 | 0.016 | 0.027 | -0.090 | -0.012 | 0.089 |
| 12:0 | 0.083 | 0.853 | 0.085 | 0.084 | 0.284 | -0.251 | -0.139 | 0.297 |
| *iso*-13:0 | -0.005 | 0.890 | 0.179 | -0.033 | -0.025 | 0.296 | 0.096 | -0.035 |
| *ante*-13:0 | 0.122 | 0.851 | 0.222 | -0.219 | 0.109 | 0.159 | 0.105 | -0.339 |
| 13:0 | 0.299 | 0.820 | 0.240 | -0.023 | 0.194 | -0.006 | 0.137 | -0.229 |
| *br*-14:0 | 0.321 | 0.709 | 0.231 | -0.121 | 0.476 | 0.027 | -0.061 | 0.177 |
| 14:0 | -0.019 | 0.375 | 0.057 | 0.213 | 0.436 | 0.211 | -0.023 | 0.094 |
| 14:1 | -0.462 | 0.378 | -0.018 | -0.082 | 0.334 | 0.226 | 0.406 | 0.017 |
| *iso*-15:0 | 0.066 | 0.144 | 0.178 | 0.157 | 0.856 | 0.026 | 0.076 | -0.064 |
| *ante*-15:0 | -0.441 | 0.231 | 0.532 | 0.316 | 0.107 | 0.245 | 0.238 | 0.261 |
| 15:0 | 0.249 | 0.378 | 0.598 | 0.025 | 0.402 | 0.181 | 0.028 | 0.108 |
| *iso*-16:0 | 0.179 | 0.535 | 0.417 | 0.014 | 0.274 | 0.368 | 0.082 | -0.212 |
| 16:0 | 0.071 | -0.008 | 0.310 | 0.742 | 0.219 | 0.176 | -0.009 | 0.209 |
| 16:1w9 | -0.407 | 0.395 | 0.374 | 0.249 | 0.103 | 0.178 | 0.471 | -0.116 |
| 16:1w7 | -0.438 | -0.267 | 0.167 | 0.807 | -0.140 | 0.114 | -0.023 | -0.152 |
| *iso*-17:0 | -0.350 | 0.312 | 0.460 | 0.440 | 0.311 | 0.082 | -0.034 | 0.156 |
| 16:1w5 | -0.343 | 0.074 | 0.219 | 0.685 | 0.351 | 0.154 | 0.080 | -0.104 |
| *ante*-17:0 | -0.011 | 0.495 | 0.636 | 0.137 | 0.077 | 0.558 | 0.109 | 0.028 |
| 17:0 | 0.603 | 0.148 | 0.544 | 0.030 | 0.426 | 0.134 | -0.232 | -0.140 |
| 16:2 | -0.181 | -0.050 | -0.024 | 0.164 | 0.073 | 0.608 | 0.014 | -0.004 |
| 17:1 | 0.005 | 0.248 | 0.902 | 0.142 | 0.219 | -0.199 | -0.086 | 0.067 |
| 18:0 | 0.351 | -0.232 | 0.220 | -0.077 | -0.114 | -0.230 | -0.012 | 0.200 |
| 18:1w9 | 0.066 | 0.118 | 0.721 | 0.179 | -0.053 | -0.062 | -0.007 | -0.067 |
| 18:1w7 | -0.397 | 0.433 | 0.442 | 0.229 | 0.159 | 0.071 | 0.177 | -0.052 |
| 18:2w6 | -0.242 | 0.186 | 0.633 | 0.237 | 0.149 | 0.407 | 0.166 | -0.041 |
| 19:0 | 0.864 | 0.160 | 0.164 | -0.025 | 0.091 | -0.020 | -0.311 | -0.109 |
| 20:0 | 0.861 | 0.291 | 0.076 | -0.212 | 0.025 | -0.016 | -0.147 | -0.125 |
| 20:1 | 0.106 | 0.141 | -0.216 | -0.196 | 0.135 | 0.121 | -0.119 | 0.096 |
| 21:0 | 0.614 | 0.514 | -0.105 | 0.043 | -0.068 | -0.124 | 0.242 | 0.131 |
| 22:0 | 0.898 | 0.213 | -0.118 | -0.044 | 0.040 | 0.145 | 0.287 | -0.060 |
| 24:0 | 0.851 | 0.011 | 0.101 | -0.160 | 0.058 | -0.166 | 0.305 | 0.067 |
| 25:0 | 0.913 | -0.081 | -0.176 | -0.161 | 0.063 | -0.037 | -0.153 | 0.042 |
| 26:0 | 0.811 | -0.095 | -0.116 | -0.137 | 0.001 | -0.252 | -0.221 | 0.115 |
| Eigenvalue | 10.067 | 8.187 | 3.114 | 1.825 | 1.602 | 1.259 | 1.092 | 1.011 |
| %of variance | 30.505 | 24.811 | 9.438 | 5.531 | 4.854 | 3.815 | 3.309 | 3.063 |
| Cumulative % of variance | 30.505 | 55.315 | 64.753 | 70.284 | 75.138 | 78.953 | 82.262 | 85.325 |

### 6.3.4.2 Fatty alcohols

Factor analysis of fatty alcohols has been carried out. The analysis generated three factors which together account for 81.71% of the variance. Table 6.6 shows the rotated factor matrix, eigenvalues, percentage of variance and cumulative percentage of variance.

Table 6.6: Rotated factor matrix for fatty alcohols

| Variable | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| C12 | 0.738 | 0.435 | -0.083 |
| C14 | 0.616 | 0.412 | -0.409 |
| *iso*-C15 | 0.427 | 0.852 | 0.180 |
| *ante*-C15 | 0.635 | 0.041 | -0.141 |
| C15 | 0.309 | 0.896 | -0.161 |
| C16 | 0.360 | 0.832 | -0.048 |
| *iso*-C17 | 0.804 | 0.514 | -0.057 |
| *ante*-C17 | 0.817 | 0.501 | -0.066 |
| C17 | 0.688 | 0.681 | 0.014 |
| C18 | 0.810 | 0.233 | -0.265 |
| C19 | 0.214 | 0.107 | 0.738 |
| C20 | -0.193 | 0.100 | 0.693 |
| C21 | -0.140 | -0.316 | 0.647 |
| C22 | -0.083 | -0.414 | 0.620 |
| C23 | -0.254 | 0.134 | 0.841 |
| C24 | -0.255 | -0.089 | 0.839 |
| Phytol | 0.940 | 0.284 | -0.180 |
| 20:1 | 0.944 | 0.262 | -0.104 |
| Eigenvalue | 9.507 | 3.575 | 1.626 |
| % of variance | 52.814 | 19.863 | 9.035 |
| Cumulative % of variance | 52.814 | 72.677 | 81.712 |

Factor 1 is characterised by high loadings of short chain fatty alcohols ($C_{12}$, $C_{14}$, $C_{15}$, $C_{16}$, $C_{17}$ and $C_{18}$), branched fatty alcohols (*iso*-$C_{15}$, *anteiso*-$C_{15}$, *iso*-$C_{17}$ and *anteiso*-$C_{17}$), phytol and monounsaturated 20:1. Therefore this factor represents marine and bacterial inputs, and account for 52.81% of the total variance and becomes the main factor. Again, marine and bacterial inputs are associated with factor 2, which accounts for 19.86% of the total variance. This factor is characterised by high loadings of short chain ($C_{12}$, $C_{14}$, $C_{15}$, $C_{16}$ and $C_{17}$) and branched fatty alcohols (*iso*-$C_{15}$, *iso*-$C_{17}$ and *anteiso*-$C_{17}$). The difference between factor 2 and factor 1 is that factor 2 does not have high loadings of phytol, monounsaturated 20:1, *anteiso*-$C_{15}$ and C18. Factor 3 (which accounts for 9.04% of the

total variance) is mainly associated with high loadings of long chain fatty alcohols ($C_{19}$, $C_{20}$, $C_{21}$, $C_{22}$, $C_{23}$ and $C_{24}$). Hence this factor represents terrestrial inputs.

### 6.3.4.3 Sterols

Factor analysis has also been carried out on sterols in the Loch Riddon core. Three factors have been generated from this analysis which together account for 81.85% of variance. The rotated factor matrix, eigenvalues, percentage of variance, cumulative percentage of variance is given in Table 6.7.

Table 6.7: Rotated factor matrix for sterols

| Variable | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| st1 | 0.940 | 0.188 | 0.218 |
| cop | 0.955 | 0.271 | 0.113 |
| epi | 0.925 | 0.033 | 0.294 |
| st2 | 0.947 | 0.126 | 0.253 |
| st3 | 0.539 | -0.471 | 0.138 |
| chol | 0.867 | 0.406 | 0.251 |
| cholest | 0.878 | 0.177 | 0.365 |
| brass | 0.882 | 0.268 | 0.308 |
| st4 | 0.885 | 0.232 | 0.361 |
| st5 | 0.871 | 0.427 | 0.106 |
| camp | 0.826 | 0.137 | 0.098 |
| st6 | 0.084 | 0.485 | 0.556 |
| stig | 0.219 | -0.053 | 0.427 |
| sito | -0.364 | -0.294 | 0.011 |
| st7 | 0.168 | 0.714 | 0.112 |
| dino | 0.769 | 0.565 | 0.105 |
| C30trienol | -0.159 | -0.205 | 0.006 |
| Eigenvalue | 10.768 | 1.683 | 1.465 |
| % of variance | 63.339 | 9.899 | 8.615 |
| Cumulative % of variance | 63.339 | 73.238 | 81.853 |

The first factor, which accounts for 63.34% of the total variance, is the major factor. It is characterised by high loadings of st1, coprostanol, epicoprostanol, st2, st3, cholesterol, cholestanol, brassicasterol, st4, st5, campesterol and dinosterol. Therefore this factor represents marine and sewage inputs. Meanwhile factor 2 (which accounts for 9.90% of the total variance) is possibly associated with marine and bacterial inputs. This factor has high loadings of cholesterol and dinosterol as well as the reduction product compounds (for

example st6 and st7 are formed from campesterol and β-sitosterol in the *in situ* reduction). Factor 3 is characterised by mixture of compound sources with high loadings of cholestanol, brassicasterol, st4, st6 and stigmasterol. This factor accounts for 8.62% of the total variance.

### 6.3.4.4 Mixed compounds

Extraction of seven factors (based on Kaiser-Guttman rule) was performed in this analysis, with Maximum Likelihood method and Varimax rotation. Table 6.8 showed the factor structure matrix after the Varimax rotation and the significant loadings are identified by underlining the factor loadings greater than or equal to 0.30 in absolute value. Table 6.8 also showed the eigenvalues, percentage of variance and cumulative percentage of variance.

In the Loch Riddon set, 7 Varimax rotated factors accounted for 86.15% of the total variance in the data: 37.48%, 14.61%, 11.41%, 10.06%, 5.41%, 4.09% and 3.09% respectively. The proportion of an individual compound contributes to end member's (source) total composition is found by dividing the absolute value of the variable's loading for that factor by the sum of the absolute values of all the loadings for that factor (normalised loading). For example, the loading for cholesterol in factor 1 is 0.944, the sum of all factor 1 loadings is 13.841, and therefore cholesterol contributes 0.947/13.841 = 0.0682 or 6.82% of the factor 1 end member. Thus the composition of that factor's end member is obtained. The percentage composition of factor 1, factor 2 and factor 3 are shown in Figure 6.33a, 6.34a and 6.35a. Composition of factor 4, 5 and 6 are not shown here. Factor scores for subjects (sample) were also obtained from this analysis. Factor scores can be used to see the contribution of certain factor to the sample. In this study, squared scores were used to determine the changes of factor contribution down the core. Figure 6.33b, 6.34b and 6.35b showed the distribution of factor 1, factor 2 and factor 3 down the Loch Riddon core.

Table 6.8: Rotated factor matrix for mixed compounds

| Variables | Factor 1 | Factor2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 |
|---|---|---|---|---|---|---|---|
| C14 | 0.469 | 0.564 | -0.318 | -0.092 | -0.112 | 0.350 | -0.390 |
| I-C15 | 0.217 | 0.926 | 0.177 | -0.123 | -0.027 | -0.131 | 0.083 |
| C15 | 0.098 | 0.934 | -0.172 | -0.101 | -0.093 | -0.076 | -0.041 |
| C16 | 0.197 | 0.919 | -0.054 | 0.009 | -0.057 | 0.088 | -0.029 |
| C17 | 0.477 | 0.807 | 0.047 | -0.085 | -0.088 | -0.000 | -0.107 |
| C18 | 0.731 | 0.465 | -0.261 | 0.147 | 0.040 | 0.292 | -0.142 |
| C19 | 0.079 | 0.268 | 0.520 | 0.016 | 0.442 | 0.376 | 0.293 |
| C20 | -0.242 | 0.043 | 0.848 | -0.112 | 0.127 | 0.105 | -0.255 |
| C21 | -0.110 | -0.228 | 0.414 | 0.090 | 0.726 | 0.162 | 0.008 |
| C22 | -0.005 | -0.327 | 0.436 | 0.127 | 0.823 | -0.024 | 0.021 |
| C24 | -0.264 | -0.149 | 0.671 | -0.074 | 0.262 | -0.156 | 0.263 |
| 14:0 | -0.021 | 0.023 | 0.015 | 0.512 | -0.569 | -0.071 | -0.105 |
| iso15:0 | 0.118 | -0.241 | 0.083 | 0.511 | -0.404 | -0.019 | 0.109 |
| ante15:0 | 0.578 | 0.054 | -0.242 | 0.566 | -0.143 | 0.178 | -0.270 |
| 15:0 | 0.005 | -0.149 | 0.024 | 0.907 | 0.043 | -0.022 | -0.010 |
| 16:0 | 0.316 | 0.416 | -0.242 | 0.457 | -0.151 | -0.084 | 0.213 |
| 16:1w7 | 0.628 | 0.486 | -0.453 | -0.061 | -0.120 | -0.095 | -0.042 |
| ante17:0 | 0.104 | -0.104 | -0.380 | 0.785 | 0.047 | 0.195 | -0.145 |
| 17:0 | -0.262 | 0.014 | 0.102 | 0.722 | 0.149 | -0.088 | 0.188 |
| 18:0 | -0.252 | 0.236 | 0.135 | 0.027 | 0.243 | -0.018 | 0.075 |
| 22:0 | -0.703 | -0.040 | 0.008 | 0.329 | 0.156 | -0.019 | 0.382 |
| 24:0 | -0.585 | 0.016 | 0.163 | 0.291 | 0.252 | -0.019 | 0.352 |
| St1 | 0.945 | 0.264 | -0.108 | 0.018 | -0.045 | -0.012 | -0.086 |
| Cop | 0.954 | 0.213 | -0.172 | 0.034 | -0.025 | 0.042 | 0.033 |
| Chol | 0.944 | 0.134 | -0.161 | 0.075 | -0.110 | 0.167 | -0.020 |
| Brass | 0.883 | 0.287 | -0.244 | 0.019 | -0.158 | 0.130 | -0.132 |
| St5 | 0.923 | 0.163 | -0.188 | 0.156 | 0.117 | 0.145 | 0.023 |
| Camp | 0.853 | 0.257 | 0.170 | -0.093 | 0.094 | -0.043 | 0.210 |
| Stig | 0.206 | 0.521 | 0.384 | -0.103 | 0.039 | 0.252 | 0.254 |
| Sito | -0.282 | 0.009 | 0.884 | -0.008 | 0.140 | -0.080 | 0.043 |
| St7 | 0.368 | -0.078 | 0.006 | -0.016 | 0.206 | 0.897 | -0.014 |
| Dino | 0.895 | -0.022 | -0.156 | 0.086 | -0.010 | 0.343 | 0.170 |
| C30trienol | -0.126 | -0.009 | 0.373 | -0.037 | 0.142 | -0.315 | 0.243 |
| Eigenvalue | 12.368 | 4.820 | 3.765 | 3.320 | 1.786 | 1.349 | 1.020 |
| % of variance | 37.479 | 14.606 | 11.408 | 10.061 | 5.412 | 4.088 | 3.092 |
| Cumulative % of variance | 37.479 | 52.085 | 63.492 | 73.554 | 78.966 | 83.053 | 86.146 |

Factor 1 explained 37.48% of the total variance. It is composed mainly of the mixture of marine and bacterial/sewage derived compounds, such as st1, coprostanol, cholesterol, brassicasterol, dinosterol, 16:1ω7 acid, *anteiso*15:0, *iso*17:0 and $C_{18}$ (Figure 6.33a). Campesterol also has high composition in factor 1. These compounds are abundant in the

upper section of the core as shown in Figure 6.33b. Factor 1 decreased with depth, suggesting the degradation of these compounds. Marine lipids degrade more rapidly than the terrestrial compounds. These results coincided with the PCA and cluster analysis where marine and bacterial/sewage compounds dominated the upper part of the core.
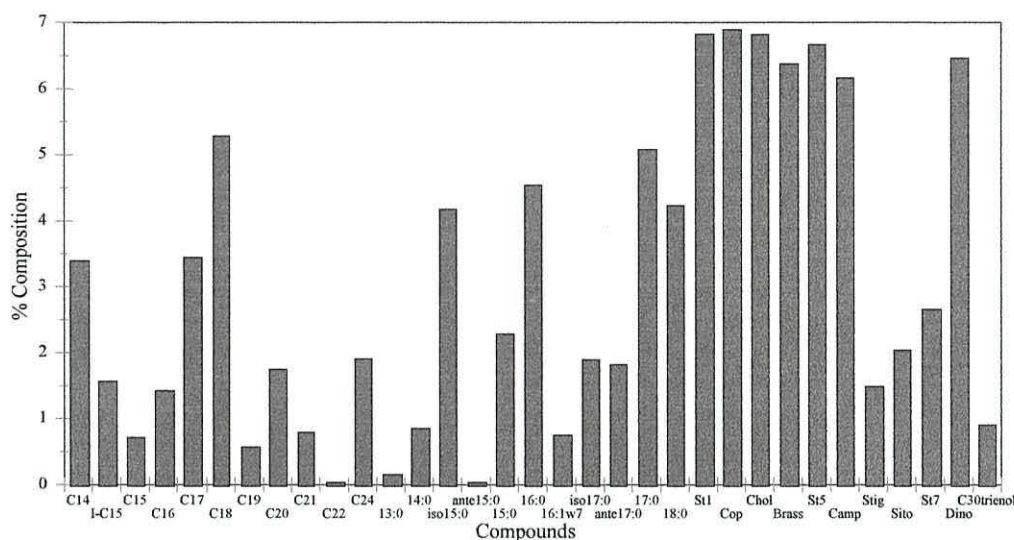


Figure 6.33a: Composition of factor 1 of the Loch Riddon sediments
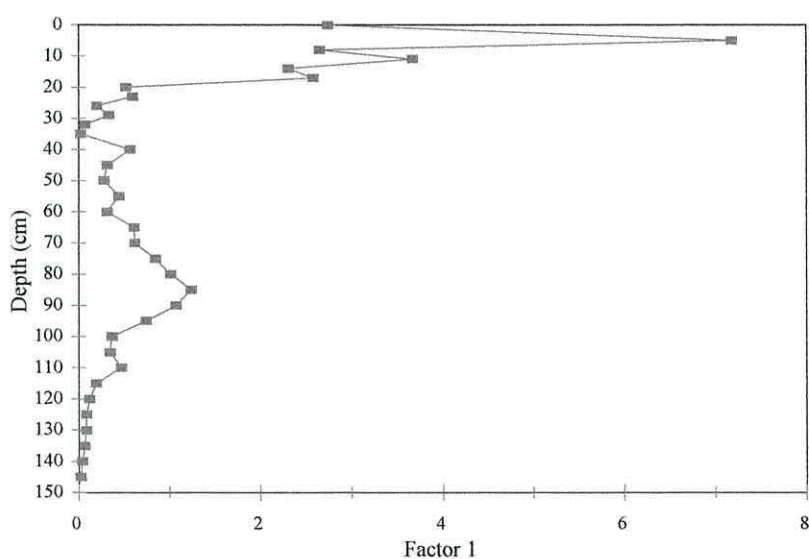


Figure 6.33b: Changes of factor 1 throughout the Loch Riddon core

14.61% of the variance was explained by factor 2. It is composed mainly of short chain fatty alcohols, as well as branched acid *iso*-$C_{15}$ (Figure 6.34a). Stigmasterol and 16:0 acids also have high composition in factor 2. These compounds are mainly derived from marine
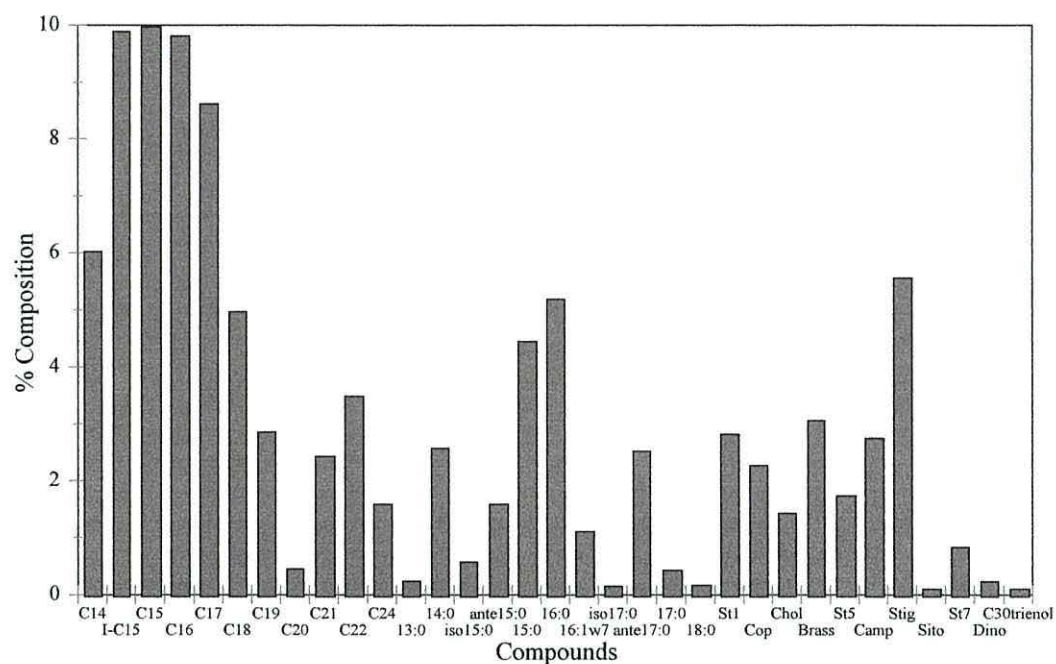


Figure 6.34a: Composition of factor 2 of the Loch Riddon sediments
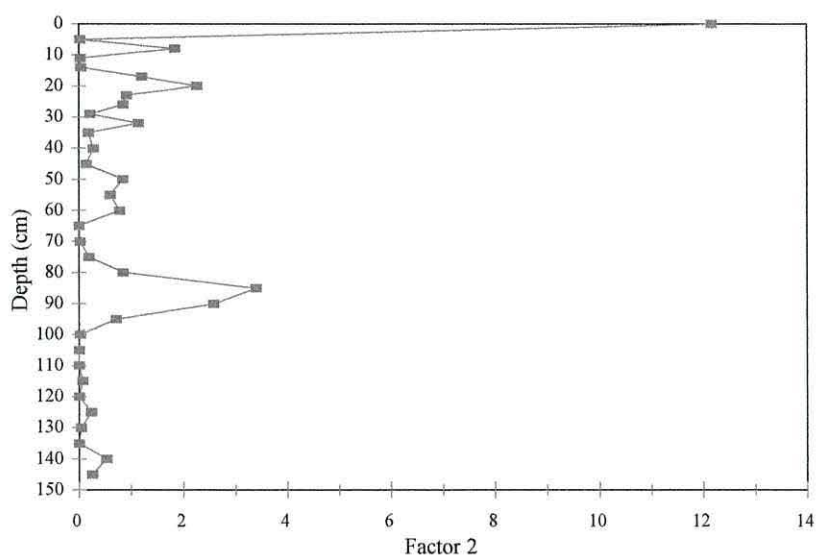


Figure 6.34b: Changes of factor 2 throughout the Loch Riddon core

organisms and bacterial biomass and were found in significant amount in the surface sample (Figure 6.34b). Fatty alcohols were dominant in this factor, unlike in factor 1 where sterols were dominant. Factor 2 also decreased with depth and increased at 85-90 cm depth suggesting the increase of bacterial activity at that depth.

Factor 3 accounted for 11.41% of the variance in the data. It is composed mainly of long chain fatty alcohols and sterol compounds such as β-sitosterol and represents a terrestrial source (Figure 6.35a). These compounds influenced the deeper section of the core as shown in Figure 6.35b. The same samples had the highest value of ASI and SSI ratios. These results were also coincided with the PCA and cluster analysis supporting that this factor represents a terrestrial source.
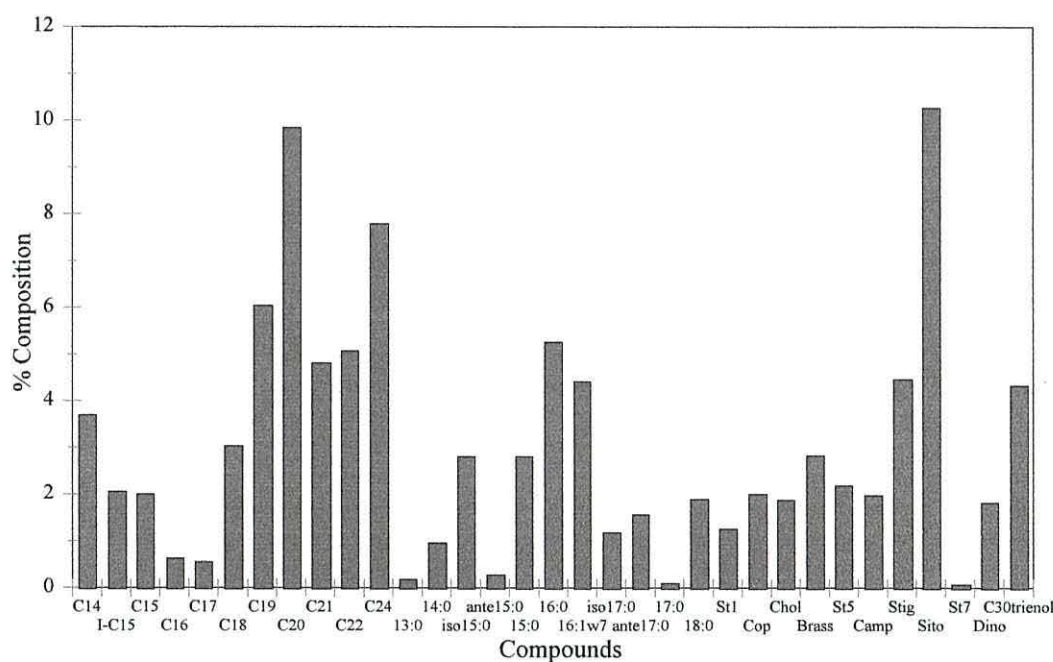
Figure 6.35a: Composition of factor 3 of the Loch Riddon sediments
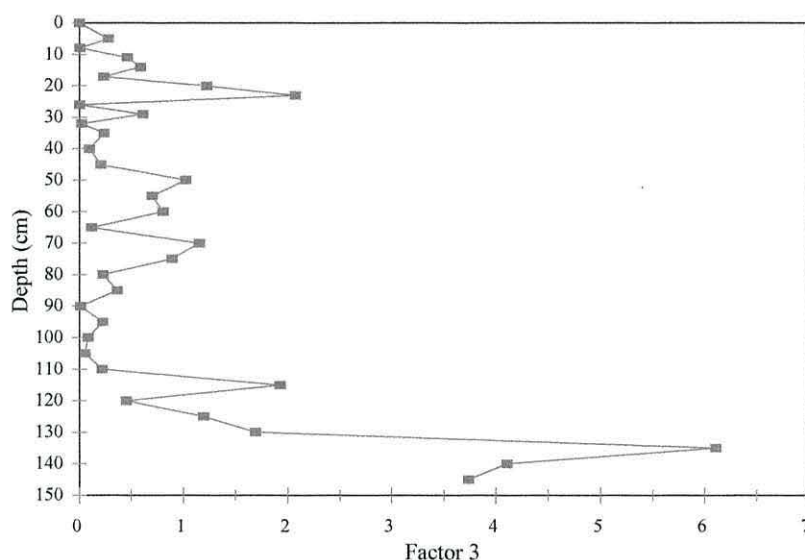
Figure 6.35b: Changes of factor 3 throughout the Loch Riddon core

## 6.4 Discussion

Analyses of fatty acid, fatty alcohol and sterol compositions help to evaluate the relative importance of various organic matter inputs in deposited sediments. Thirty-three fatty acids, eighteen fatty alcohols and seventeen sterols were found from the Loch Riddon core to investigate their temporal variability.

The biomarker approach has been widely used to assess organic matter sources in different depositional environments (Volkman, 1986; Saliot *et al.*, 1991; Canuel and Martens, 1996; Mudge and Norris, 1997; Duan, 2000; Shi *et al.*, 2001; Grossi *et al.*, 2003). Short chain fatty acids and fatty alcohols are major components in the surface sediment samples but decrease in deep samples. However, longer chain fatty acids and fatty alcohols are minor in surface sediment samples, and increase in deeper samples (Figure 6.2 and Figure 6.8). In general, long chain saturated fatty acids and fatty alcohols are associated with vascular plant tissues and thought to originate from terrestrial sources while short chain homologues have been attributed to aquatic source organisms (Fukushima and Ishiwatari, 1984; Saliot *et al.*, 1991; Carrie *et al.*, 1998; Rohjans *et al.*, 1998; Mudge *et al.*, 1998). The presence of

polyunsaturated fatty acids has been used as an indicator of viable algal cells (Volkman *et al.*, 1989; Dunstan *et al.*, 1994) and their abundance in some depositional environments was taken as indicating a fresh algal input to surface sediments (Canuel and Martens, 1996). Only polyunsaturated 18:2ω6 acids was found in the Loch Riddon core. Meanwhile, monounsaturated 16:1ω7 acids was found in high concentrations but decreased down the core. Monounsaturated fatty acids (16:1ω7) are major fatty acids in many species of algae (Volkman *et al.*, 1989; Dunstan *et al.*, 1994) but 18:1ω7 is a typical bacteria-specific fatty acid (Parkes and Taylor, 1983). Some short chain saturated ($C_{14}$-$C_{18}$) and monounsaturated 18:1ω9 have mixed sources, including algae, zooplankton, bacteria, benthic animals and marsh plants (Cranwell and Volkman, 1981).

As sediment depth increases, decreases are observed in short/long ratios of fatty acids and fatty alcohols, percentage of monounsaturated and polyunsaturated fatty acids as well as percentage of branched fatty acids and fatty alcohols in the Loch Riddon core. These changes may be due to diagenesis. The decrease of short chain fatty acids and fatty alcohols and the increase of longer chain compounds down the core reflecting the preferential utilisation of marine organic matter as unsaturated and shorter chain compounds are primarily substrates for marine organisms (Nishimura and Baker, 1987; Colombo *et al.*, 1997; Mudge *et al.*, 1998). Short chain fatty acids and fatty alcohols are less stable and degrade more rapidly than the long chain compounds, and unsaturated acids degrade more than the saturated compounds. Therefore, the general decrease of short chain compounds is probably caused by microbial and chemical degradation during early diagenesis. The increase of longer chain compounds indicates an increased contribution of terrestrial organic matter in Loch Riddon from the non-coniferous forests surrounding this area.

The ASIs and SSIs can be used to estimate the input of terrestrial derived materials to sediments (Mudge and Norris, 1997). These ratios increase with the core depth, and nearly all of the ASI values were < 1.0 indicating the dominance of marine fatty alcohols in the core. β-sitosterol/cholesterol ratios appeared to be the strongest SSI in the Loch Riddon core, increasing by a factor of 12 between the surface and bottom samples (Figure 6.16). Therefore β-sitosterol can be assumed to be originated from terrigenous sources in this

system. Correlation matrix between ASIs and SSIs can be used to indicate any relationships between the indices. However, these indices were not positively correlated in the Loch Riddon showing the ASI or SSI alone cannot be used as an indicator of terrestrially derived organic matter.

Branched fatty acids and fatty alcohols were also detected in the Loch Riddon core. These fatty acids probably originate from bacteria (Parkes, 1987; Saliot *et al.*, 1991; Thoumelin *et al.*, 1997). The relatively high concentrations of branched fatty acids in the core reflect bacterial contributions to the sediments. Branched fatty alcohols were relatively low in concentrations suggesting that there were another group of bacteria population in the core with smaller bacterial input than the former group. In the Loch Riddon core samples, the percentage of branched fatty acids decreases downs the core with a peak at 32-35 cm (Figure 6.6). The percentage of branched fatty alcohols also decreased towards the deeper sections with maxima at the surface and at 20-23 cm (Figure 6.10). These observations were probably associated with an increase of microbial activity. Similar distribution of percentage branched compounds with the profile of odd/even ratios were also observed in this study reinforcing their link to bacterial activity.

Cholesterol is thought to originate primarily from zooplankton, although they are often common in algae as well (Volkman, 1986; Volkman *et al.*, 1987). The $C_{28}$ sterol, brassicasterol was detected in the core, and has previously been identified as major constituent of diatoms (Volkman, 1986). 24 norcholesta-5,22(E)-dien-3β-ol, was also found in this study. This sterol is not unique to marine organisms but is often found in the marine environment (Volkman, 1986; Yunker *et al.*, 1995). Nichols *et al.* (1993) reported that st2 (cholesta-5,22(E)-dien-3β-ol) was a good biomarker for diatoms. This sterol is also found in the Loch Riddon core. In general, $C_{29}$ sterols are thought to originate from higher plants, but they may also derive from diverse algal species (Volkman, 1986). Most $C_{29}$ sterols in the Loch Riddon core are likely derived from terrestrial higher plants, because long chain fatty acids in these samples, which represent contributions from higher plants, are high in abundance. Marine sterols (24 norcholesta-5,22(E)-dien-3β-ol, cholesta-5,22(E)-dien-3β-ol, cholesterol and brassicasterol) decrease in concentration while $C_{29}$ sterols increase with depth (Figure 6.15). 24 norcholesta-5,22(E)-dien-3β-ol and cholesta-5,22(E)-dien-3β-ol were not found in the core from 40cm downward. It is apparent that

marine sterols have been subjected to degradation with depth while sterols from higher plants survive. Similar observations can be seen with the fatty acids and fatty alcohols.

5β-coprostanol has been shown to be a reliable marker for sewage pollution (Sherwin *et al.*, 1993; Jeng and Han, 1994) and also has been used to show sewage pollution history from a sediment core (Venkatesan and Mirsadeghi, 1992; Jeng and Han, 1994; Jeng and Han, 1996). In the Loch Riddon core, the 5β-coprostanol was only observed from the depth 40-45 cm and upward. The depth 40-45 cm suggests an increase in population around this region due to the rapid growth of industrialisation with disposal of associated sewage wastes. Epicoprostanol/coprostanol ratios can be used to indicate the degree of treatment as epicoprostanol is generally present in treated sewage sludge (Mc Calley *et al.*, 1981; Mudge *et al.*, 1999). In the Loch Riddon core epicoprostanol can only be observed from 17-20 cm upwards. Therefore this depth suggests the time when sewage treatment plants become operational with marine disposal of the treated sludge. 5β -coprostanol concentrations has similar distribution with concentration of pyrene and fluoranthene, which are the polycyclic aromatic hydrocarbons that can be considered to reflect inputs from the combustion of organic matter, such as wood and fossil fuels (Killops and Killops, 1993; Pereira *et al.*, 1999). This may suggest a link between these compounds to the extent of human influence around this area. Concentration of these hydrocarbons started to increase 250 years ago due to the industrial evolution (from 55cm depth of the core). Therefore, the sewage starts increasing at around 200 years ago. One hundred years later, the treatment of sewage started as shown by the epicoprostanol/coprostanol ratios.

PCA was the first multivariate analyses carried out on this data set. PCA was conducted on individual chemical group as well as on combination of fatty acids, fatty alcohols and sterols. PCA separates fatty acids by primary source such as marine/algae, bacterial and terrestrial plants. Based on the PCA model, long chain fatty acids have principally terrestrial sources in the sediment core. Long chain fatty acids project well to the right in the PCA plot and these compounds are thought to be derived from higher-land plants (Carrie *et al.*, 1998; Mudge *et al.*, 1998). Saturated $C_{19}$ also clustered with the terrestrial compounds. This compound may not be degraded or $C_{19}$ is having terrigenous source. Short chain, polyunsaturated and 16:1ω7 fatty acids principally have a marine and algal source in the PCA model. Bacteria are the major source of branched fatty acids (Parkes,

1987; Killops and Killops, 1993). Two groups of bacterial derived compounds were observed in the PCA model suggesting that there are two different bacterial populations present in this core. $18:1\omega7$ acid, which is also abundant in bacteria, was grouped with one of the bacterial derived compounds reinforcing their link to the bacterial biomass.

In the PCA model, phytol and 20:1 fatty alcohol are the strongest indicators of marine inputs. Phytol is a potential marker for chlorophyll (Killops and Killops, 1993) and 20:1 alkenol is produced abundantly by zooplankton (Sargent *et al.*, 1977, 1981; Graeve and Kattner, 1992). A marine origin may also be ascribed to the shorter length fatty alcohols $C_{12}$, $C_{14}$ and $C_{18}$. These fatty alcohols have an origin primarily in zooplankton wax esters (Sargent *et al.*, 1977; Wakeham and Canuel, 1990; Grimalt and Albaiges, 1990). The terrigenous saturated alcohols (long chain) have strong negative loadings in the PCA model. $C_{19}$- $C_{21}$ are also loaded with the longer chain alcohols suggesting that they may have been from terrestrial inputs or they were not readily degraded in the sediments. Similar with PCA carried out on fatty acids, there were two bacterial groups in the PCA model.

PCA separates the sterols into their primary sources like terrestrial, marine/algae and bacteria/sewage. These observations correspond to PCA applied to fatty acids and fatty alcohols individually. In the PCA model, 4 sterols have principally terrestrial inputs. They are the $C_{29}$ sterols such as β-sitosterol, stigmasterol and campesterol. Another sterol is a $C_{30}$ sterol. β-sitosterol projects well to the left and is one of the strongest indicators of the higher plant inputs. Sterols such as st1, st2, cholesterol and brassicasterol have marine/algae source in the Loch Riddon core. These compounds project to the right with positive loadings in the PCA model. Therefore PCA makes a clear separation between the marine sterols and the higher plant sterols. The PCA model also shows that there are two groups of bacterial derived compounds. First was sewage derive compounds such as coprostanol and epicoprostanol, while st7 (sitostanol), which is a compound produced in an *in situ* reduction of β-sitosterol was grouped separately from the first bacterial group. Therefore it can be suggested that this compound represents the second bacterial population, as the *in situ* reduction may be associated with anaerobic bacteria.

PCA was also conducted on the mix compounds of fatty acids, fatty alcohols and sterols. PCA showed the compound separation from marine and bacterial derived compounds to the terrestrially derived organic matter. $C_{30}$ sterol, β-sitosterol, long chain fatty acids and fatty alcohols are thought to be terrestrial in origin. These compounds project to the left of the PCA plot. Meanwhile, stigmasterol and campesterol were clustered together with $C_{15}$ and $C_{16}$ fatty alcohols as well as saturated 18:0 fatty acids. Saturated 18:0 fatty acid and $C_{16}$ fatty alcohol are major constituents in the Loch Riddon core. These compounds represent marine inputs, suggesting that stigmasterol and campesterol also have marine origin. Marine derived compounds are clustered together with bacterial/sewage derived compounds. The upper parts of the core contain marine and bacterial derived organic matter while terrestrially derived compounds influence the bottom part. Group 1 bacteria dominated the surface sample together with the marine markers. Hence, they may be aerobic bacteria.

In the Loch Riddon core, the top samples of the core were used to characterise the biomarker signatures (*X*-block) in performing PLS. PLS then uses the multivariate relationship between variables to quantify the amount of top sample materials in the rest of the core (*Y*-block). The decrease of organic matter can be seen throughout the core (from the fit diagram). These results may be due to the degradation of marine derived organic matter, indicating that marine lipids degrade more rapidly than terrigenous markers. When PLS was carried out with sediment samples from the Clyde Sea (S1, S2 and S10 to characterise marine markers; S12, S13 and S21 to characterise sewage markers; S31, S32 and S33 to characterise terrestrial signatures), the same observation was discovered. Marine signatures are high at the surface and decrease with depth. It is likely to be due to degradation of more labile compounds such as short chain and unsaturated compounds. Terrestrial materials on the other hand were constant down the core suggesting that degradation for these compounds did not take place and the terrestrial inputs were constant throughout the sediment deposition. Plots of the *X*-block (t1) and *Y*-block (u1) show that S1, S2 and S10 were appropriate signatures for characterising the marine markers, while S12, S13 and S21 as well as S31, S32 and S33 might not be the perfect signatures for sewage and terrestrial markers.

Cluster analysis was another multivariate analysis conducted on the Loch Riddon data, where it was used to classify the core section with the geochemical data. Using the Ward's method with hierarchical clustering, 2 cluster groups were identified in cluster analysis of fatty acids, fatty alcohols and sterols individually. The first cluster group represents a mixture of marine and bacterial derived compounds, while the second cluster group contains the terrestrial derived lipids. Saturated 19:0 acid was also clustered together with long chain fatty acids in the fatty acid cluster analysis. In the fatty alcohol cluster analysis, $C_{19}$-$C_{21}$ were grouped together with the long chain fatty alcohols. Long chain fatty acids and fatty alcohols are generally associated with the higher plants and are considered as indicator of terrigenous inputs (Fukushima and Ishiwatari, 1984; Saliot *et al.*, 1991; Carrie *et al.*, 1998). Therefore saturated 19:0 acid and $C_{19}$-$C_{21}$ fatty alcohols may have terrestrial origin or they are not readily degraded in the sediment core. The same results were observed in the PCA analysis. Branched chain fatty acids and fatty alcohols are associated with bacterial communities. The cluster analysis of fatty acids shows 2 groups of bacterial derived compounds occurred in the sediment samples and these observations are supported by the PCA. Marine and sewage derived sterols were also clustered together while the higher plant sterols were grouped in a different cluster. 24 norcholesta-5,22(E)-dien-3β-ol and cholesta-5,22(E)-dien-3β-ol are the strongest indicator of marine sterols as they were clustered together with the greatest similarity. Cluster analysis was also carried out on the mixed compounds of fatty acids, fatty alcohols and sterols. Two cluster groups with 4 smaller clusters were observed. The first cluster group has terrestrially origin compounds in one group and marine and bacterial derived compounds in the other. The second cluster group is associated with marine and bacterial/sewage markers. Cluster analysis was also carried out to reveal specific linkage between core depths. In the Loch Riddon core, the first 20 cm depth is characterised by marine and bacterial/sewage organic matter, which are grouped in the first cluster. While the second cluster group consists of deeper samples and dominated by terrestrial derived compounds. Based on this study, cluster analysis is appropriate enough to be carried out in the biomarker studies as it separates the compounds into their respective source and can characterised the samples accordingly.

The use of factor analysis attempts to explain the correlation between a set of variables in terms of a small number of underlying factors. Factors are supposed to contain the essential information in a larger set of variables or objects. Factor analysis was carried out

in this study to interpret the distribution of fatty alcohols, fatty acids and sterols. Three factors were extracted from factor analysis carried out on fatty alcohols and sterols, while fatty acids and mixed compounds were extracted with eight and seven factors respectively. Terrestrial derived compounds representing terrestrial inputs became the major factor in fatty acid factor analysis and factor 3 with the fatty alcohols and mixed compounds. Meanwhile, marine and bacterial derived compounds which representing marine and bacterial inputs were obtained from every set of data. This was factor 1 in the factor analyses carried out on fatty alcohols, sterols and mixed compounds, meaning that it explained the most information compared to the other factors. Marine and bacterial inputs became the second factor on fatty acid factor analysis. Marine derived compounds always mixed with the bacterial derived compounds and became one factor in the analysis. Therefore, factor analysis is not suitable to be conducted on core samples compared to the surface samples, as they cannot separate these compounds accordingly. Thus, all the data, from all 4 factor analyses carried out is reduced to 2 major systems: bacterial/marine and terrestrial.

Little fresh algal input was observed in the Loch Riddon based on the PUFA found in the core. Preferential utilisations were also observed on the marine derived compounds and changes in sources were occurred for the terrestrial and sewage signatures. The age of the core might be more than 800 years. A lot of things happened during that time. For example, land clearances in Scotland a long time ago may affect the contribution of terrestrial organic matter into Loch Riddon. Industrialisation and increasing of population in Scotland may affect anthropogenic inputs in the Loch Riddon. Therefore the integration of the biomarker approaches and multivariate analyses tell us about such events by elucidating sources of biomarker compounds.

**6.5 Conclusions**

Most of the lipids in the upper part of the core are derived from marine organisms but the microbial or chemical processes in the sediments rapidly degrade these compounds. The short/long ratios of fatty acids and fatty alcohols clearly show that marine derived organic matter is the major constituent of the lipids in the core sediments even though pine forests surround the area. Terrestrial derived organic matter gradually increases with depth due to contribution from higher plants. Terrestrial organic matters are also better preserved in the

sediments compared to marine organic matter. Therefore, the deeper part of the core is characterised by terrestrially derived materials. This is supported by ASI and SSI ratios. Individual and combined PCA confirmed these observations by separating terrestrial markers from marine and bacterial markers. The coprostanol/cholesterol ratios show the increasing input of coprostanol from the past due to increasing population and human activities. This study also demonstrates that PAHs can be used to track the history of PAH contamination from the past to the present time. PLS path modelling shows the contribution of organic matter from the top samples along the core. The cluster analysis differentiated the core into two regions. The first cluster group defines mainly the top samples, characterised by marine and bacterial/sewage compounds, while the second cluster group represented the deeper section of the core dominated by terrigenous inputs. Factor analysis reduced the lipids into two clear sources: bacterial/marine and terrestrial.

**CHAPTER 7: GENERAL DISCUSSION**

The use of selected compounds and/or ratios can explain a lot about the distribution and source inputs in marine environments. Of the fatty alcohol ratios, the ASI provided the best indicator of terrestrial *vs.* marine derived organic matter. $C_{14}$ appears to be the strongest marine marker in the Mawddach Estuary as well as in the Loch Riddon sediment core, and $C_{16}$ for the Conwy core. From the ASI, $C_{24}$ appears to be the strongest terrestrial marker in both cores and $C_{22}$ in the Mawddach Estuary. Similar to the ASIs, the SSIs can be used as an indicator of terrestrially derived organic matter. The ergosterol/cholesterol ratios appear to particularly useful in identifying the extent of terrestrially derived organic matter in the Mawddach Estuary and in the Conwy core. β-sitosterol/cholesterol appeared to be the strongest index in the Loch Riddon core. Samples collected from the sea lochs have a higher β-sitosterol/cholesterol ratios showing that β-sitosterol has been successfully used to trace the terrestrial matter. The ASIs in the Clyde Sea sediment samples have ratios < 1.0 throughout the sampling sites indicating that β-sitosterol, campesterol and stigmasterol have also sources in algae (Volkman, 1986).

The short/long ratios show the changes of fatty acids and fatty alcohols in the sampling sites. These ratios increase from the freshwater samples to the marine samples in the Mawddach Estuary. In both cores, these ratios decrease down the core indicating the degradation of marine derived compounds.

The varieties of fatty alcohols and fatty acids ratios used are indicators of processes or secondary production. The odd/even and branched/precursor ratios indicate regions where enhanced bacterial activity may be expected due to the presence of sewage derived organic matter. At the same time, the coprostanol/cholesterol ratio is able to indicate the sewage discharge point throughout the sampling sites. Epicoprostanol/coprostanol ratios can be used to indicate the degree of sewage treatment.

The usefulness of multivariate statistical techniques in extracting the maximum information from complex mixtures of lipid compounds has been highlighted in recent literature (Yunker *et al.*, 1995; Aboul-Kassim and Simoneit, 1996; Mudge and Norris, 1997; Mudge *et al.*, 1998, 1999; Simeonov *et al.*, 2000). Individual compounds and ratios

using two or more compounds can provide some information regarding the origin of organic matter in the sediments; multivariate statistical analyses are able to use more of the compounds in a single analysis. Therefore, multivariate techniques such as PCA, PLS, factor analysis and cluster analyses are invaluable.

PCA is applied to detect the "hidden" structure of the dataset, trying to explain the influence of latent factors on the data distribution. PCA is an adequate method for comparing the biomarker distribution throughout the sampling sites in the Mawddach Estuary and in the Loch Riddon core. PCA manage to separate the compounds into their geochemical sources and PCA allow clear differentiation between samples. In the Loch Riddon data, PCA was able to show different bacteria groups that occurred in the sediment core. This analysis was also able to show the "path" through sites. In the Mawddach Estuary, PCA showed clear separation from marine and bacterial derived compounds to the terrestrially derived organic matter, as well as separating the marine samples from the freshwater samples. In the Conwy core, however, PCA was not able to separate the marine and bacterial derived compounds. These compounds were clustered together in PCA of single chemical group as well as in the mixed compounds. The Conwy core was 50cm in depth and the top 30cm contain marine and bacterial compounds. Within the Clyde Sea sediment samples, only PCA conducted on fatty acids showed clear separation of biomarkers according to their geochemical sources. Fatty acids were more abundant in these sediments compared to fatty alcohols and sterols. Compound separation was not clear in the PCA and it might be due to the current movements in the Clyde Sea that transports the compounds from their sources to other places.

PLS can quantify the changes in a system if signatures are available. In an estuarine system such as Mawddach Estuary, the marine and terrestrial end members can be used as signatures. Samples rich in certain classes of organic matter can also be used as signatures. For example, samples collected near the sewage outlets can be used as sewage signatures. In the Conwy core, there are no real signatures. Hence, the PLS was not carried out on these data. Good end members were available in the Clyde Sea sediment samples to conduct PLS analysis. Top samples of the Loch Riddon core were used as biomarker signatures showing that there are changes from the surface. Samples collected from the Clyde Sea sediment samples then were used as signatures for the PLS analysis with the

Loch Riddon data, as Loch Riddon core was collected from this environment. Some signatures are appropriate for PLS analysis and some are not.

The PLS models that have been created reflects underlying geochemical relationships. The model allows the predictions of biomarker transportations throughout the sampling sites to be made. PLS modelling also allows finding out a more detailed connection of compound sources for the sediment diagenesis. PLS that was conducted on the Mawddach and Clyde Sea sediment samples clearly show the signature transportation via river runoff, current movements and tidal exchange. With core samples, the change from the surface was observed. These changes could be due to changes in source or loss of degradable compounds, which tend to be the marine markers. Figure 7.1 shows the concentration of β-sitosterol and cholesterol as well as the ratio of β-sitosterol/cholesterol in the Loch Riddon core. The concentration of cholesterol decreases down the core, while the concentration of β-sitosterol increases. The β-sitosterol/cholesterol ratios were < 1.0 in the upper part of the core (0 - 20 cm) and became 1.0 at 20 - 23 cm depth. The ratios were then > 1.0 from 26 cm downwards. Therefore it shows suggests a change in source, where there is a real increase of β-sitosterol concentration with increasing depth. Unless there is a change in grain size, for example, muddier at the base of the core or increased compaction of the sediment meaning several year's worth of β-sitosterol is compressed into smaller fraction of the sediment. This might not be the case as cholesterol decreases with depth and remains relatively constant in lower regions. The change of source in this case might be because of land clearances as concentration of β-sitosterol is relatively low at the upper part of the core. The concentration of cholesterol increases upward (from 50 cm depth) and coincident with the increase of the sewage markers. Therefore it shows another example of change in source in this core sediment samples.

The ratio of β-sitosterol/cholesterol in the Loch Riddon core shows an agreement with Figure 7.2 where bottom samples were used as signatures. The fit diagram may superficially look like terrestrial signatures because of the loss of compounds due to degradation. If 145-150 cm is assumed as "terrestrial", then there is no overlap with 0-5 cm suggesting a reduction in terrestrial input with time. But this is not true as terrestrial derived compounds were also found in the 0-5 fractions. Therefore, the 145-150 cm fraction might not be the true terrestrial signatures but degradation signature. In the 1500's
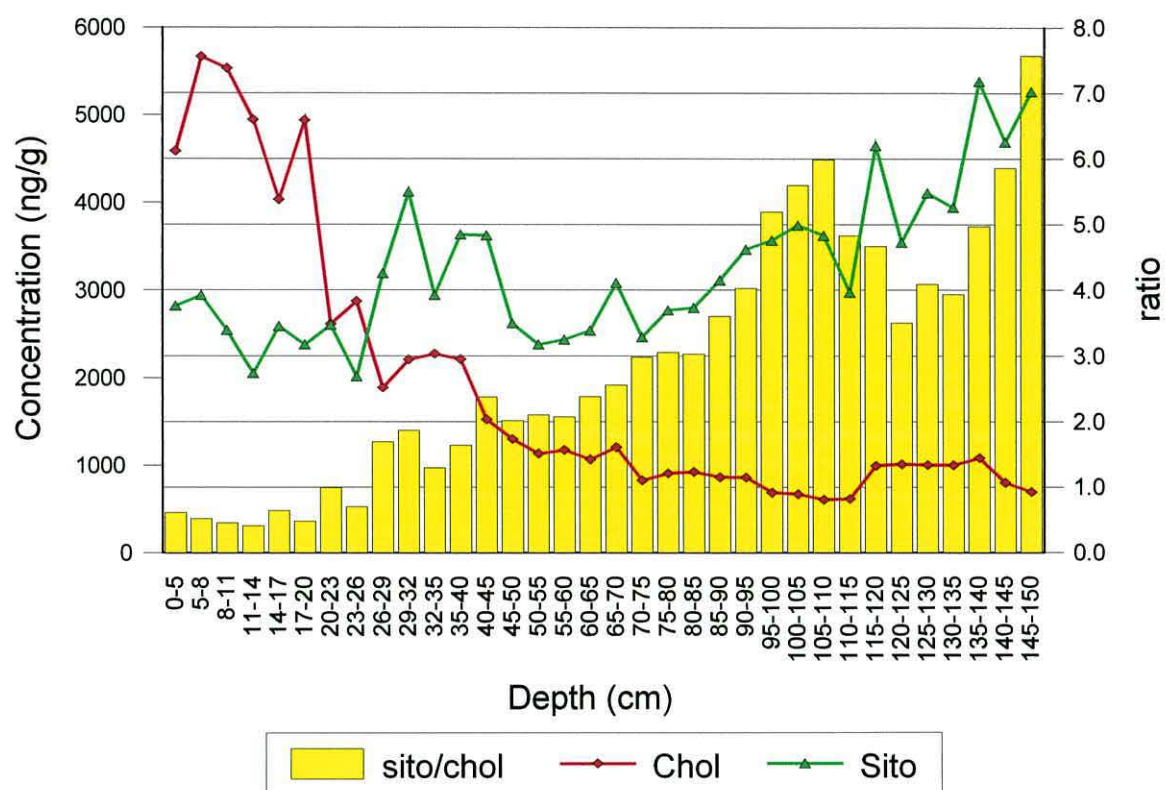
Figure 7.1: Changes in concentration of cholesterol and β-sitosterol as well as β-sitosterol/cholesterol ratios in sediment core of Loch Riddon
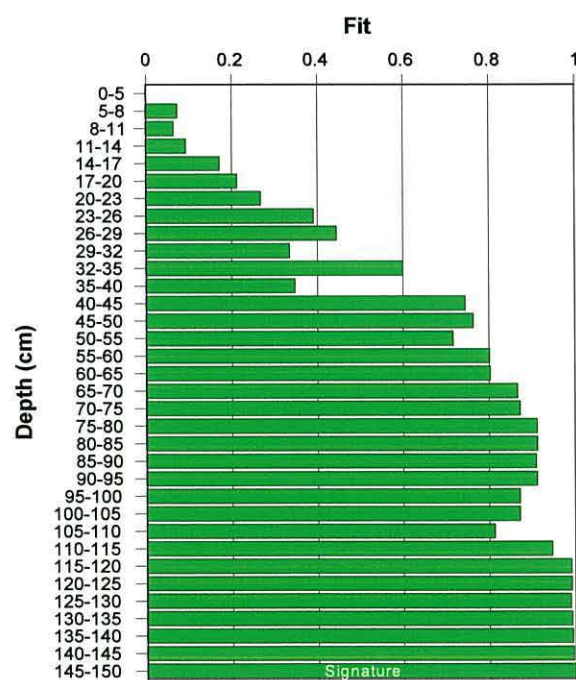


Figure 7.2: The fit diagram in the PLS model with the bottom samples as signatures

there were major forest clearances in Scotland. By 1640, timber was scarce due to the rising demands of fuel, tanning, shipping, mining and clearing for farmed crops (Cawdorcastle, 2000). Therefore, the loss of terrestrial big plants reduces the β-sitosterol inputs to the Loch Riddon.

Figure 7.3a shows the fit diagram with a terrestrial signature using all compounds. The terrestrial compounds were constant down the core. When the "marine" signature compounds (short chain fatty alcohols and fatty acids) were removed (Figure 7.3b), the fit increased, as signatures were rich in the longer compounds. These two diagrams show that PLS is sensitive depends on the compounds used. The fit diagram in Figure 7.3c was similar as Figure 7.3b, but in Figure 7.3c the marine sterols have been removed. This implies more terrestrial derived compounds at the top of the core. The degradation of signatures did not occur as the more readily degradable compounds have been removed. Figure 7.3d shows the fit diagram with marine signatures without the short chain fatty alcohols and fatty acids as well as the marine sterols. Generally, it was poorly fitted, as the compounds excluded are those that make up the marine signatures. These results indicate that those compounds are derived from marine inputs.

Score contribution at 17-20 cm depth and 130-135 cm depth were derived from the fit diagram of terrestrial signatures without the short chain fatty acids and fatty alcohols as well as the marine sterols (Figure 7.4). The 17-20 cm depth was enriched in marine compounds such as brassicasterol, dinosterol and phytol. The 130-135 cm depth, on the other hand, were definitely influenced by terrestrial derived compounds such as long chain fatty alcohols, long chain fatty acids and β-sitosterol. Sitostanol (st7) shows a marine tendency. It might be due to β-sitosterol transported to marine environment then reduced to 5α-sitostanol. Therefore, sitostanol may co-vary with either marine or bacterial derived compounds (especially anaerobic bacteria).
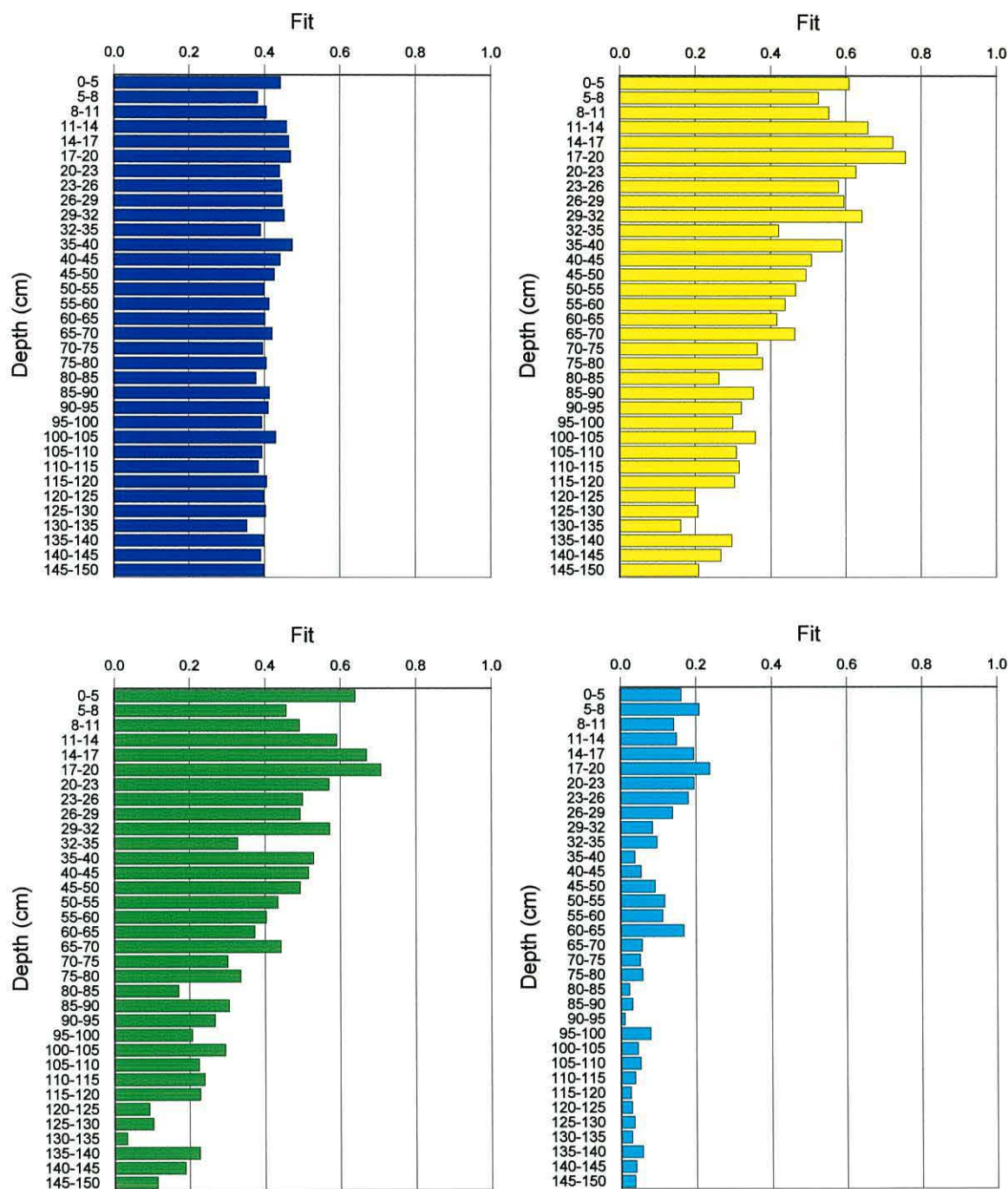
Figure 7.3: The fit diagram: a) Terrestrial signatures with all compounds b) Terrestrial compounds without the short chain compounds c) Terrestrial signatures without the short chain compounds and the $C_{26}$ and $C_{27}$ sterols d) Marine signatures without the short chain compounds and the $C_{26}$ and $C_{27}$ sterols
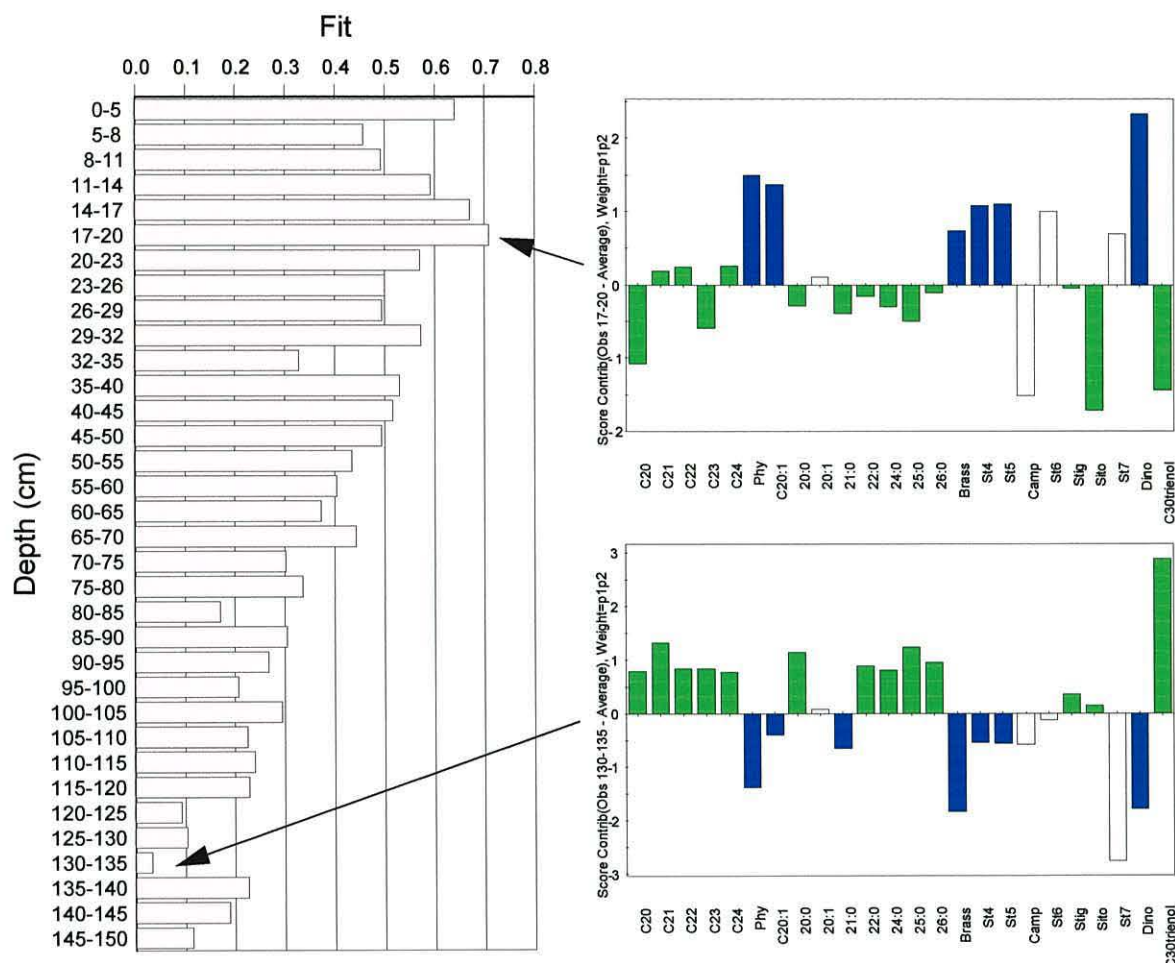
Figure 7.4: Score contribution from the fit diagram with terrestrial signatures

Cluster analysis was carried out to reveal specific linkage between sampling sites being an indication of similarities and dissimilarities between their variables. Based on this study, cluster analysis is appropriate enough to be carried out in the biomarker studies as it separates the compounds into their respective source and characterised the samples accordingly. For example, in the Loch Riddon core, the first 20cm is characterised by marine and bacterial/sewage organic matter and are grouped in separate cluster from the deeper samples that were dominated by terrestrial derived compounds. Within the Clyde Sea sediment samples, the results of cluster analysis were more complicated. But the separation of compounds can also be seen even though not as clearer as in the Loch Riddon cluster analysis. This may be due to that all these samples were collected from

marine environments and were dominated by marine derived compounds. The open sea samples, however, were clustered together and separated from the sea loch samples, which were rich with terrigenous inputs.

Factor analysis offers a powerful means of detecting similarities among compounds (variables) or samples. The technique uses the extraction of the eigenvalues and eigenvectors from the matrix of correlations or covariance. Therefore, factor analysis is a multivariate technique designed to analyse the inter-relationships within a set of variables or objects.

Factor analysis and principal component analysis have similar purpose, which is to reduce the original variables into fewer composite variables, called factors or principal components. Principal component analysis is used to find optimal ways of combining variables into a small number of subsets, while factor analysis may be used to identify the structure underlying such variables. In factor analysis, interest centres mainly on the common factor, where factor analysis analyses only the common variance of the observed variables. Meanwhile principal component analysis considers the total variance and makes no difference between common variance. Hence, it is generally used in a descriptive fashion (Krzanowski and Marriott, 1995).

Factor analysis is not widely used in organic geochemical studies. One reason for this is that visual pattern recognition such in principal component analysis and cluster analysis may be sufficient enough and there may be no need for quantitative results. Limitations in the various factor analysis methods can be another reason. Some of the results produced by factor analysis methods cannot be interpreted in a quantitative manner. Factor analysis is also computationally demanding and more complicated than other analysis such as principal component analysis. Another reason that factor analysis is not widely used is that it also places a much heavier demand on the user who may have difficulty in choosing the input parameters for the factor analysis and difficulty in interpretation of the results (Howard, 1991; Rapp, 1991). One of the problems is deciding the optimal number of factors required because some factors represent systematic and random errors and should not be included. By choosing too few factors (under factoring), one or more determinable common factors will be omitted. Meanwhile by choosing too many factors, the factors

obtained may be due to fortuitous correlations among the attributes. Sample size is another problem in factor analysis. Correlation coefficients tend to be unstable if the sample size is not large. It is generally unwise to conduct a factor analysis on a sample with less than 50 observations. Therefore in this study, factor analysis is not appropriate, as the sample size is less than 50.

With some data one analysis method will give a more interpretable result, whereas with other data the alternative method will prove better. Principal component analysis will always yield a result, but it is not necessary that the components are possible to interpret. In this kind of study principal component analysis is a better method to be used. Factor analysis should be regarded as alternative to principal component analysis. If the communalities of the attributes are all equal, then factor analysis cannot give better results than principal component analysis (Howard, 1991).

**CHAPTER 8: CONCLUSIONS**

On the basis of the biomarker distributions, the geochemical ratios and the multivariate statistical techniques, it can be concluded that:

- The marine samples in the Mawddach Estuary are characterised by relatively high concentrations of short chain fatty acids, fatty alcohols and marine sterols. Meanwhile the freshwater samples are characterised by an increasing dominance of terrestrially derived materials with high concentration of long chain fatty acids, fatty alcohols and the higher plant sterols. These results are corresponded with the ASIs, SSIs and short/long ratios. Samples that are near the sewage discharge points have high concentrations of bacterial fatty acids and fatty alcohols. These samples are dominated by sewage derived material as shown by coprostanol/cholesterol ratios.

- In the Mawddach samples, PCA confirmed the above observations. PCA performed on individual group compounds showed a separation between geochemically source input of compounds and samples.

- The Clyde Sea samples are characterised by marine derived organic matter. This can be seen on the results from the ASIs, SSIs and short/long ratios. The River Clyde sample (S13) is different from other samples as it contains the greatest amount of sewage and bacterial markers. Again, PCA confirmed these observations.

- In general, the marine and bacterial derived materials decrease with depth in a core by early diagenesis and terrestrial derived materials appear stable to early diagenesis. Therefore, the upper part of the core is characterised by marine and bacterial biomarkers and the deeper section is characterised by terrestrially derived organic matter indicating that marine lipids degrade more rapidly than terrestrial derived organic matter. This is clearly shown by the ASIs, SSIs and short/long ratios. Individual and combined PCA confirmed these observations by separating terrestrial markers from marine and bacterial markers.

- The coprostanol/cholesterol ratios showed the increasing input of coprostanol from the past due to increasing population and human activities.

- PLS modelling can be used to see the contribution or transportation of compounds from one environment to another. PLS path modelling also shows the contribution of organic matter from samples along the core as well as the decrease of organic matter.

- Cluster analysis with Ward's method differentiated the Clyde Sea sediment samples and the Loch Riddon core into two regions. Cluster group I consist of samples collected from the open seas and characterised by marine and bacterial lipids, while cluster group II represents samples collected within the lochs and are characterised by terrestrial and sewage derived compounds. Meanwhile cluster group I in the core defines mainly the top samples, characterised by marine and bacterial/sewage compounds, while cluster group II consists the deeper section of the core dominated by terrigenous inputs.

- Factor analysis reduced the data set from the Mawddach and the core into 3 and 2 factors. There are 3 significant sources in the Mawddach: marine/bacterial, sewage and terrestrial. Meanwhile in the sediment core there are 2 clear sources: bacterial/marine and terrestrial.

- PCA is the best method to use in lipid biomarker studies as it separates the compounds into their geochemical source and groups the samples accordingly.

- Factor analysis and cluster analysis can be used as alternative methods to PCA. Factor analysis can be a good method if the user understands the underlying objectives of the method and select the input parameters correctly.

- Normalisation of the compounds to the total organic carbon can be done for future research. Again, as the statistics used this study is concentration independent, this normalisation is not always necessary.

**Proposed titles of papers to be published**

1. Distribution and sources of lipid biomarkers in the surface sediments of the Mawddach Estuary: combination of multivariate analyses and biomarker approaches.

   Journal to be submitted to: Marine Pollution Bulletin.

2. Source input elucidation using multivariate analyses in the Clyde Sea.

   Journal to be submitted to: Marine Environmental Research.

3. The use of multivariate statistics in temporal studies.

   Journal to be submitted to: Environmental Forensics.

# REFERENCES

Aboul-Kassim, T.A.T. and Simoneit, B.R.T. (1996) Lipid geochemistry of surficial sediments from the coastal environment of Egypt. I. Aliphatic hydrocarbons-characterisation and sources. *Mar. Chem.* **54**, 135-158.

Albers, C. S., Kattner, G. and Hagen, W. (1996) The composition of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepod: Evidence of energetic adaptations. *Mar. Chem.* **55**, 347-358.

Andrews, J.E., Greenway, A.M. and Dennis, P.F. (1998) Combined carbon isotope and C/N ratios as indicators of source and fate of organic matter in a poorly flushed, tropical estuary: Hunts Bay, Kingston Harbour, Jamaica. *Estuarine, Coastal and Shelf Science* **46**, 743-756.

Antia, N.J., Lee, R.F., Nevenzel, J.C. And Cheng, J.Y. (1974) Wax ester production by the marine cryptomonad *Chroomonas salina* grown photoheterotrophically on glycerol. *J. Protozool.* **21**, 768-771.

Banaimoon, S.A. (1992) Fatty acids in marine macroalgae from Southern Yemen (Hadramout) including occurrence of eicosatetraenoic (20:4) and eicosapentaenoic (20:5) acids. *Botanica Marina* **35**, 165-168.

Barrett, S.M., Volkman, J.K., Dunstan, G.A. and Leroi, J.M. (1995) Sterols of 14 species of marine diatoms. *J. Phycol.* **31**, 360-369.

Bartlett, P.D. (1987) Degradation of coprostanol in an experimental system. *Mar. Pollut. Bull.* **18**, 27-29.

Berge, J.P., Gouygou, J.P., Dubacq, J.P. and Durand, P. (1995) Reassessment of lipid composition of the diatom, *Skeletonema costatum*. *Phytochemistry* **39**, 1017-1021.

Bertone, S., Giacomini, M., Ruggiero, C., Piccarolo, C. and Calegari, L. (1996) Automated systems for identification of heterotrophic marine bacteria on the basis of their fatty acid composition. *App. Environ. Microbiol.* **62**, 2122-2132.

Bohlin, L., Sjostrand, U., Sodano, G. and Djerassi, C. (1982) Sterols in marine invertebrates. 33. Structures of five new 3β-(hydroxymethyl)-A-norsteranes: indirect evidence for transformation of dietary precursors in sponges. *J. Org. Chem.* **47**, 5309-5314.

Boon, J.J., De Leeuw, J.W. and Schenck, P.A. (1975) Organic geochemistry of Walvis Bay diatomaceous ooze. I. Occurrence and significance of fatty acids. *Geochim. Cosmochim. Acta* **39**, 1559-1565.

Borchjensen, C. and Mollerup, J. (1996) Supercritical fluid chromatography of fish, shark and seal oils. *Chromatographia* **42**, 252-258.

Bradshaw, S. A., Ohara, S. C. M., Corner, E. D. S. and Eglinton, G. (1991) Effects on dietary lipids of the marine bivalve *Scrobicularia plana* feeding in different modes. *J. Mar. Biol. Ass. U. K.* **71**, 635-653.

Brassell, S. C. and Eglinton, G. (1986) Molecular geochemical indicators in sediments. *In*: Sohn, M. L. (ed.) *Marine Organic Geochemistry*. American Chemical Society, Washington DC. 427p.

Burd, F. (1989) *The saltmarsh survey of Great Britain. An inventory of British saltmarshes*. Peterborough, Nature Conservancy Council, (Research and survey in nature conservation, No. 17).

Canuel, E. A. and Martens, C. S. (1996) Reactivity of recently deposited organic matter: degradation of lipid compounds near the sediment-water interface. *Geochim. Cosmochim. Acta* **60**, 1793-1806.

Carpenter, S.R., Elser, M.M. and Elser, J.J. (1986) Chlorophyll production, degradation and sedimentation: implication for pale limnology. *Limnol. Oceanogr.* **31**, 112-124.

Carrie, R. H., Mitchell, L. and Black, K. D. (1998) Fatty acids in surface sediments at the Hebridean shelf edge, west of Scotland. *Org. Geochem.* **29**, 1583-1593.

Caudales, R., Moreau, R.A., Wells, J.M. and Antoine, A.D. (1993) Cellular lipid and fatty acid composition of Cyanobionts from *Azolla caroliniana*. *Symbiosis* **14**, 191-200.

Caudales, R. and Wells, J.M. (1992) Differentiation of free living *Anabaena* and *Nostoc* cyanobacteria o the basis of fatty acid composition. *International Journal of Systematic Bacteriology* **42**, 246-251.

Claustre, H., Marty, J., Cassiani, L. and Dagaut, J. (1989) Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea: ecological implications. *Mar. Micro. Food Webs* **3**, 51-66.

Colombo, J. C., Silverberg, N. and Gearing, J. N. (1997) Lipid biogeochemistry in the Laurentian Trough: I - fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. *Org. Geochem.* **25**, 211-225.

Conte, M.H., Madureira, L.A.S., Eglinton, G., Keen, D. and Rendall C. (1994) Millimeter-scale profiling of abyssal marine sediments – role of bioturbation in early sterol diagenesis. *Org. Geochem.* **22**, 979-990.

Cranwell, P.A. and Volkman, J.K. (1981) Alkyl and steryl esters in a recent lacustrine sediment. *Chem. Geol.* **32**, 29-43.

Currie, B. R. and Johns, R. B. (1989) An organic geochemical analysis of terrestrial biomarkers in a transect of the Great Barrier Reef Lagoon. *Aust. J. Mar. Freshwat. Res.* **40**, 275-284.

Dachs, J., Bayona, J. M., Fillaux, J., Saliot, A. and Albaigés, J. (1999) Evaluation of anthropogenic and biogenic inputs into the western Mediterranean using molecular markers. *Mar. Chem.* **65**, 195-210.

Dembitsky, V.M., Pechenkinashubina, E.E. and Rozentsvet, O.A. (1991) Glycolipids and fatty acids of some seaweeds and marine grasses from the Black Sea. *Phytochemistry* **30**, 2279-2283.

Dittmar, T. and Lara, R.J. (2001) Molecular evidence for lignin degradation in sulphate-reducing mangrove sediments (Amazônia, Brazil). *Geochim. Cosmochim. Acta* **65**, 1417-1428.

Duan, Y. (2000) Organic geochemistry of recent marine sediments from the Nansha Sea, China. *Org. Geochem.* **31**, 159-167.

Dunstan, G. A., Volkman, J. K., Barrett, S. M., Leroi, J. -M. and Jeffrey, S. W. (1994) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). *Phytochem.* **35**, 155-161.

Dunstan, G. A., Volkman, J. K., Jeffrey, S.W. and Barrett, S.M. (1992) Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. *Journal of Marine Biology and Ecology* **161**, 115-134.

Dureth, S., Herrman, R. and Pecher, K. (1986) Tracing faecal pollution by coprosranol and intestinal bacteria in an ice-covered Finnish lake loaded with both industrial and domestic sewage. *Wat. Air Soil Pollut.* **28**, 131-149.

Eglinton, G. and Hamilton, R.J. (1967) Leaf epicuticular waxes. *Science* **156**, 1322.

Eglinton, G., Parkes, R.J. and Zhao, M. (1993) Lipid biomarkers in biogeochemistry: Future roles? *Mar. Geol.* **113**, 141-145.

Ertel, J.R. and Hedges, J.I. (1985) Sources of sedimentary humic substances: vascular plant debris. *Geochim. Cosmochim. Acta* **49**, 2097-2107.

Escalona, R.L., Rosales, M.T.L. and Mandelli, E.F. (1980) On the presence of faecal steroids in sediments from two Mexican harbor. *Bull. Environ. Contam. Toxicol.* **24**, 289-295.

Fahl, K. and Kattner, G. (1993) Lipid content and fatty acid composition of algal communities in sea ice and water from the Weddell Sea (Antarctica). *Polar Biology* **13**, 405-409.

Fahl, K. and Stein, R. (1999) Biomarkers as organic-carbon-source and environmental indicators in the Late Quartenary Arctic Ocean: Problems and perspectives. *Mar. Chem.* **63**, 293-309.

Falcão, M.M. (1996) *Nutrient dynamics in the Ria Formosa Lagoon: effects of the interaction of the lagoon with its interfaces in the cycling of nitrogen, phosphorus and silica*. Ph.D Thesis, University of Algarve. 223p. (in Portuguese).

Farrington, J.W. and Quinn, J.Q. (1973) Biochemistry of fatty acids in recent sediments from Narragansett Bay, Rhode Island. *Geochim. Cosmochim. Acta* **37**,259-268.

Fisher, E., Oldfield, F., Wake, R., Boyle, J., Appleby, P. and Wolff, G.A. (2003) Molecular marker records of land use change. *Org. Geochem.* **34**, 105-119.

Floreto, E.A.T. Teshima, S. and Ishikawa, M. (1996) Effects of nitrogen and phosphorus in the growth and fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta). *Botanica Marina* **39**, 69-74.

Francois, R., Altabet, M. A., Yu, E.-F., Sigman, D.M., Bacon, M.P., Frank, M., Bohrmann, G., Bareille, G. and Labeyrie, L.D. (1997) Contribution of Southern Ocean surface-water stratification to low atmospheric $CO_2$ concentrations during the last glacial period. *Nature* **389**, 929-935.

Freudenthal, T., Wagner, T., Wenzhöfer, F., Zabel, M. and wafer, G. (2001) Early diagenesis of organic matter from sediments of the eastern subtropical Atlantic: Evidence from stable nitrogen and carbon isotopes. *Geochim. Cosmochim. Acta* **65**, 1795-1808.

Fukushima, K. and Ishiwatari, R. (1984) Acid and alcohol compositions of wax esters in sediments from different environments. *Chem. Geol.* **47**, 41-56.

Furlong, E.T. and Carpenter, R. (1988) Pigment preservation and remineralization in oxic coastal marine sediments. *Geochim. Cosmochim. Acta* **52**, 87-99

Gagosian, R. B., Nigrelli, G. E. and Volkman, J. K. (1983) Vertical transport and transformation of biogenic organic compounds from a sediment trap experiment off the coast of Peru. *In*: Suess, E. and Thiede, J. (Eds.). *Coastal Upwelling, its Sediment Record. Part A: Responses of the Sedimentary Regime to Present Coastal Upwelling*. Plenum Press, New York, pp. 241-272.

Gerlach, R.W., Kowalski, B.R. and Wold, H.O.A. (1979) Partial least-squares path modelling with latent variables. *Anal. Chim. Acta* **112**, 417-421.

Goad, L.J. and Goodwin, T.W. (1972) The biosynthesis of plant sterols. *Prog. Phytochem.* **3**, 113-198.

Gomez-Belinchon, J.I., Llop, R., Grimalt, J.O. and Albaiges, J. (1988) The decomposing of hydrocarbons and fatty acids in the dissolved and particulate water phases of a deltaic environment. *Mar. Chem.* **25**, 325-348.

Goñi, M. A. and Hedges, J. I. (1995) Sources and reactivities of marine-derived organic matter in coastal sediments as determined by alkaline CuO oxidation. *Geochim. Cosmochim. Acta* **59**, 2965-2981

Goñi, M. A. and Thomas, K.A. (2000) Sources and transformation of organic matter in surface soils and sediments from a tidal estuary (North Inlet, South Carolina, USA). *Estuaries* **23**, 548-564.

Goñi, M. A., Ruttenberg, K.C. and Eglinton, T.I. (1997) Sources and contribution of terrigenous organic carbon to surface sediments in the Gulf of Mexico. *Nature* **389**, 275-278.

Goñi, M. A., Ruttenberg, K.C. and Eglinton, T.I. (1998) A reassessment of the sources and importance of land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* **62**, 3055-3075.

Goñi, M. A., Yunker, M. B., Macdonald, R. W. and Eglinton, T. I. (2000) Distribution and sources of organic biomarkers in arctic sediments from the Mackenzie River and Beaufort Shelf. *Mar. Chem.* **71**, 23-51.

Gordon, E.S. and Goñi, M. A. (2003) Sources and distribution of terrigenous organic matter delivered by the Atchafalaya River to sediments in the northern Gulf of Mexico. *Geochim. Cosmochim. Acta* **67**, 2359-2375.

Graeve, M., Hagen, W. and Kattner, G. (1994) Herbivorous or omnivorous: on the significance of lipid composition as trophic markers in Antarctic copepods. *Deep Sea Research Part A: Oceanographic Research Papers* **41**, 915-924.

Graeve, M. and Kattner, G. (1992) Species-specific differences in intact wax esters of *Calanus hyperboreus* and *C. Finmarchicus* from Fram Strait - Greenland Sea. *Mar. Chem.* **39**, 269-281.

Graham, M.C., Eaves, M.A., Farmer, J.G., Dobson, J. and Fallick, A.E. (2001) A study of carbon and nitrogen stable isotope and elemental ratios as potential indicators of source and fate of organic matter in sediments of the Forth Estuary, Scotland. *Estuarine Coastal and Shelf Science* **52**, 375-380.

Grimalt, J.O. and Albaiges, J. (1990) Characterization of the depositional of the Ebro Delta (western Mediterranean) by the study of sedimentary lipid markers. *Mar. Geol.* **95**, 207-224.

Grimalt, J.O., Fernandez, P., Bayona, J.M. and Albaiges, J. (1990) Assessment of fecal sterols and ketones as indicators of urban sewage inputs into coastal waters. *Environ. Sci. Technol.* **24**, 357-363.

Grimalt, J.O. and Olive, J. (1993) Source input elucidation in aquatic systems by factor and principal component analysis of molecular marker data. *Anal. Chim. Acta* **278**, 159-176.

Grossi, V., Caradec, S. and Gilbert, F. (2003) Burial and reactivity of sedimentary microalgal lipids in bioturbated Mediterranean coastal sediments. *Mar. Chem.* **81**, 57-69.

Gustafson, O., Haghseta, F., Chan, C., MacFarlane, J. and Gschwend, P. M. (1997) Quantification of the dilute sedimentary phase: implication for PAH speciation and bioavailability. *Environ. Sci. Tech.* **31**, 203-209.

Haddad, R. I., Martens, C. S. and Farrington, J. W. (1992) Quantifying early diagenesis of fatty acids in a rapidly accumulating coastal marine sediments. *Adv. Org. Geochem.* **19**, 205-216.

Haig, A. J. N. (1986) Use of the Clyde Estuary and Forth for disposal of effluents. *Proc. R. Soc. Edinburgh*, Sect. B 90, 393-406.

Hama, T. (1991) Production and turnover rates of fatty acids in marine particulate matter through phytoplankton photosynthesis. *Mar. Chem.* **33**, 213-227.

Hama, J. and Handa, N. (1992) Diel photosynthetic production of cellular organic matter in natural phytoplankton populations, measured with 13C and gas chromatography mass spectrometry 2. Fatty acids and amino acids. *Mar. Biol.* **112**, 183-190.

Harvey, H. R. (1994) Fatty acids and sterols as source markers of organic matter in sediments of the North Carolina continental slope. *Deep Sea Res. II* **41**, 783-796.

Harvey, H. R. and Macko, S. A. (1997) Catalysts or contributers? Tracking bacterial mediation of early diagenesis in the marine water column. *Org. Geochem.* **26**, 531-544.

Hedges, J.I., Clark, W.A., Quay, P.D., Richey, J.E., Devol, A.H. and Santos, U.M. (1986) Composition and fluxes of particulate organic material in the Amazon River. *Limnol. Oceanogr.* **31**, 717-738.

Hedges, J.I. and Parker, P.L. (1976) Land-derived organic matter in surface sediments from the Gulf of Mexico. *Geochim. Cosmochim. Acta* **40**, 1019-1029.

Hedges, J.I. and Mann, D.C. (1979) The lignin geochemistry of marine sediments from the southern Washington coast. *Geochim. Cosmochim. Acta* **43**, 1809-1818.

Hedges, J.I. and Prahl, F.G. (1993) Early diagenesis: consequences for applications of molecular markers. *In*: M.H. Engel and S.A. Macko (Editors), *Organic Geochemistry: Principles and Applications*. Plenum. New York, NY, pp. 237-253.

Hinrichs, K. -U., Schneider, R. R., Müller, P. J. and Rullkötter, J. (1999) A biomarker perspective on paleoproductivity variations in two late Quarternary sediment sections from the Southeast Atlantic Ocean. *Org. Geochem.* **30**, 341-366.

Howard, P. J. A. (1991) *An Introduction to Environmental Pattern Analysis*. Pathernon Publishing Group Ltd. 254p.

Huang, M.Y. and Meinschein, W.G. (1976) Sterols as source indicators of organic materials in sediments. *Geochim. Cosmochim. Acta* **40**, 323-330.

Huang, M.Y. and Meinschein, W.G. (1979) Sterols as ecological indicators. *Geochim. Cosmochim. Acta* **43**, 739-745.

Hudson, E. D., Parrish, C. C. and Helleur, R. J. (2001) Biogeochemistry of sterols in plankton, settling particles and recent sediments in a cold ocean ecosystem (Trinity Bay, Newfoundland). *Mar. Chem.* **76**, 253-270.

Irwin, H. and Meyer, T. (1989) Lacustrine organic facies. A biomarker study using multivariate statistical analysis. *Org. Geochem.* **16**, 197-210.

Ishiwatari, I., Ogura, K. and Horie, S. (1980) Organic geochemistry of a lacustrine sediment (Lake Haruna, Japan). *Chem. Geol.* **29**, 261-280

Ishiwatari, R., Yamada, K., Matsumoto, K., Houtatsu, M. and Naraoka, H. (1999) Organic molecular and carbon isotopic records of the Japan Sea over the past 30 kyr. *Paleoceanography* **14**, 260-270.

Jaffe, R., Wolff, G.A., Cabrera, A.C. and Chitty, H.C. (1995) The biochemistry of lipids in rivers of the Orinoco Basin. *Geochim. Cosmochim. Acta* **59,** 4507-4522.

Jeng, W. L. and Han, B. C. (1994) Sedimentary coprostanol in Kaohsiung Harbour and the Tan-Shui Estuary, Taiwan. *Mar. Poll. Bull.* **28**, 494-499.

Jeng, W.L. and Han, B.C. (1996) Coprostanol in a sediment core from the anoxic Tan-Shui Estuary, Taiwan. *Estuar.Coast. Shelf Sci.* **42**, 727-735.

Kaiser, K. L. E. and Esterby, S. R. (1991) Regression and cluster analysis of the acute toxicity of 207 chemicals to six species of biota and octanol/water partition coefficient. *Sci. Total Environ.* **109-110**, 499-514.

Kakela, R. and Hyvarinen, H. (1993) Fatty acid composition of fats around the mystacial and superciliary vibrissae differ from that of blubber in the Sairnaa Ringed Seal (*Phoca-hispida saimensis*). *Comparative Biochemistry and Physiology Part B, Biochemistry and Molecular Biology* **105**, 547-552.

Kattner, G., Graeve, M. and Hagen, W. (1994) Ontogenic and seasonal changes in lipid and fatty acid alcohol compositions of the dominant Antarctic copepods *Calanus propinquuts*, *Calanoides acutus* and *Rhincalanus gigas*. *Mar. Biol.* **118**, 637-644.

Kawamura, K. and Ishiwatari, R. (1984) Fatty acid geochemistry of a 200m sediment core from Lake Biwa, Japan. Early diagenesis and paleoenvironmental information. *Geochim. Cosmochim. Acta* **48**, 251-266.

Kelly, A. G. (1995) Accumulation and persistence of chlorobiphenyls, organochlorine pesticides and faecal sterols at the garroch head sewage sludge disposal site, Firth of Clyde. *Environ. Pollut.* **88**, 207-217.

Kharlamenko, V.I., Zhukova, N.V., Khotimchenko, S.V., Svetashev, V.I. and Kamenev, G.M. (1995) Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kratemaya Bright, Yankich Island Kurile Islands). *Mar. Ecol. Prog. Ser.* **120**, 231-241.

Khotimchenko, S. V. (1995) Uncommon 16:1ω5 acid from *Dictyota dichotoma* and fatty acids of some brown algae of Dictyptaceae. *Phytochem.* **38**, 1411-1415.

Killops, S.D. and Killops, V.J. (1993) *An Introduction to Organic Geochemistry*. Longman Scientific and Technical. New York. 265 p.

Kruskal, J.B. and Wish, M. (1984) *Multidimensional scaling*. Beverly Hills: Sage Publications. 93p.

Krzanowski, W. J. and Marriott, F. H. C. (1995) *Multivariate Analysis*. Part 2. Classification, covariance structure and repeated measurements. Edward Arnold. London-Sydney-Auckland. 280p.

Lajat, M., Saliot, A. and Schimmelmann, A. (1990) Free and bound lipids in recent (1835-1987) sediments from Santa Barbara Basin. *Org. Geochem.* **16**, 793-803.

Laureillard, J. and Saliot, A. (1993) Biomarkers in organic matter produced in estuaries: a case study of the Krka estuary (Adriatic Sea) using the sterol marker series. *Mar. Chem.* **43**, 247-261.

Leavitt, P.R. and Carpenter, S.R. (1990) Aphotic degradation in the hypolimnion: implications for sedimentation studies and paleolimnology. *Limnol. Oceanogr.* **28**, 520-534.

Lee, R. F. And Hirota, J. (1976) Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. *Limnol. Oceanogr.* **18**, 227-239.

Lee, C. (1994) Controls on carbon preservation – new perspectives. *Chem. Geol.* **114**, 285-288.

Leeming, R. and Nichols, P. D. (1998) Determination of the sources and distribution of sewage and pulp-fibre-derived pollution in the Derwent Estuary, Tasmania, using sterol biomarkers. *Mar. Freshwater Res.* **49**, 7-17.

Mannino, A. and Harvey, H. R. (1999) Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: Sources and diagenetic patterns. *Geochim. Cosmochim. Acta* **63**, 2219-2235.

Matsuda, H. and Koyama, T. (1977) Early diagenesis of fatty acids in lacustrine sediments. I. Identification and distribution of fatty acids in recent sediments from a freshwater lake. *Geochim. Cosmochim. Acta* **41**,777-783.

Matsumoto, K., Yamada, K. and Ishiwatari, R. (2001) Sources of 24-ethylcholest-5-en-3β-ol in Japan Sea sediments over the past 30,000 years inferred from its carbon isotopic composition. *Org. Geochem.* **32**, 259-270.

McCaffrey, M.A., Farrington, J.W. and Repeta, D.J. (1991) The organic geochemistry of Peru margin surface sediments. II. Paleoenvironmental implications of hydrocarbon and alcohol profiles. *Geochim. Cosmochim. Acta* **55**, 483-498.

McCalley, D. V., Cooke, M. and Nickless, G. (1981) The effect of sewage treatment on fecal sterols. *Water Res.* **15**, 1019-1025.

Meglen, R.R. (1992) Examining large databases: a chemometric approach using principal analysis. *Mar. Chem.* **39**, 217-237.

Méjanelle, L., Laureillard, J., Fillaux, J., Saliot, A. and Lamberty, C. (1995) Winter distribution of algal pigments in small- and large-size particles in the northeastern Atlantic. *Deep-Sea Res.* **42**, 117-133.

Meyers, P. A. and Ishiwatari, I. (1993) Lacustrine organic geochemistry: an overview of indicators of organic matter sources and diagenesis in Lake sediments. *Org. Geochem.* **20**, 867-900.

Meyers, P. A., Leenheer, M. J., Eadie, B. J. and Maule, S. J. (1984) Organic geochemistry of suspended and settling particulate matter in Lake Michigan. *Geochim. Cosmochim. Acta.* **48**, 443-452.

Millie, D.F., Paerl, H.W. and Hurley, J.P. (1993) Microalgal pigment assessments using high performance liquid chromatography; a synopsis of organismal and ecological applications. *Can. J. Fish. Aqua. Sci.* **50**, 2513-2527.

Minchin, P.R. (1987) An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio* **69**, 89-108.

Morris, R.J. and Culkin, F. (1976) Marine lipids: analytical technique and fatty acid ester analyses. *Oceanogr. Mar. Biol. Ann. Rev.* **14**, 391-433.

Mudge, S. M. (2002) Aspects of hydrocarbon fingerprinting using PLS – New data from Prince William Sound. *Environmental Forensics.* **3**, 323-329.

Mudge, S. M. and Bebianno, M. J. (1997) Sewage contamination following an accidental spillage in the Rio Formosa, Portugal. *Mar. Poll. Bull.* **34**, 163-170.

Mudge, S. M., Bebianno, M. J., East, J. A. and Barreira, L, A. (1999) Sterols in the Ria Formosa Lagoon, Portugal. *Wat. Res.* **33**, 1038-1048.

Mudge, S. M., East, J. A., Bebianno, M. J. and Barreira, L, A. (1998) Fatty acids in the Ria Formosa Lagoon, Portugal. *Org. Geochem.* **29**, 963-977.

Mudge, S. M., Goodchild, I. D. and Wheeler, M. (1995) Vegetable oil spills on salt marshes. *Chemistry and Ecology* **10**, 127-135.

Mudge, S. M. and Lintern, D. G. (1999) Comparison of sterol biomarkers for sewage with other measures in Victoria Harbour, B. C., Canada. *Estuar. Coast. Shelf Sci.* **48**, 27-38

Mudge, S. M. and Norris, C. E. (1997) Lipid biomarkers in the Conwy Estuary (North Wales, U.K.): a comparison between fatty alcohols and sterols. *Mar Chem.* **57**, 61-84.

Nadjek, M. (1993) Factors influencing fatty acid and hydrocarbon composition of sedimenting particles in the northeastern Adriatic sea. *Mar. Chem.* **41**, 299-310.

Nekola, J.C. (2003) Large-scale terrestrial gastropod community composition patterns in the Great Lakes region of North America. *Biodiversity Research* **9**, 55-71.

Newell, S. Y. and Fell, J. W. (1992) Ergosterol content of living and submerged, decaying leaves and twigs of red mangrove. *Can. J. Microbiol.* **38**, 979-982.

Newton, A. (1995) *The water quality of the Ria Formosa Lagoon, Portugal*. Ph.D Thesis, University of Wales, Bangor.

Nichols, P. D. and Espey, Q. I. (1991) Characterisation of organic matter at the air-sea interface, in subsurface water and in bottom sediments near the Malabar sewage outfall in Sydney's coastal region. *Aust. J. Mar. Freshwat. Res.* **42**, 327-348.

Nichols, P. D., Klumpp, D.W., and Johns, R.B. (1982) Lipid components of the seagrasses *Posidonia australis* and *Heterozostera tasmanica* as indicators of carbon source. *Phytochemistry* **21**, 1613-1621.

Nichols, P. D., Leeming, R., Rayner, M. S., Latham, V., Ashbolt, N. J. and Tumer, C. (1993a) Comparison of the fecal sterol coprostanol and fecal bacterial groups in inner-shelf waters and sediments near Sydney, Australia. *J. Chromat.* **643**, 189-195.

Nichols, D.S., Nichols, P. D. and Sullivan, C.W. (1993b) Fatty acid, sterol and hydrocarbon composition of Antarctic sea-ice diatom communities during the spring bloom in McMurdo Sound. *Antarctic Sci.* **5**, 271-278.

Nichols, P.D., Palmisano, A.C., Smith, G.A. and White, D.C. (1986) Lipids of the Antarctic sea ice diatom *Nitzschia cylindrus*. *Phytochem.* **25**, 1649-1653.

Nishimura, M. and Baker, E. W. (1987) Compositional similarities of non-solvent extractable fatty acids from recent marine sediments deposited in differing environments. *Geochim. Cosmochim. Acta* **51**, 1365-1378.

Onstad, G.D., Cnfield, D.E., Quay, P.D. and Hedges, J.I. (2000) Sources of particulate organic matter in rivers from the continental USA: Lignin phenol and stable carbon isotope compositions. *Geochim. Cosmochim. Acta* **64**, 3539-3546.

Parameswaran, P. S., Das, B. and Kamat, S. Y. (1994) Lipid contents of the sponge *Haliconia sp. Indian. J. Chem.* **33**, 99-101.

Parkes, R. J. (1987) Analysis of microbial communities within sediments using biomarkers. *In*: Fletcher, M., Gray, T. R. G. and Jones, J. G. (Eds.). *Ecology of Microbial Communities*. Cambridge University Press, London, 440 pp.

Parkes, R.J. and Taylor, J. (1983) The relationships between fatty acid distributions and bacterial respiratory types in contemporary sediments. *Estuar. Coast. Shelf Sci.* **16**, 173-189.

Patterson, G. W., Gladu, P. K., Wikfors, G. H. and Lusby, W. R. (1992) Unusual tetraene sterols in some phytoplankton. *Lipids* **27**, 154-156.

Pearson, A., Eglinton, T. and McNichols, A. P. (2000) An organic tracer for surface ocean radiocarbon. *Paleoceanography* **15**, 541-550.

Peng, X., Zhang, G., Mai, B., Min, Y. and Wang, Z. (2002) Spatial and temporal trend of sewage pollution indicated by coprostanol in Macao Estuary, southern China. *Mar. Poll. Bull.* **45**, 295-299.

Pereira, M. G. S. (1999) *Bacterial degradation of linseed and sunflower oils in salt marsh sediments*. PhD Thesis. University of Wales, Bangor. 199p.

Pereira, W. E., Hostettler, F. D., Luoma, S. N., van Green, A., Fuller, C. C. and Anima, R. J. (1999) Sedimentary record of anthropogenic and biogenic polycyclic aromatic hydrocarbons in San Francisco Bay, California. *Mar. Chem.* **64**, 99-113.

Perry, G.J., Volkman, J.K., Johns, R.B. and Bavor, H.J. (1979) Fatty acids of bacterial origin in contemporary marine sediments. *Geochim. Cosmochim. Acta* **43**, 1715-1725.

Pinturier-Geiss, L., Laureillard, J., Riaux-Gobin, C., Fillaux, J. and Saliot, A. (2001) Lipids and pigments in deep-sea surface sediments and interfacial particles from the Western Crozet Basin. *Mar. Chem.* **75**, 249-266.

Piovetti, L., Deffo, P., Valls, R. and Peiffer, G. (1991) Determination of sterols and diterpenoids from brown-algae (Cystoseiraceae). *J. Chromatogr.* **588**, 99-105.

Piretti, M.V., Pagliuca, G., Boni, L., Pistocchi, R., Diamante, M. and Gazzotti, T. (1997) Investigation of 4-methyl sterols from cultured dinoflagellate algal strains. *J. Phycol.* **33**, 61-67.

Pocklington, R., Leonard, J.O. and Crewe, N.F. (1987) Le coprostanol comme indicator de la contamination fecale dans l'eau de mer et les sediment marin. *Oceanol. Acta* **10**, 83-89.

Pond, D.W. and Harris, R.P. (1996) The lipid composition of the Coccolithophore *Emiliana huxleyi* and its possible ecophysiological significance. *J. Mar. Biol. Ass. U.K.* **76**, 579-594.

Ponomarenko, L. P., Makarieva, T. N., Stonik, V. A., Dmitrenok, A. S. and Dmitrenok, P. S. (1995) Sterol composition of *Linneus torquatus* (Nemertini) Anopla. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **III**, 575-577.

Poodle, T. (1986) Freshwater inflows to the Firth of Clyde. *Proc. R. Soc. Edinburgh*, Sect. B 90, 55-66.

Prahl, F.G., Ertel, J.R., Goñi, M. A., Sparrow, M.A. and Eversmeyer, B. (1994) Terrestrial organic carbon contributions to sediments on the Washington margin. *Geochim. Cosmochim. Acta* **58**, 3035-3048.

Quemeneur, M. and Marty, Y. (1994) Fatty acids and sterols in domestic wastewaters. *Wat. Res.* **28**, 1217-1226.

Rajendran, N., Fujiyoshi, E. and Matsuda, O. (1991) Fatty acids and fatty alcohols of zooplankton from coastal upwelling area of Hyuga Nada, Japan. *Bull. of the Japanese Society of Fisheries* **57**, 2277-2284.

Rajendran, N., Matsuda, O., Rajendran, R. and Urushigawa, Y. (1997) Comparative description of microbial community structure in surface sediments of eutrophic bays. *Mar. Poll. Bull.* **34**, 26-33.

Rao, K.S., Dominic, R., Singh, K., Kaluwin, C., Rivett, D.E. and Jones, G.P. (1990) Lipid, fatty acid, amino acid and mineral composition of 5 edible plant leaves. *Journal of Agriculture and Food Chemistry* **38**, 2137-2139.

Rapp, J. B. (1991) A statistical approach to the interpretation of aliphatic hydrocarbons distributions in marine sediments. *Chem. Geol.* **93**, 163-177.

Readman, J.W., Preston, M.R. and Mantoura, R.F.C. (1986a) An integrated technique to quantify sewage, oil and PAH pollution in estuarine and coastal environments. *Mar. Pollut. Bull.* **19**, 298-308.

Readman, J.W., Mantoura, R.F.C., Llewellyn, C.A., Preston, M.R. and Reeves, A.D. (1986b) The use of pollutant and biogenic markers as source discriminants of organic inputs to estuarine sediments. *J. Environ. Anal. Chem.* **27**, 29-54.

Reemtsma, T., Haake, B., Ittekkot, V., Nair, R. R. and Brockmann, U. H. (1990) Downward flux of particulate fatty acids in the central Arabian sea. *Mar. Chem.* **29**, 183-202.

Reitan, K. I., Rainuzzo, J. R. and Olsen, Y. (1994) Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *J. Phycol.* **30**, 972-979.

Reynhardt, E.C. and Reinderer, M. (1994) Structures and molecular dynamics of plant waxes. 2. Cuticular waxes from leaves of *Fagus sylvatica* L. and *Hordeum vulgare* L. *European Biophysics Journal* **23**, 59-76.

Rohjans, D., Brocks, P., Scholz-Böttcher, B. M. and Rullkötter, J. (1998) Lipid biogeochemistry of surface sediments in the Lower Saxonian Wadden Sea, northwest Germany, and the effect of the strong winter 1995-1996. *Org. Geochem.* **29**, 1507-1516.

Rowland, S.J. and Robson, J.N. (1990) The widespread occurrence of highly branched acyclic $C_{20}$, $C_{25}$ and $C_{30}$ hydrocarbons in recent sediments and biota - a review. *Mar. Environ. Res.* **30**, 191-216.

Rütters, H., Sass, H., Cypionka, H. and Rullkötter, J. (2002) Microbial communities in a Wadden Sea sediment core - clues from analyses of intact glyceride lipids, and released fatty acids. *Org. Geochem.* **33**, 803-816.

Sajbidor, J., Lamacka, M., Breirerova, E., Chrastina, A., Pokreisz, P. and Certik, M. (1994) Effect of salt stress on fatty acid alterations in some strains of *Dipodascopsis* and *Dipodascus* spp. *World Journal of Microbiology and Biotechnology* **10**, 184-186.

Saliot, A., Laureillard, J., Scribe, P. and Sicre, M.A. (1991) Evolutionary trends in the lipid biomarker approach for investigating the biogeochemistry of organic matter in the marine environment. *Mar. Chem.* **36**, 233-248.

Sargent, J.R., Gatten, R.R. and Henderson, R.J. (1981) Marine wax esters . *Pure Appl. Chem.* **53**, 867-871.

Sargent, J.R., Gatten, R.R. and McIntosh, R. (1977) Wax esters in the marine environment - their occurrence, formation, transformation and ultimate fates. *Mar. Chem.* **5**, 573-584.

Scribe, P., Barreau, C., Pepe, C., Fuche, C., Dagaut, J., and Saliot, A. (1991) Fluvial inputs of organic matter from the Rhône River to the Northwestern basin of the Mediterranean Sea: sterol composition of organic matter. *Water Pollut. Res. Rep.* **20**, 259-267.

Sherwin, M.R., Van Vleet, E.S., Fossato, V.U. and Dolci, F. (1993) Coprostanol (5β-cholestan-3β-ol) in lagoonal sediments and mussels in Venice, Italy. *Mar. Pollut. Bull.* **26**, 501-507.

Shi, W., Sun, M. Y., Molina, M. and Hodson, R. E. (2001) Variability in the distribution of lipid biomarkers and their molecular isotopic composition in Altamaha estuarine sediments: implications for the relative contribution of organic matter from various sources. *Org. Geochem.* **32**, 453-467.

Simeonov, V., Massart, D. L., Andreev, G. and Tsakovski, S. (2000) Assessment of metal pollution based on multivariate statistical modeling of 'hot spot' sediments from the Black Sea. *Chemosphere* **41**, 1411-1417.

Skerratt, J. H., Nichols, P. D., McMeekin, T. A. and Burton, H. (1995) Seasonal and interannual changes in planktonic biomass and community structure in Eastern Antarctica using signature lipids. *Mar. Chem.* **51**, 93-113.

Smith, D.J., Eglinton, G. And Morris, R.J. (1983) The lipid chemistry of an interfacial sediment from the Peru Continental Shelf: Fatty acids, alcohols, aliphatic ketones and hydrocarbons. *Geochim. Cosmochim. Acta* **47**, 2225-2232.

Solic, M. and Krstulovic, N. (1992) Separate and combined effects of solar radiation, temperature, salinity and pH on the survival of faecal coliforms in seawater. *Mar. Poll. Bull.* **24**, 411-416.

Soma, Y., Tanaka, A., Soma, M. and Kawai, T. (1996) Photosynthetic pigments and perylene in the sediments of southern basin of Lake Baikal. *Org. Geochem.* **24**, 553-561.

Strom, S.L., Morello, T.A. and Bright, K. J. (1998) Protozoan size influences algal pigment degradation during grazing. *Mar. Ecol.: Prog. Ser.* **164**, 189-197.

Sun, M. Y., Aller, R.C. and Lee, C. (1994) Spatial and temporal distributions of sedimentary chloropigments as indicators of benthic processes in Long Island Sound. *J. Mar. Res.* **52**, 149-176.

Sun, M. Y. and Wakeham, S.G. (1994) Molecular evidence for degradation and preservation of organic matter in the anoxic Black Sea Basin. *Geochim. Cosmochim. Acta* **58**, 3395-3406.

Sun, M. Y. and Wakeham, S.G. (1999) Diagenesis of planktonic fatty acids and sterols in Long Island Sound sediments: Influences of a phytoplankton bloom and bottom water oxygen content. *J. Mar. Res.* **57**, 357-385.

Suzuki, T. and Matsuyama, Y. (1995) Determination of free fatty acids in marine phytoplankton causing red tides by fluorometric high performance liquid chromatography. *Journal of the American Oil Chemistry Society* **72**, 1211-1214.

Thoumelin, G., Bodineau, L. and Wartel, M. (1997) Origin and transport of organic matter across the Seine estuary: Fatty acid and sterol variation. *Mar. Chem.* **58**, 59-71.

Van Vleet, E.S. and Quinn, J.G. (1979) Early diagenesis of fatty acids and isoprenoid alcohols in estuarine and coastal sediments. *Geochim. Cosmochim. Acta* **43**, 289-303.

Venkatesan, M.I. and Kaplan, I.R. (1987) The lipid geochemistry of Antarctic sediments: Bransfield Strait. *Mar. Chem.* **21**, 347-375.

Venkatesan, M.I. and Kaplan, I.R. (1990) Sedimentary coprostanol as an index of sewage addition in Santa Monica Basin, Southern California. *Environ. Sci. Technol.* **24**, 208-214.

Venkatesan, M. I. and Mirsadeghi, F. H. (1992) Coprostanol as sewage tracer in McMurdoch Sound, Antarctica. *Mar. Poll. Bull.* **25**, 328-333.

Venkatesan, M.I. and Santiago, C.A. (1989) Sterols in ocean sediments: Novel tracers to examine habitats of cetaceans, pinnipeds, penguins and humans. *Mar. Biol.* **102**, 431-437.

Voß, M. and Struck, U. (1997) Stable nitrogen and carbon isotopes as indicator of eutrophication of the Oder river (Baltic Sea). *Mar. Chem.* **59**, 35-49.

Volkman, J.K. (1986) A review of sterol markers for marine and terrigenous organic matter. *Org. Geochem.* **9**, 83-99.

Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L. and Gelin, F. (1998) Microalgal biomarkers: A review of recent research developments. *Org. Geochem.* **29**, 1163-1179.

Volkman, J.K., Farrington, J.W. and Gagosian, R.B. (1987) Marine and terrigenous lipids in coastal sediments from the Peru upwelling region at 15°S: Sterols and triterpene alcohols. *Org. Geochem.* **11**, 463-477.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I. and Garland, C.D. (1989) Fatty acids and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Biol.Ecol.* **128,** 219-240.

Volkman, J.K., Gillan, F.T. and Johns, R.B. (1981) Sources of neutral lipids in a temperate intertidal sediment. *Geochim. Cosmochim. Acta* **45**, 1817-1828.

Volkman, J.K., Johns, R.B., Gillan, G.T. and Perry, G.J. (1980) Microbial lipid of an intertidal sediment. I. Fatty acids and hydrocarbon. *Geochim. Cosmochim. Acta* **44**, 1133-1143.

Volkman, J. K., Rohjans, D., Rullkötter, J., Scholz-Böttcher, B. M. and Liebezeit, G. (2000) Sources and diagenesis of organic matter in tidal flat sediments from the German Wadden Sea. *Continental Shelf Res.* **20**, 1139-1158.

Wakeham, S. G. (1987) Steroid geochemistry in the oxygen minimum zone of the eastern tropical North Pacific Ocean. *Geochim. Cosmochim. Acta* **51**, 3051-3069.

Wakeham, S. G. and Canuel, E. A. (1990) Fatty acids and sterols of particulate matter in a brackish and seasonally coastal salt pond. *Org. Geochem.* **16**, 703-713.

Wakeham, S. G. and Ertel, J. R. (1988) Diagenesis of organic matter in suspended particles and sediments in the Cariaco Trench. *Org. Geochem.* **13**, 815-822

Wakeham, S. G. and Lee, C. (1989) Organic geochemistry of particulate matter in the ocean: the role of particles in oceanic sedimentary cycles. *Org. Geochem.* **14**, 83-96.

Walker, R.W., Wun, C.K. and Litsky, W. (1982) Coprostanol as an indicator of faecal pollution. *Crit. Rev. Environ. Control* **12**, 91-112.

Weete, J. D. (1973) Sterols of the fungi: Distribution and biosynthesis. *Phytochem.* **12**, 1843-1864.

Westerhausen, L., Poynter, J., Eglinton, G., Erlenkeuser, H. and Sarnthein, M. (1993) Marine and terrigenous origin of organic matter in modern sediments of the equatorial east Atlantic: the $\delta^{13}C$ and molecular record. *Deep Sea Res. I* **40**, 1087-1121.

Wold, H. (1966) *In*: Krishnaiah, P.R. (Ed.) *Multivariate Analysis*. Academic Press, New York, p. 391.

Yde, M., DeWulf, E., DeMaeyer-Cleempoel, S. and Quaghebeur, D. (1982) Coprostanol and bacterial indicators of faecal pollution in the Scheldt Estuary. *Bull. Environ. Contam. Toxicol.* **28**, 129-134.

Yunker, M.B., MacDonald, R.W., Veltkamp, D.J. and Cretrey, W.J. (1995) Terrestrial and marine biomarkers in a seasonally ice-covered Arctic estuary - integration of multivariate and biomarker approaches. *Mar. Chem.* **49**, 1-50.

Zegouagh, Y., Derenne, S., Largeau, C. and Saliot, A. (1996) OM sources and early diagenetic alterations in Arctic surface sediments (Lena River delta and Laptev Sea, Eastern Siberia): I. Analysis of the carboxylic acids released via sequential treatments. *Org. Geochem.* **24**, 841-857.

http://www.cawdorcastle.com

**APPENDICES**

# Appendix 1: Concentration (ng g$^{-1}$ WW) of fatty acids from the Mawddach samples

| Site | 12:0 | 13:0 | br-14:0 | 14:0 | iso-15:0 | ante-15:0 | 15:0 | br-16:0 | 16:0 | 16:1w11 | 16:1w9 | 16:1w7 | iso-17:0 | ante-17:0 | 17:0 | 16:2 | 17:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 54386 | 1731 | 344 | 51074 | 1614 | 726 | 17559 | 433 | 40518 | 846 | 11174 | 58923 | 560 | 10020 | 13934 | 2466 | 4503 |
| 2 | 34986 | 1990 | 602 | 33229 | 571 | 2164 | 14916 | 8463 | 138284 | 762 | 6820 | 47026 | 1079 | 2939 | 6344 | 980 | 3484 |
| 3 | 24138 | 959 | 1401 | 42401 | 32451 | 31454 | 31983 | 17867 | 198086 | 0 | 3787 | 22444 | 19242 | 20348 | 8958 | 68 | 1299 |
| 4 | 10178 | 470 | 562 | 16044 | 7983 | 9731 | 7642 | 6662 | 54372 | 662 | 3266 | 25938 | 7673 | 4629 | 5108 | 136 | 3034 |
| 5 | 10614 | 556 | 797 | 7753 | 14555 | 14513 | 45773 | 10415 | 94854 | 0 | 1522 | 19800 | 9344 | 11283 | 3927 | 88 | 531 |
| 6 | 11383 | 495 | 776 | 8291 | 13948 | 13467 | 49243 | 9149 | 66702 | 103 | 1445 | 20649 | 10601 | 10689 | 5019 | 13 | 265 |
| 7 | 3908 | 174 | 672 | 20172 | 19131 | 16742 | 47922 | 7490 | 70810 | 20 | 1343 | 20414 | 10078 | 11160 | 10667 | 79 | 247 |
| 8 | 14536 | 265 | 350 | 28675 | 12064 | 8532 | 52199 | 6426 | 74035 | 195 | 1431 | 11711 | 2237 | 1680 | 11921 | 0 | 648 |
| 9 | 1914 | 199 | 990 | 3634 | 16691 | 18191 | 36508 | 19393 | 44382 | 164 | 1427 | 2602 | 11968 | 10403 | 12204 | 28 | 134 |
| 10 | 146 | 17 | 67 | 244 | 326 | 175 | 447 | 210 | 11190 | 0 | 12 | 1801 | 408 | 249 | 1375 | 0 | 6 |
| 11 | 831 | 104 | 600 | 3486 | 3286 | 10887 | 27381 | 14694 | 39933 | 1018 | 7049 | 3853 | 7541 | 13778 | 11847 | 115 | 3610 |
| 12 | 640 | 128 | 449 | 1733 | 2278 | 5945 | 14097 | 10696 | 31818 | 0 | 678 | 3739 | 1836 | 4277 | 597 | 1758 | 2293 |
| 13 | 749 | 53 | 197 | 8518 | 8044 | 9412 | 8902 | 8566 | 42920 | 502 | 3418 | 20085 | 12978 | 6278 | 8018 | 117 | 305 |
| 14 | 547 | 27 | 240 | 5967 | 22586 | 24914 | 20731 | 17516 | 39527 | 0 | 854 | 1622 | 19325 | 18631 | 2817 | 1052 | 1826 |
| 15 | 723 | 90 | 232 | 3865 | 11556 | 14439 | 26391 | 4529 | 87778 | 0 | 10969 | 32805 | 5380 | 1533 | 13629 | 700 | 2961 |
| 16 | 57 | 5 | 12 | 1533 | 733 | 315 | 306 | 692 | 5138 | 0 | 362 | 477 | 20 | 39 | 337 | 0 | 263 |
| 17 | 312 | 11 | 37 | 2444 | 1448 | 1274 | 3093 | 1295 | 92301 | 0 | 125 | 6503 | 188 | 881 | 1886 | 15 | 760 |
| 18 | 30 | 2 | 58 | 132 | 132 | 401 | 602 | 757 | 11316 | 0 | 259 | 453 | 56 | 55 | 169 | 0 | 3 |
| 19 | 153 | 19 | 434 | 3813 | 2013 | 2603 | 7358 | 10401 | 45645 | 0 | 797 | 11211 | 364 | 801 | 1723 | 4654 | 3490 |
| 20 | 232 | 12 | 599 | 2612 | 11613 | 9650 | 3158 | 3756 | 83515 | 0 | 263 | 835 | 9890 | 1245 | 1797 | 505 | 457 |
| 21 | 266 | 8 | 403 | 1717 | 1878 | 2471 | 1659 | 16108 | 15617 | 0 | 812 | 3588 | 426 | 773 | 396 | 742 | 389 |
| 22 | 148 | 5 | 551 | 1456 | 139 | 46 | 2988 | 21289 | 40797 | 0 | 358 | 5095 | 3758 | 251 | 1219 | 0 | 198 |
| 23 | 0 | 5 | 1417 | 396 | 539 | 512 | 966 | 759 | 6346 | 0 | 152 | 749 | 3020 | 2716 | 920 | 18 | 177 |
| 24 | 0 | 0 | 0 | 1500 | 973 | 132 | 400 | 930 | 88794 | 0 | 1401 | 16105 | 807 | 1089 | 1464 | 32 | 890 |
| 25 | 0 | 0 | 0 | 1505 | 1019 | 112 | 486 | 1053 | 59888 | 0 | 707 | 10837 | 213 | 3083 | 3208 | 0 | 1023 |

| Site | 16:3 | 18:0 | 18:1w11 | 18:1w9 | 18:1w7 | 18:2w6 | 18:2 | 18:3w3 | 20:0 | 20:1 | 21:0 | 20:4w6 | 22:0 | 20:5w3 | 24:0 | 25:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 670 | 30471 | 0 | 31143 | 1485 | 5882 | 1410 | 8622 | 7318 | 2925 | 0 | 8070 | 0 | 2436 | 1760 | 1600 |
| 2 | 568 | 23847 | 0 | 13246 | 1992 | 8550 | 0 | 5168 | 1967 | 728 | 0 | 6379 | 0 | 20880 | 1933 | 1757 |
| 3 | 96 | 30456 | 0 | 2348 | 2694 | 996 | 0 | 795 | 14980 | 0 | 0 | 1056 | 23700 | 731 | 1760 | 1931 |
| 4 | 0 | 9271 | 0 | 2091 | 354 | 1936 | 0 | 2655 | 6159 | 1455 | 0 | 1495 | 8115 | 2614 | 6029 | 901 |
| 5 | 0 | 19949 | 0 | 231 | 1863 | 444 | 0 | 700 | 14987 | 0 | 1521 | 504 | 21654 | 169 | 25302 | 548 |
| 6 | 52 | 24681 | 0 | 223 | 3162 | 612 | 0 | 1038 | 4323 | 12 | 368 | 734 | 4795 | 225 | 2550 | 422 |
| 7 | 48 | 28119 | 33 | 146 | 2348 | 303 | 0 | 418 | 7966 | 0 | 800 | 513 | 1059 | 137 | 18191 | 226 |
| 8 | 0 | 21886 | 0 | 6202 | 1532 | 6187 | 0 | 89 | 7475 | 0 | 0 | 981 | 16941 | 1954 | 20665 | 1666 |
| 9 | 0 | 27553 | 41 | 198 | 2380 | 499 | 0 | 305 | 3311 | 19 | 5219 | 1078 | 20328 | 76 | 15493 | 1993 |
| 10 | 0 | 6618 | 0 | 633 | 400 | 300 | 0 | 174 | 423 | 0 | 0 | 26 | 2348 | 194 | 2384 | 353 |
| 11 | 33 | 24339 | 1237 | 12321 | 1401 | 7935 | 0 | 166 | 4711 | 442 | 3742 | 2707 | 27059 | 1243 | 1531 | 3215 |
| 12 | 270 | 15605 | 0 | 2316 | 1220 | 5976 | 0 | 706 | 5494 | 510 | 0 | 1800 | 10234 | 738 | 730 | 89 |
| 13 | 242 | 46260 | 0 | 14445 | 287 | 16791 | 0 | 3010 | 5118 | 1558 | 1612 | 665 | 4891 | 1143 | 11999 | 4813 |
| 14 | 0 | 15954 | 0 | 741 | 2831 | 443 | 0 | 764 | 3413 | 69 | 118 | 911 | 2190 | 201 | 27295 | 4852 |
| 15 | 1108 | 87911 | 1509 | 20853 | 932 | 12115 | 0 | 3540 | 38489 | 1302 | 4108 | 5798 | 40473 | 1539 | 38553 | 4347 |
| 16 | 0 | 5771 | 0 | 1117 | 107 | 408 | 0 | 3003 | 3547 | 0 | 0 | 96 | 280 | 96 | 3886 | 517 |
| 17 | 0 | 45218 | 0 | 1068 | 483 | 1468 | 0 | 3211 | 19722 | 0 | 0 | 1007 | 17566 | 729 | 22129 | 2336 |
| 18 | 20 | 9958 | 0 | 758 | 23 | 456 | 0 | 1540 | 3451 | 0 | 0 | 16 | 4249 | 40 | 4081 | 451 |
| 19 | 32 | 55010 | 0 | 15935 | 210 | 3627 | 0 | 14802 | 29797 | 0 | 0 | 986 | 27060 | 412 | 42138 | 3542 |
| 20 | 0 | 35082 | 0 | 4298 | 1266 | 215 | 0 | 220 | 29231 | 0 | 0 | 131 | 15614 | 143 | 40727 | 2786 |
| 21 | 655 | 9703 | 0 | 3581 | 333 | 2092 | 0 | 2070 | 1986 | 306 | 841 | 552 | 11807 | 426 | 6670 | 171 |
| 22 | 0 | 6704 | 0 | 3243 | 392 | 3693 | 0 | 4062 | 4663 | 0 | 702 | 714 | 7193 | 518 | 11315 | 439 |
| 23 | 0 | 2921 | 0 | 981 | 520 | 639 | 0 | 634 | 570 | 0 | 874 | 0 | 569 | 292 | 2362 | 141 |
| 24 | 0 | 62807 | 0 | 40024 | 651 | 11731 | 0 | 2679 | 51926 | 0 | 1087 | 1514 | 44754 | 182 | 59387 | 6417 |
| 25 | 0 | 63803 | 0 | 28107 | 658 | 3505 | 66 | 12015 | 41836 | 0 | 0 | 662 | 25362 | 174 | 59138 | 6281 |

Appendix 2: Concentration (ng g$^{-1}$ WW) of fatty alcohols from the Mawddach samples

| Site | C9 | C12 | C13 | br C14 | C14 | iso-C15 | ante-C15 | C15 | C16 | iso-C17 | ante-C17 | C17 | C18 | iso-C19 | ante-C19 | C19 | C20 |
|------|-----|-----|-----|--------|-----|---------|----------|-----|------|---------|----------|-----|-----|---------|----------|-----|-----|
| 1 | 0 | 52 | 47 | 2 | 49 | 3 | 0 | 0 | 96 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 119 |
| 2 | 0 | 56 | 37 | 1 | 52 | 3 | 2 | 0 | 88 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 116 |
| 3 | 4 | 6 | 33 | 30 | 30 | 32 | 27 | 27 | 84 | 41 | 17 | 25 | 215 | 7 | 11 | 12 | 161 |
| 4 | 0 | 14 | 16 | 0 | 24 | 4 | 6 | 0 | 76 | 0 | 0 | 9 | 78 | 0 | 0 | 0 | 74 |
| 5 | 0 | 18 | 0 | 25 | 25 | 29 | 92 | 15 | 48 | 39 | 35 | 23 | 195 | 0 | 0 | 0 | 152 |
| 6 | 0 | 26 | 0 | 18 | 23 | 29 | 74 | 21 | 54 | 33 | 28 | 28 | 38 | 0 | 0 | 0 | 164 |
| 7 | 0 | 17 | 0 | 33 | 11 | 42 | 18 | 19 | 29 | 32 | 37 | 28 | 162 | 0 | 0 | 0 | 163 |
| 8 | 0 | 0 | 0 | 7 | 19 | 8 | 18 | 0 | 43 | 38 | 30 | 27 | 179 | 0 | 0 | 0 | 189 |
| 9 | 0 | 0 | 0 | 30 | 25 | 30 | 59 | 26 | 60 | 2 | 8 | 16 | 229 | 0 | 0 | 42 | 98 |
| 10 | 0 | 24 | 14 | 0 | 34 | 28 | 31 | 60 | 64 | 9 | 25 | 15 | 273 | 0 | 9 | 23 | 101 |
| 11 | 0 | 15 | 0 | 0 | 44 | 3 | 4 | 9 | 59 | 0 | 0 | 8 | 204 | 0 | 0 | 12 | 71 |
| 12 | 6 | 13 | 12 | 0 | 14 | 5 | 6 | 60 | 111 | 39 | 17 | 49 | 335 | 0 | 8 | 23 | 326 |
| 13 | 0 | 0 | 7 | 0 | 14 | 6 | 11 | 14 | 68 | 0 | 0 | 9 | 249 | 0 | 0 | 0 | 101 |
| 14 | 0 | 0 | 0 | 16 | 48 | 68 | 49 | 71 | 75 | 43 | 23 | 20 | 302 | 7 | 10 | 27 | 156 |
| 15 | 0 | 0 | 0 | 0 | 40 | 0 | 2 | 38 | 75 | 0 | 0 | 10 | 291 | 0 | 0 | 30 | 176 |
| 16 | 0 | 0 | 0 | 0 | 15 | 40 | 20 | 69 | 128 | 15 | 16 | 59 | 364 | 0 | 0 | 42 | 212 |
| 17 | 0 | 0 | 0 | 0 | 22 | 23 | 6 | 29 | 59 | 4 | 11 | 2 | 495 | 0 | 0 | 37 | 172 |
| 18 | 0 | 0 | 0 | 0 | 14 | 3 | 3 | 0 | 50 | 0 | 0 | 0 | 220 | 0 | 0 | 0 | 81 |
| 19 | 0 | 0 | 0 | 0 | 16 | 2 | 2 | 12 | 74 | 0 | 0 | 10 | 281 | 0 | 0 | 0 | 151 |
| 20 | 0 | 0 | 10 | 3 | 20 | 24 | 6 | 34 | 81 | 29 | 18 | 26 | 264 | 0 | 0 | 13 | 273 |
| 21 | 0 | 0 | 0 | 0 | 53 | 0 | 3 | 22 | 88 | 0 | 0 | 24 | 99 | 0 | 0 | 0 | 242 |
| 22 | 0 | 0 | 20 | 28 | 28 | 41 | 20 | 22 | 134 | 37 | 29 | 26 | 276 | 22 | 11 | 80 | 747 |
| 23 | 0 | 0 | 0 | 0 | 15 | 3 | 3 | 0 | 99 | 0 | 0 | 5 | 47 | 0 | 0 | 0 | 632 |
| 24 | 0 | 0 | 0 | 3 | 20 | 0 | 0 | 69 | 56 | 0 | 0 | 8 | 43 | 0 | 0 | 0 | 502 |
| 25 | 0 | 0 | 0 | 0 | 15 | 2 | 3 | 0 | 98 | 0 | 0 | 8 | 40 | 0 | 0 | 0 | 432 |

| Site | C21 | C22 | C23 | C24 | C25 | C26 | br C27 | C27 | C28 | C29 | C30 | Phytol | 20:1 |
|------|-----|-----|-----|-----|-----|-----|--------|-----|-----|-----|-----|--------|------|
| 1 | 0 | 17 | 0 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 145 | 18 |
| 2 | 0 | 24 | 0 | 25 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 30 | 34 |
| 3 | 7 | 83 | 0 | 125 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 26 | 12 |
| 4 | 0 | 39 | 0 | 104 | 5 | 0 | 0 | 0 | 7 | 0 | 0 | 14 | 8 |
| 5 | 8 | 134 | 9 | 213 | 6 | 102 | 5 | 0 | 0 | 0 | 0 | 37 | 10 |
| 6 | 8 | 119 | 6 | 21 | 6 | 8 | 3 | 0 | 12 | 0 | 0 | 35 | 9 |
| 7 | 7 | 100 | 0 | 16 | 6 | 27 | 0 | 0 | 17 | 0 | 0 | 4 | 0 |
| 8 | 3 | 92 | 0 | 58 | 6 | 160 | 0 | 21 | 30 | 0 | 0 | 13 | 8 |
| 9 | 18 | 85 | 16 | 94 | 3 | 220 | 5 | 0 | 0 | 0 | 0 | 7 | 17 |
| 10 | 7 | 78 | 0 | 17 | 0 | 56 | 0 | 9 | 3 | 0 | 0 | 50 | 41 |
| 11 | 0 | 115 | 13 | 47 | 6 | 44 | 0 | 0 | 8 | 0 | 7 | 14 | 11 |
| 12 | 10 | 109 | 12 | 264 | 20 | 53 | 0 | 0 | 7 | 0 | 0 | 77 | 38 |
| 13 | 8 | 144 | 14 | 37 | 5 | 60 | 0 | 0 | 6 | 0 | 0 | 2 | 3 |
| 14 | 22 | 257 | 15 | 185 | 27 | 325 | 6 | 6 | 13 | 11 | 4 | 37 | 23 |
| 15 | 14 | 204 | 8 | 336 | 15 | 276 | 0 | 10 | 1 | 0 | 0 | 6 | 2 |
| 16 | 13 | 408 | 12 | 318 | 25 | 284 | 0 | 11 | 9 | 0 | 16 | 15 | 40 |
| 17 | 8 | 279 | 0 | 116 | 1 | 188 | 0 | 0 | 0 | 0 | 0 | 23 | 10 |
| 18 | 0 | 235 | 0 | 50 | 0 | 64 | 0 | 5 | 40 | 8 | 0 | 9 | 0 |
| 19 | 23 | 246 | 18 | 186 | 11 | 76 | 0 | 16 | 67 | 16 | 7 | 18 | 14 |
| 20 | 36 | 502 | 27 | 186 | 16 | 129 | 0 | 0 | 18 | 2 | 0 | 21 | 34 |
| 21 | 21 | 559 | 15 | 190 | 0 | 35 | 0 | 0 | 29 | 0 | 14 | 9 | 22 |
| 22 | 37 | 618 | 20 | 203 | 21 | 174 | 0 | 0 | 52 | 5 | 0 | 18 | 26 |
| 23 | 14 | 636 | 0 | 61 | 12 | 108 | 0 | 6 | 73 | 0 | 6 | 19 | 21 |
| 24 | 22 | 701 | 17 | 358 | 3 | 122 | 0 | 5 | 80 | 7 | 9 | 25 | 18 |
| 25 | 9 | 677 | 18 | 349 | 143 | 144 | 0 | 14 | 111 | 13 | 10 | 6 | 0 |

Appendix 3: Concentration (ng g$^{-1}$ WW) of sterols from the Mawddach samples

| Site | st1 | cop | epi | st2 | st3 | chol | cholest | brass | st4 | st5 | ergo | st6 | camp | st7 | stig | st8 | st9 | st10 | st11 | sito | st12 | st13 | dino | st14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 64 | 17 | 7 | 233 | 27 | 1391 | 15 | 69 | 868 | 0 | 13 | 0 | 19 | 0 | 23 | 813 | 0 | 0 | 0 | 252 | 435 | 332 | 167 | 376 |
| 2 | 57 | 18 | 11 | 188 | 22 | 1274 | 8 | 130 | 471 | 0 | 15 | 0 | 11 | 0 | 23 | 530 | 0 | 0 | 0 | 255 | 382 | 166 | 149 | 306 |
| 3 | 94 | 298 | 101 | 121 | 60 | 1468 | 88 | 191 | 67 | 66 | 40 | 156 | 13 | 25 | 60 | 75 | 0 | 0 | 13 | 494 | 254 | 2174 | 88 | 43 |
| 4 | 67 | 125 | 48 | 84 | 0 | 1311 | 28 | 118 | 58 | 0 | 16 | 59 | 33 | 0 | 15 | 373 | 0 | 0 | 0 | 444 | 209 | 180 | 30 | 428 |
| 5 | 47 | 160 | 58 | 0 | 0 | 1193 | 37 | 103 | 349 | 0 | 23 | 0 | 19 | 0 | 16 | 583 | 0 | 7 | 0 | 116 | 664 | 178 | 47 | 610 |
| 6 | 0 | 77 | 35 | 0 | 0 | 1384 | 32 | 106 | 321 | 0 | 27 | 0 | 33 | 35 | 51 | 101 | 71 | 29 | 34 | 65 | 192 | 275 | 37 | 224 |
| 7 | 0 | 227 | 61 | 132 | 7 | 1556 | 141 | 103 | 454 | 7 | 18 | 113 | 52 | 0 | 57 | 636 | 0 | 67 | 0 | 386 | 638 | 301 | 226 | 98 |
| 8 | 0 | 383 | 25 | 88 | 51 | 1219 | 70 | 100 | 120 | 27 | 74 | 58 | 63 | 0 | 119 | 105 | 24 | 0 | 0 | 392 | 613 | 111 | 110 | 565 |
| 9 | 0 | 48 | 38 | 20 | 0 | 1153 | 27 | 38 | 89 | 22 | 38 | 32 | 29 | 0 | 27 | 118 | 0 | 36 | 16 | 92 | 267 | 43 | 38 | 23 |
| 10 | 0 | 51 | 37 | 0 | 0 | 1364 | 17 | 32 | 123 | 17 | 18 | 23 | 30 | 0 | 30 | 69 | 34 | 44 | 28 | 149 | 872 | 394 | 0 | 48 |
| 11 | 0 | 105 | 37 | 91 | 0 | 1172 | 71 | 89 | 184 | 16 | 36 | 36 | 97 | 0 | 74 | 112 | 57 | 24 | 0 | 360 | 1181 | 148 | 63 | 50 |
| 12 | 0 | 150 | 37 | 70 | 27 | 1301 | 67 | 156 | 127 | 27 | 96 | 71 | 140 | 30 | 124 | 259 | 28 | 23 | 0 | 326 | 5377 | 1936 | 0 | 162 |
| 13 | 41 | 97 | 56 | 0 | 29 | 1280 | 23 | 27 | 92 | 59 | 36 | 26 | 36 | 34 | 51 | 170 | 39 | 45 | 52 | 438 | 642 | 80 | 26 | 134 |
| 14 | 0 | 157 | 44 | 42 | 0 | 1272 | 73 | 26 | 92 | 35 | 32 | 21 | 33 | 49 | 124 | 0 | 31 | 38 | 42 | 206 | 847 | 260 | 26 | 36 |
| 15 | 0 | 34 | 25 | 26 | 26 | 1292 | 42 | 23 | 113 | 13 | 42 | 0 | 19 | 30 | 90 | 158 | 28 | 29 | 30 | 151 | 660 | 159 | 42 | 201 |
| 16 | 0 | 59 | 34 | 53 | 39 | 1430 | 27 | 30 | 159 | 37 | 23 | 26 | 59 | 0 | 116 | 187 | 64 | 35 | 0 | 188 | 708 | 292 | 44 | 0 |
| 17 | 31 | 46 | 22 | 18 | 0 | 1490 | 36 | 16 | 90 | 23 | 111 | 39 | 129 | 36 | 184 | 91 | 20 | 0 | 0 | 671 | 1479 | 256 | 10 | 63 |
| 18 | 0 | 18 | 13 | 24 | 36 | 1212 | 19 | 29 | 155 | 15 | 27 | 17 | 72 | 20 | 94 | 186 | 34 | 0 | 0 | 743 | 2413 | 568 | 21 | 26 |
| 19 | 0 | 63 | 38 | 46 | 46 | 1299 | 26 | 45 | 227 | 18 | 37 | 0 | 110 | 68 | 129 | 188 | 0 | 32 | 0 | 928 | 1035 | 159 | 58 | 39 |
| 20 | 0 | 21 | 10 | 10 | 18 | 1342 | 23 | 12 | 328 | 17 | 29 | 25 | 90 | 46 | 86 | 122 | 14 | 0 | 0 | 578 | 1095 | 72 | 18 | 43 |
| 21 | 0 | 44 | 27 | 41 | 0 | 1296 | 22 | 0 | 88 | 0 | 24 | 25 | 99 | 0 | 103 | 120 | 0 | 0 | 0 | 1047 | 824 | 71 | 0 | 38 |
| 22 | 40 | 77 | 53 | 61 | 83 | 1325 | 23 | 76 | 188 | 0 | 76 | 26 | 104 | 13 | 108 | 143 | 82 | 30 | 40 | 1029 | 827 | 74 | 0 | 22 |
| 23 | 0 | 48 | 23 | 25 | 27 | 1275 | 15 | 34 | 170 | 0 | 97 | 34 | 108 | 50 | 157 | 145 | 28 | 0 | 0 | 795 | 895 | 220 | 0 | 91 |
| 24 | 0 | 8 | 4 | 0 | 0 | 1376 | 15 | 0 | 176 | 0 | 90 | 41 | 108 | 14 | 152 | 112 | 15 | 0 | 0 | 1324 | 954 | 77 | 0 | 24 |
| 25 | 0 | 7 | 4 | 0 | 0 | 1277 | 7 | 13 | 99 | 0 | 93 | 46 | 129 | 21 | 159 | 108 | 15 | 0 | 0 | 1332 | 1467 | 120 | 0 | 80 |

Appendix 4: Concentration (ng g$^{-1}$ DW) of fatty acids from the Conwy core

| Depth(cm) | 12:0 | 13:0 | br-14 | 14:0 | iso-15 | ante-15 | 15:0 | br-16 | 16:0 | 16:1w11 | 16:1w9 | 16:1w7 | iso-17 | 16:1w5 | ante-17 | 17:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-2 | 16 | 183 | 1136 | 1882 | 1934 | 2752 | 563 | 962 | 9232 | 4622 | 194 | 3199 | 920 | 4513 | 364 | 174 |
| 2-4 | 343 | 74 | 521 | 1152 | 3070 | 2541 | 983 | 689 | 9008 | 272 | 370 | 4558 | 797 | 2284 | 463 | 195 |
| 4-6 | 210 | 76 | 635 | 1088 | 2050 | 1882 | 1204 | 862 | 7188 | 275 | 295 | 3561 | 892 | 2086 | 477 | 224 |
| 6-8 | 85 | 18 | 173 | 241 | 449 | 444 | 355 | 218 | 1606 | 50 | 50 | 931 | 220 | 452 | 90 | 48 |
| 8-10 | 226 | 39 | 430 | 452 | 1006 | 1069 | 433 | 338 | 3235 | 133 | 162 | 1578 | 354 | 1666 | 135 | 161 |
| 10-12 | 1131 | 193 | 1205 | 1846 | 3560 | 3042 | 1950 | 1176 | 12512 | 609 | 300 | 6221 | 1528 | 3124 | 562 | 312 |
| 12-14 | 839 | 133 | 890 | 1870 | 2467 | 2329 | 1910 | 1340 | 10299 | 628 | 436 | 5478 | 1549 | 2925 | 572 | 346 |
| 14-16 | 896 | 130 | 884 | 2127 | 2748 | 2684 | 1979 | 1284 | 10922 | 646 | 350 | 5585 | 1676 | 3259 | 551 | 324 |
| 16-18 | 375 | 79 | 439 | 1036 | 1188 | 1329 | 764 | 460 | 4946 | 352 | 145 | 2515 | 803 | 1308 | 260 | 146 |
| 18-20 | 1146 | 191 | 1009 | 2463 | 2623 | 2785 | 1684 | 974 | 10959 | 806 | 404 | 5094 | 1705 | 2619 | 384 | 251 |
| 20-22 | 2245 | 302 | 1891 | 4819 | 4513 | 4523 | 2343 | 2098 | 24549 | 2747 | 1217 | 12673 | 3468 | 8093 | 1200 | 775 |
| 22-24 | 336 | 63 | 305 | 888 | 845 | 853 | 546 | 481 | 5662 | 349 | 360 | 2955 | 706 | 1224 | 180 | 159 |
| 24-26 | 769 | 172 | 772 | 2743 | 2280 | 1997 | 1512 | 1267 | 13417 | 1241 | 469 | 6502 | 1742 | 3355 | 516 | 371 |
| 26-28 | 647 | 209 | 907 | 1735 | 2361 | 2118 | 1591 | 1131 | 12186 | 803 | 372 | 5269 | 1318 | 2382 | 468 | 214 |
| 28-30 | 873 | 233 | 984 | 2950 | 1644 | 2411 | 2763 | 1668 | 17369 | 1621 | 782 | 8123 | 1301 | 4224 | 696 | 396 |
| 30-32 | 686 | 186 | 675 | 2729 | 1681 | 1786 | 882 | 1305 | 16359 | 520 | 206 | 6761 | 1048 | 1702 | 633 | 242 |
| 32-34 | 496 | 139 | 415 | 2138 | 1043 | 1158 | 601 | 968 | 13132 | 399 | 143 | 5331 | 853 | 1108 | 518 | 114 |
| 34-36 | 738 | 218 | 775 | 3311 | 2645 | 2335 | 1324 | 1283 | 15573 | 281 | 224 | 6408 | 1422 | 938 | 633 | 109 |
| 36-38 | 171 | 34 | 148 | 447 | 453 | 414 | 291 | 214 | 3295 | 39 | 40 | 1315 | 448 | 695 | 136 | 62 |
| 38-40 | 161 | 20 | 130 | 332 | 353 | 379 | 191 | 218 | 2092 | 86 | 39 | 820 | 264 | 441 | 155 | 38 |
| 40-42 | 143 | 22 | 134 | 229 | 380 | 399 | 178 | 148 | 2023 | 106 | 48 | 738 | 268 | 422 | 80 | 36 |
| 42-44 | 163 | 25 | 307 | 431 | 590 | 610 | 356 | 288 | 3465 | 182 | 101 | 1049 | 381 | 634 | 99 | 50 |
| 44-46 | 280 | 49 | 371 | 535 | 1044 | 1165 | 501 | 385 | 6146 | 305 | 138 | 1663 | 550 | 950 | 206 | 96 |
| 46-48 | 140 | 30 | 119 | 596 | 566 | 695 | 370 | 414 | 4461 | 234 | 133 | 1215 | 343 | 558 | 158 | 57 |
| 48-50 | 33 | 5 | 60 | 129 | 278 | 300 | 185 | 59 | 1483 | 50 | 63 | 383 | 104 | 202 | 37 | 21 |

| Depth(cm) | 16:2 | 17:1 | 16:3 | 18:0 | 16:3 | 18:1w9 | 18:1w7 | 18:2w6 | 18:2 | 18:3w3 | 20:0 | 21:0 | 22:0 | 22:1w11 | 20:5w3 | 24:0 | 25:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-2 | 18 | 34 | 14 | 2459 | 109 | 65 | 713 | 13 | 279 | 1 | 8 | 30 | 325 | 191 | 1731 | 402 | 42 |
| 2-4 | 12 | 294 | 167 | 3088 | 152 | 895 | 2356 | 339 | 8 | 136 | 811 | 31 | 563 | 656 | 598 | 1205 | 88 |
| 4-6 | 10 | 298 | 111 | 2348 | 73 | 819 | 2356 | 355 | 11 | 175 | 836 | 22 | 535 | 611 | 544 | 622 | 62 |
| 6-8 | 1 | 8 | 6 | 485 | 17 | 163 | 452 | 88 | 0 | 45 | 186 | 6 | 136 | 132 | 137 | 225 | 17 |
| 8-10 | 4 | 92 | 63 | 1581 | 20 | 265 | 1594 | 364 | 52 | 87 | 270 | 12 | 373 | 156 | 214 | 535 | 21 |
| 10-12 | 28 | 368 | 230 | 2934 | 111 | 980 | 3319 | 555 | 129 | 389 | 1085 | 31 | 965 | 652 | 860 | 1199 | 156 |
| 12-14 | 22 | 480 | 111 | 3197 | 165 | 975 | 2940 | 344 | 21 | 248 | 779 | 22 | 518 | 480 | 972 | 573 | 86 |
| 14-16 | 16 | 405 | 138 | 3105 | 148 | 1007 | 3072 | 285 | 31 | 195 | 1028 | 30 | 719 | 651 | 954 | 971 | 131 |
| 16-18 | 9 | 136 | 75 | 1612 | 59 | 503 | 1331 | 111 | 12 | 67 | 568 | 20 | 439 | 235 | 332 | 759 | 45 |
| 18-20 | 15 | 268 | 162 | 3285 | 122 | 1065 | 2565 | 459 | 18 | 137 | 1281 | 41 | 691 | 414 | 672 | 673 | 87 |
| 20-22 | 27 | 778 | 276 | 11267 | 343 | 690 | 8544 | 1042 | 69 | 538 | 3636 | 54 | 1892 | 1064 | 1616 | 2337 | 247 |
| 22-24 | 8 | 35 | 21 | 2305 | 61 | 365 | 1487 | 159 | 5 | 26 | 962 | 28 | 463 | 176 | 284 | 978 | 90 |
| 24-26 | 17 | 287 | 153 | 6479 | 78 | 2172 | 3977 | 758 | 47 | 277 | 2275 | 68 | 1050 | 446 | 730 | 2183 | 196 |
| 26-28 | 20 | 181 | 111 | 4045 | 108 | 2561 | 2901 | 509 | 34 | 259 | 1060 | 43 | 730 | 452 | 739 | 1785 | 87 |
| 28-30 | 17 | 343 | 171 | 4567 | 160 | 3547 | 3051 | 801 | 37 | 738 | 2620 | 105 | 2112 | 714 | 1002 | 1700 | 177 |
| 30-32 | 5 | 127 | 40 | 3859 | 114 | 1286 | 2623 | 303 | 29 | 465 | 2749 | 103 | 2268 | 436 | 824 | 1612 | 212 |
| 32-34 | 9 | 109 | 49 | 3138 | 85 | 1045 | 2283 | 248 | 25 | 197 | 2239 | 79 | 1874 | 440 | 496 | 1334 | 176 |
| 34-36 | 16 | 75 | 32 | 5069 | 119 | 641 | 3551 | 245 | 24 | 230 | 3221 | 116 | 3180 | 548 | 575 | 2018 | 293 |
| 36-38 | 3 | 48 | 23 | 847 | 12 | 438 | 585 | 43 | 20 | 109 | 460 | 20 | 694 | 95 | 106 | 806 | 89 |
| 38-40 | 8 | 27 | 15 | 794 | 11 | 281 | 578 | 45 | 8 | 27 | 182 | 24 | 638 | 136 | 151 | 318 | 40 |
| 40-42 | 3 | 33 | 10 | 583 | 9 | 496 | 347 | 25 | 26 | 35 | 327 | 14 | 451 | 58 | 59 | 711 | 89 |
| 42-44 | 5 | 54 | 11 | 1086 | 10 | 936 | 590 | 32 | 34 | 49 | 481 | 19 | 503 | 91 | 90 | 719 | 93 |
| 44-46 | 6 | 76 | 23 | 1513 | 18 | 1103 | 809 | 71 | 40 | 30 | 752 | 51 | 1225 | 160 | 178 | 1596 | 194 |
| 46-48 | 4 | 94 | 8 | 815 | 7 | 802 | 315 | 43 | 23 | 27 | 352 | 26 | 483 | 125 | 198 | 765 | 90 |
| 48-50 | 1 | 19 | 3 | 369 | 1 | 300 | 155 | 22 | 7 | 10 | 205 | 17 | 275 | 21 | 23 | 396 | 45 |

## Appendix 5: Concentration (ng g$^{-1}$ DW) of fatty alcohols from the Conwy core

| Depth(cm) | C14 | i-C15 | a-C15 | C15 | C16 | i-C17 | a-C17 | C17 | C18 | i-C19 | C19 | C20 | C21 | C22 | C23 | C24 | C25 | C26 | C27 | C28 | C29 | Phytol | 20:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-2 | 46 | 60 | 39 | 75 | 431 | 15 | 21 | 45 | 188 | 17 | 23 | 364 | 45 | 485 | 21 | 139 | 6 | 69 | 0 | 0 | 0 | 185 | 128 |
| 2-4 | 15 | 16 | 12 | 19 | 98 | 14 | 12 | 10 | 83 | 1 | 4 | 90 | 17 | 126 | 13 | 164 | 6 | 34 | 0 | 7 | 0 | 110 | 16 |
| 4-6 | 12 | 12 | 8 | 12 | 68 | 7 | 26 | 8 | 53 | 3 | 1 | 57 | 11 | 79 | 5 | 117 | 0 | 38 | 0 | 6 | 0 | 112 | 14 |
| 6-8 | 36 | 17 | 18 | 19 | 82 | 14 | 20 | 12 | 47 | 14 | 3 | 120 | 11 | 90 | 14 | 192 | 0 | 33 | 0 | 6 | 0 | 118 | 18 |
| 8-10 | 17 | 9 | 7 | 9 | 102 | 20 | 28 | 8 | 91 | 13 | 2 | 101 | 14 | 67 | 8 | 158 | 0 | 37 | 0 | 9 | 0 | 111 | 8 |
| 10-12 | 18 | 9 | 6 | 9 | 92 | 6 | 9 | 7 | 63 | 5 | 2 | 86 | 12 | 91 | 8 | 162 | 1 | 78 | 0 | 11 | 0 | 91 | 7 |
| 12-14 | 30 | 11 | 12 | 11 | 103 | 9 | 21 | 11 | 99 | 10 | 3 | 99 | 18 | 117 | 13 | 258 | 0 | 92 | 0 | 13 | 0 | 144 | 5 |
| 14-16 | 29 | 11 | 15 | 13 | 113 | 16 | 24 | 14 | 110 | 11 | 0 | 110 | 22 | 123 | 13 | 312 | 0 | 93 | 0 | 13 | 0 | 183 | 4 |
| 16-18 | 24 | 8 | 11 | 8 | 87 | 10 | 34 | 9 | 78 | 6 | 0 | 76 | 15 | 95 | 9 | 220 | 0 | 81 | 0 | 12 | 0 | 75 | 6 |
| 18-20 | 22 | 7 | 5 | 6 | 77 | 6 | 14 | 7 | 62 | 10 | 0 | 58 | 11 | 64 | 7 | 150 | 0 | 55 | 0 | 0 | 0 | 75 | 8 |
| 20-22 | 32 | 11 | 13 | 11 | 95 | 18 | 23 | 13 | 129 | 12 | 8 | 168 | 22 | 138 | 10 | 203 | 3 | 112 | 0 | 31 | 0 | 112 | 13 |
| 22-24 | 31 | 12 | 8 | 14 | 74 | 0 | 0 | 16 | 117 | 4 | 0 | 154 | 24 | 129 | 14 | 342 | 0 | 83 | 0 | 0 | 0 | 47 | 9 |
| 24-26 | 32 | 15 | 7 | 15 | 98 | 0 | 0 | 20 | 161 | 0 | 0 | 224 | 36 | 173 | 13 | 306 | 0 | 114 | 0 | 0 | 0 | 65 | 9 |
| 26-28 | 25 | 12 | 8 | 13 | 81 | 0 | 0 | 17 | 139 | 0 | 0 | 247 | 30 | 141 | 11 | 364 | 0 | 97 | 0 | 18 | 0 | 56 | 11 |
| 28-30 | 26 | 12 | 2 | 12 | 74 | 0 | 0 | 16 | 119 | 0 | 0 | 240 | 27 | 161 | 14 | 327 | 0 | 82 | 0 | 21 | 0 | 67 | 5 |
| 30-32 | 10 | 6 | 3 | 7 | 64 | 0 | 0 | 4 | 67 | 0 | 7 | 153 | 18 | 79 | 7 | 206 | 7 | 76 | 5 | 10 | 0 | 13 | 2 |
| 32-34 | 8 | 5 | 0 | 3 | 45 | 0 | 0 | 5 | 52 | 0 | 3 | 246 | 15 | 68 | 5 | 187 | 8 | 62 | 8 | 18 | 3 | 19 | 2 |
| 34-36 | 14 | 6 | 0 | 5 | 62 | 0 | 0 | 6 | 72 | 0 | 4 | 167 | 21 | 88 | 7 | 223 | 12 | 80 | 9 | 19 | 4 | 23 | 4 |
| 36-38 | 15 | 4 | 0 | 5 | 67 | 0 | 0 | 5 | 75 | 0 | 5 | 214 | 21 | 100 | 8 | 330 | 12 | 83 | 13 | 16 | 6 | 23 | 1 |
| 38-40 | 12 | 2 | 0 | 4 | 56 | 0 | 0 | 4 | 189 | 0 | 7 | 224 | 21 | 100 | 11 | 497 | 10 | 83 | 8 | 14 | 1 | 22 | 2 |
| 40-42 | 11 | 2 | 0 | 2 | 43 | 0 | 0 | 7 | 179 | 0 | 6 | 245 | 22 | 97 | 10 | 505 | 9 | 83 | 9 | 13 | 3 | 9 | 3 |
| 42-44 | 14 | 2 | 0 | 2 | 49 | 0 | 0 | 5 | 265 | 0 | 5 | 407 | 21 | 130 | 8 | 505 | 12 | 101 | 6 | 19 | 2 | 11 | 1 |
| 44-46 | 16 | 2 | 0 | 3 | 68 | 0 | 0 | 6 | 325 | 0 | 10 | 559 | 29 | 178 | 15 | 620 | 16 | 135 | 12 | 22 | 1 | 27 | 3 |
| 46-48 | 18 | 2 | 0 | 3 | 48 | 0 | 0 | 5 | 308 | 0 | 7 | 607 | 25 | 159 | 18 | 712 | 18 | 119 | 9 | 18 | 3 | 8 | 1 |
| 48-50 | 11 | 5 | 0 | 1 | 59 | 0 | 0 | 5 | 345 | 0 | 8 | 626 | 42 | 210 | 22 | 939 | 21 | 152 | 9 | 24 | 2 | 11 | 1 |

i = *iso*
a = *anteiso*

Appendix 6: Concentration (ng g$^{-1}$ DW) of sterols from the Conwy core

| Depth(cm) | st1 | st2 | cop | epi | st3 | st4 | chol | cholest | brass | st5 | st6 | ergo | st7 | camp | st8 | stig |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-2 | 347 | 220 | 1512 | 469 | 1096 | 285 | 5078 | 920 | 245 | 865 | 515 | 605 | 1083 | 1220 | 210 | 562 |
| 2-4 | 117 | 51 | 627 | 144 | 91 | 66 | 1504 | 410 | 195 | 128 | 0 | 28 | 37 | 87 | 0 | 209 |
| 4-6 | 116 | 61 | 363 | 26 | 74 | 58 | 875 | 94 | 94 | 81 | 0 | 131 | 33 | 76 | 0 | 108 |
| 6-8 | 112 | 70 | 528 | 39 | 21 | 126 | 1163 | 107 | 151 | 33 | 0 | 129 | 0 | 32 | 0 | 167 |
| 8-10 | 82 | 32 | 288 | 34 | 19 | 23 | 871 | 67 | 142 | 34 | 0 | 104 | 0 | 125 | 0 | 100 |
| 10-12 | 92 | 31 | 347 | 31 | 21 | 21 | 927 | 52 | 0 | 92 | 149 | 92 | 17 | 33 | 16 | 87 |
| 12-14 | 102 | 32 | 303 | 37 | 0 | 0 | 1164 | 136 | 0 | 34 | 0 | 37 | 0 | 109 | 0 | 79 |
| 14-16 | 103 | 30 | 551 | 27 | 0 | 0 | 1268 | 170 | 0 | 96 | 0 | 42 | 0 | 157 | 0 | 181 |
| 16-18 | 97 | 35 | 284 | 29 | 0 | 0 | 940 | 122 | 0 | 83 | 0 | 50 | 0 | 90 | 0 | 106 |
| 18-20 | 128 | 64 | 400 | 47 | 0 | 38 | 1078 | 164 | 0 | 63 | 0 | 81 | 0 | 241 | 0 | 239 |
| 20-22 | 131 | 85 | 358 | 51 | 0 | 35 | 747 | 176 | 0 | 366 | 0 | 218 | 0 | 281 | 0 | 188 |
| 22-24 | 98 | 109 | 324 | 37 | 0 | 193 | 940 | 190 | 0 | 311 | 0 | 275 | 0 | 294 | 0 | 109 |
| 24-26 | 114 | 164 | 435 | 54 | 0 | 375 | 1363 | 338 | 0 | 437 | 0 | 337 | 0 | 482 | 0 | 230 |
| 26-28 | 132 | 111 | 376 | 56 | 0 | 328 | 1348 | 247 | 0 | 450 | 0 | 189 | 0 | 525 | 0 | 220 |
| 28-30 | 127 | 69 | 283 | 41 | 0 | 0 | 1259 | 233 | 0 | 351 | 0 | 222 | 0 | 366 | 0 | 292 |
| 30-32 | 55 | 40 | 85 | 27 | 0 | 68 | 451 | 88 | 0 | 426 | 69 | 139 | 990 | 275 | 34 | 130 |
| 32-34 | 38 | 18 | 43 | 17 | 0 | 41 | 357 | 66 | 0 | 193 | 53 | 158 | 943 | 239 | 39 | 127 |
| 34-36 | 36 | 31 | 81 | 24 | 0 | 39 | 439 | 95 | 0 | 167 | 53 | 315 | 1005 | 221 | 66 | 238 |
| 36-38 | 33 | 23 | 60 | 31 | 0 | 43 | 401 | 102 | 0 | 154 | 28 | 211 | 1077 | 262 | 60 | 305 |
| 38-40 | 14 | 70 | 26 | 22 | 0 | 12 | 323 | 99 | 0 | 836 | 95 | 340 | 431 | 447 | 47 | 383 |
| 40-42 | 19 | 91 | 25 | 20 | 0 | 17 | 312 | 68 | 0 | 943 | 27 | 362 | 623 | 436 | 41 | 398 |
| 42-44 | 15 | 17 | 23 | 19 | 0 | 21 | 290 | 90 | 0 | 243 | 30 | 430 | 675 | 667 | 27 | 456 |
| 44-46 | 30 | 17 | 36 | 27 | 0 | 15 | 402 | 124 | 0 | 393 | 34 | 798 | 1177 | 1002 | 29 | 607 |
| 46-48 | 20 | 14 | 28 | 14 | 0 | 23 | 377 | 121 | 0 | 236 | 33 | 674 | 1121 | 719 | 102 | 560 |
| 48-50 | 17 | 18 | 22 | 16 | 0 | 17 | 269 | 179 | 0 | 585 | 33 | 1024 | 301 | 1239 | 215 | 735 |

| Depth(cm) | st9 | st10 | st11 | sito | st12 | st13 |
|---|---|---|---|---|---|---|
| 0-2 | 315 | 159 | 1797 | 3149 | 8785 | 2279 |
| 2-4 | 0 | 0 | 288 | 834 | 5724 | 1248 |
| 4-6 | 0 | 0 | 257 | 595 | 5839 | 1354 |
| 6-8 | 0 | 0 | 181 | 362 | 6039 | 1738 |
| 8-10 | 0 | 0 | 86 | 328 | 6302 | 1335 |
| 10-12 | 35 | 16 | 156 | 581 | 7929 | 1392 |
| 12-14 | 0 | 0 | 100 | 246 | 5496 | 1959 |
| 14-16 | 0 | 0 | 113 | 248 | 5490 | 1340 |
| 16-18 | 0 | 0 | 120 | 130 | 2756 | 675 |
| 18-20 | 0 | 0 | 128 | 418 | 4570 | 992 |
| 20-22 | 0 | 0 | 124 | 577 | 2200 | 1505 |
| 22-24 | 0 | 0 | 71 | 431 | 2798 | 724 |
| 24-26 | 0 | 0 | 105 | 735 | 5365 | 891 |
| 26-28 | 0 | 0 | 111 | 419 | 4206 | 718 |
| 28-30 | 0 | 0 | 158 | 521 | 4076 | 786 |
| 30-32 | 47 | 226 | 401 | 338 | 1259 | 1279 |
| 32-34 | 20 | 67 | 123 | 503 | 1182 | 566 |
| 34-36 | 17 | 75 | 922 | 355 | 2070 | 1120 |
| 36-38 | 20 | 182 | 971 | 352 | 1540 | 1429 |
| 38-40 | 19 | 41 | 498 | 340 | 2571 | 951 |
| 40-42 | 12 | 72 | 639 | 469 | 3518 | 1382 |
| 42-44 | 18 | 111 | 1032 | 450 | 1169 | 1747 |
| 44-46 | 19 | 159 | 1430 | 743 | 2848 | 1476 |
| 46-48 | 15 | 93 | 1110 | 931 | 4264 | 7518 |
| 48-50 | 39 | 59 | 352 | 1849 | 4877 | 2540 |

# Appendix 7: Concentration (ng g$^{-1}$ DW) of fatty acids from the Clyde Sea sediment samples

| Site | br12:0 | 12:0 | iso-13:0 | ante-13:0 | 13:0 | br14:0 | 14:0 | iso-15:0 | ante-15:0 | 15:0 | i-16:0 | 16:0 | 16:1 | 16:1w9 | 16:1w7 | iso-17:0 | 16:1w5 | ante-17:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 58 | 1138 | 71 | 218 | 264 | 1188 | 9353 | 807 | 3974 | 1963 | 1374 | 18327 | 50 | 616 | 8711 | 1034 | 936 | 639 |
| S2 | 84 | 2358 | 170 | 438 | 486 | 2000 | 12181 | 1826 | 897 | 1900 | 2030 | 17354 | 149 | 806 | 8240 | 1393 | 1169 | 1038 |
| S3 | 67 | 836 | 343 | 243 | 304 | 1352 | 7872 | 2915 | 3593 | 1534 | 1418 | 19491 | 148 | 252 | 7861 | 1006 | 770 | 677 |
| S4 | 48 | 6444 | 36 | 200 | 1037 | 700 | 14745 | 2623 | 1729 | 4658 | 1048 | 22680 | 468 | 465 | 10266 | 827 | 1462 | 657 |
| S5 | 94 | 488 | 368 | 204 | 308 | 159 | 12165 | 5327 | 5168 | 2695 | 2240 | 47710 | 166 | 691 | 6140 | 1819 | 1270 | 912 |
| S6 | 58 | 516 | 494 | 238 | 369 | 2230 | 16668 | 9182 | 7535 | 4295 | 3541 | 41524 | 172 | 1311 | 9191 | 3104 | 1821 | 1525 |
| S7 | 225 | 2666 | 1007 | 611 | 742 | 3163 | 12758 | 7693 | 7899 | 4834 | 3125 | 22303 | 198 | 960 | 5887 | 2732 | 1884 | 1512 |
| S8 | 78 | 742 | 205 | 133 | 151 | 635 | 4451 | 1524 | 1733 | 837 | 587 | 14427 | 29 | 205 | 992 | 466 | 330 | 249 |
| S9 | 29 | 263 | 108 | 66 | 84 | 273 | 3042 | 765 | 951 | 824 | 341 | 17967 | 19 | 61 | 756 | 333 | 208 | 140 |
| S10 | 136 | 1314 | 165 | 73 | 327 | 1355 | 11341 | 2292 | 2724 | 2249 | 1641 | 22222 | 54 | 743 | 10536 | 1253 | 1134 | 774 |
| S11 | 338 | 4575 | 232 | 611 | 993 | 2748 | 13235 | 5147 | 6439 | 3625 | 1986 | 17350 | 106 | 334 | 8458 | 1540 | 1516 | 792 |
| S12 | 71 | 485 | 161 | 548 | 139 | 3439 | 4720 | 4450 | 5991 | 3461 | 836 | 20999 | 32 | 53 | 1874 | 2295 | 261 | 2674 |
| S13 | 145 | 132 | 1084 | 1056 | 65 | 8269 | 1653 | 10492 | 12491 | 628 | 2796 | 8659 | 147 | 140 | 1079 | 6153 | 1247 | 7143 |
| S14 | 37 | 37 | 7 | 63 | 90 | 340 | 1256 | 1086 | 1984 | 821 | 480 | 4187 | 0 | 23 | 281 | 621 | 573 | 348 |
| S15 | 615 | 6980 | 1386 | 989 | 1190 | 4437 | 12743 | 263 | 7916 | 1855 | 2844 | 5287 | 24 | 716 | 1389 | 1985 | 1627 | 1156 |
| S16 | 36 | 416 | 99 | 65 | 88 | 341 | 2559 | 866 | 1040 | 571 | 371 | 11122 | 19 | 89 | 706 | 264 | 175 | 156 |
| S17 | 100 | 1458 | 475 | 231 | 391 | 1268 | 9194 | 3750 | 3465 | 2058 | 1203 | 17645 | 91 | 406 | 3739 | 1056 | 664 | 620 |
| S18 | 119 | 1032 | 431 | 277 | 261 | 1283 | 8895 | 3114 | 3926 | 3022 | 1405 | 15418 | 99 | 166 | 3185 | 1087 | 726 | 804 |
| S19 | 229 | 2306 | 516 | 322 | 331 | 1486 | 7924 | 3148 | 4359 | 2543 | 1268 | 25469 | 18 | 148 | 4784 | 1063 | 1422 | 613 |
| S20 | 310 | 802 | 823 | 555 | 375 | 2225 | 5358 | 3925 | 5150 | 2415 | 1423 | 14347 | 95 | 269 | 2492 | 986 | 820 | 588 |
| S21 | 214 | 2275 | 699 | 358 | 576 | 1763 | 10634 | 4452 | 4458 | 3557 | 1698 | 20145 | 84 | 502 | 4174 | 1300 | 898 | 678 |
| S22 | 30 | 528 | 92 | 66 | 95 | 343 | 3212 | 1059 | 1684 | 140 | 454 | 4181 | 0 | 71 | 198 | 388 | 435 | 284 |
| S23 | 38 | 492 | 121 | 79 | 130 | 384 | 3386 | 978 | 1182 | 1141 | 447 | 11319 | 18 | 103 | 891 | 346 | 219 | 196 |
| S24 | 4 | 83 | 13 | 11 | 16 | 70 | 1306 | 585 | 1081 | 183 | 272 | 3232 | 0 | 57 | 172 | 365 | 532 | 230 |
| S25 | 24 | 452 | 151 | 118 | 212 | 749 | 6803 | 2062 | 2782 | 2145 | 940 | 17397 | 74 | 127 | 2832 | 676 | 534 | 426 |
| S26 | 44 | 836 | 385 | 175 | 325 | 1337 | 8263 | 3910 | 4115 | 2834 | 1617 | 13707 | 88 | 383 | 425 | 5320 | 1293 | 764 |
| S27 | 115 | 900 | 1082 | 332 | 805 | 703 | 3599 | 5624 | 5348 | 5722 | 2981 | 19257 | 234 | 498 | 766 | 2740 | 1549 | 1295 |
| S28 | 76 | 829 | 347 | 144 | 205 | 677 | 6162 | 2494 | 1812 | 1357 | 860 | 16772 | 95 | 255 | 2944 | 682 | 474 | 292 |
| S29 | 41 | 356 | 121 | 54 | 90 | 247 | 2663 | 714 | 627 | 550 | 252 | 5770 | 12 | 63 | 1330 | 176 | 196 | 87 |
| S30 | 168 | 2251 | 538 | 357 | 478 | 1806 | 6306 | 3119 | 3875 | 4142 | 1645 | 10693 | 97 | 869 | 578 | 1274 | 1025 | 704 |
| S31 | 34 | 622 | 108 | 68 | 127 | 408 | 4174 | 877 | 1039 | 1407 | 385 | 13921 | 30 | 71 | 626 | 385 | 237 | 166 |
| S32 | 66 | 731 | 56 | 219 | 107 | 1177 | 8734 | 507 | 782 | 659 | 1240 | 21140 | 22 | 1269 | 1680 | 578 | 516 | 681 |
| S33 | 23 | 497 | 131 | 110 | 132 | 829 | 5971 | 315 | 588 | 984 | 859 | 15504 | 0 | 56 | 890 | 453 | 395 | 396 |

| Site | 17:0 | 16:2 | 17:1 | 18:0 | 18:1w9 | 18:1w7 | 18:2w6 | 19:0 | 18:3w3 | 20:0 | 20:1 | 22:0 | 22:1w11 | 24:0 | 25:0 | 26:0 | 27:0 | 28:0 |
|------|------|------|------|------|--------|--------|--------|------|--------|------|------|------|---------|------|------|------|------|------|
| S1 | 831 | 2278 | 14 | 4677 | 1724 | 1832 | 652 | 187 | 174 | 1763 | 214 | 654 | 924 | 768 | 89 | 326 | 42 | 92 |
| S2 | 865 | 1101 | 273 | 4368 | 1627 | 1935 | 849 | 207 | 166 | 1255 | 99 | 1044 | 520 | 1250 | 180 | 629 | 47 | 145 |
| S3 | 998 | 104 | 170 | 5226 | 835 | 1210 | 883 | 328 | 81 | 1527 | 114 | 912 | 367 | 955 | 115 | 317 | 39 | 102 |
| S4 | 2001 | 95 | 524 | 13412 | 2749 | 4031 | 474 | 365 | 108 | 1902 | 170 | 1388 | 1058 | 1203 | 139 | 595 | 46 | 224 |
| S5 | 2044 | 2293 | 361 | 8040 | 1494 | 3180 | 762 | 495 | 145 | 2198 | 102 | 1800 | 1592 | 2566 | 291 | 1526 | 175 | 681 |
| S6 | 2607 | 20 | 638 | 12433 | 5046 | 6450 | 996 | 570 | 190 | 2802 | 235 | 1761 | 1808 | 2524 | 278 | 1506 | 162 | 729 |
| S7 | 2258 | 320 | 419 | 12514 | 5087 | 3512 | 1063 | 693 | 285 | 4626 | 184 | 2834 | 1349 | 2802 | 236 | 719 | 63 | 186 |
| S8 | 675 | 5 | 77 | 5130 | 686 | 508 | 183 | 280 | 238 | 2213 | 26 | 1720 | 124 | 1823 | 205 | 724 | 79 | 248 |
| S9 | 713 | 144 | 37 | 2815 | 498 | 174 | 82 | 137 | 24 | 3175 | 6 | 747 | 108 | 1073 | 127 | 555 | 71 | 297 |
| S10 | 1011 | 2762 | 17 | 5671 | 4585 | 2162 | 791 | 234 | 212 | 2112 | 259 | 790 | 1120 | 931 | 108 | 396 | 51 | 112 |
| S11 | 1169 | 31 | 384 | 4532 | 2984 | 2500 | 1119 | 208 | 380 | 1719 | 84 | 1830 | 68 | 2048 | 133 | 360 | 40 | 59 |
| S12 | 813 | 27 | 84 | 5153 | 869 | 2198 | 145 | 246 | 35 | 1775 | 5 | 3056 | 489 | 4437 | 638 | 3329 | 335 | 1331 |
| S13 | 5149 | 101 | 364 | 1984 | 1367 | 2534 | 450 | 1079 | 12 | 9617 | 121 | 3675 | 736 | 5149 | 408 | 962 | 71 | 465 |
| S14 | 492 | 0 | 100 | 1667 | 1427 | 1830 | 579 | 96 | 0 | 184 | 1 | 419 | 0 | 2 | 58 | 84 | 47 | 67 |
| S15 | 1802 | 292 | 369 | 10817 | 2143 | 1795 | 421 | 382 | 423 | 2634 | 70 | 1609 | 1066 | 9187 | 79 | 158 | 10 | 37 |
| S16 | 423 | 217 | 53 | 2632 | 263 | 311 | 121 | 119 | 52 | 807 | 21 | 673 | 97 | 736 | 89 | 244 | 46 | 54 |
| S17 | 971 | 527 | 225 | 4459 | 923 | 1761 | 520 | 273 | 155 | 1836 | 92 | 3455 | 878 | 4571 | 743 | 3298 | 285 | 1380 |
| S18 | 430 | 178 | 182 | 2173 | 1409 | 1440 | 790 | 300 | 239 | 1003 | 97 | 1134 | 713 | 1007 | 98 | 267 | 16 | 72 |
| S19 | 1070 | 0 | 19 | 2172 | 1799 | 1366 | 2069 | 198 | 982 | 1330 | 232 | 1155 | 445 | 956 | 61 | 297 | 264 | 192 |
| S20 | 812 | 545 | 135 | 2176 | 741 | 965 | 430 | 248 | 139 | 1190 | 71 | 1153 | 287 | 1136 | 89 | 305 | 17 | 53 |
| S21 | 2638 | 25 | 239 | 1938 | 1194 | 1966 | 509 | 309 | 112 | 2202 | 39 | 2871 | 917 | 4558 | 643 | 2115 | 187 | 534 |
| S22 | 390 | 0 | 7 | 3279 | 705 | 745 | 452 | 129 | 116 | 1100 | 341 | 413 | 425 | 393 | 30 | 92 | 8 | 0 |
| S23 | 872 | 136 | 63 | 7348 | 373 | 422 | 159 | 393 | 46 | 3119 | 24 | 2351 | 166 | 2006 | 222 | 734 | 66 | 250 |
| S24 | 317 | 12 | 74 | 2380 | 792 | 157 | 347 | 97 | 0 | 748 | 28 | 345 | 0 | 212 | 17 | 26 | 0 | 0 |
| S25 | 1182 | 7 | 154 | 6838 | 763 | 1018 | 406 | 399 | 182 | 3857 | 37 | 2379 | 696 | 715 | 144 | 465 | 39 | 97 |
| S26 | 1176 | 279 | 308 | 10557 | 2382 | 1998 | 1029 | 387 | 50 | 4812 | 361 | 1921 | 2427 | 1795 | 124 | 983 | 61 | 181 |
| S27 | 337 | 143 | 523 | 14683 | 3293 | 4072 | 938 | 843 | 253 | 5112 | 413 | 3190 | 1491 | 2942 | 279 | 1089 | 72 | 244 |
| S28 | 735 | 403 | 208 | 3292 | 5101 | 1282 | 297 | 134 | 87 | 1584 | 44 | 1222 | 473 | 1965 | 234 | 1023 | 79 | 219 |
| S29 | 195 | 203 | 45 | 1064 | 203 | 312 | 173 | 40 | 37 | 957 | 34 | 783 | 190 | 1337 | 164 | 827 | 77 | 364 |
| S30 | 1714 | 115 | 803 | 8215 | 1283 | 1483 | 651 | 597 | 202 | 1188 | 88 | 3273 | 884 | 3201 | 273 | 658 | 41 | 118 |
| S31 | 1200 | 182 | 40 | 12383 | 286 | 285 | 133 | 714 | 45 | 6786 | 15 | 2864 | 88 | 1869 | 112 | 280 | 27 | 31 |
| S32 | 1811 | 126 | 38 | 7449 | 1155 | 492 | 158 | 721 | 24 | 5048 | 203 | 3637 | 1827 | 5192 | 577 | 1760 | 189 | 318 |
| S33 | 1214 | 0 | 34 | 5366 | 857 | 452 | 339 | 292 | 12 | 2534 | 225 | 1888 | 596 | 1020 | 249 | 800 | 118 | 146 |

## Appendix 8: Concentration (ng g$^{-1}$ DW) of fatty alcohols from the Clyde Sea sediment samples

| Site | C11 | C12 | C13 | C14 | iso-C15 | ante-C15 | C15 | C16 | iso-C17 | ante-C17 | C17 | C18 | C19 | C20 | br-C21 |
|------|-----|-----|-----|-----|---------|----------|-----|------|---------|----------|-----|------|-----|------|--------|
| S1 | 9 | 16 | 21 | 313 | 33 | 7 | 83 | 947 | 10 | 11 | 75 | 612 | 33 | 476 | 2 |
| S2 | 12 | 27 | 17 | 302 | 57 | 15 | 66 | 1013 | 13 | 10 | 70 | 747 | 53 | 649 | 5 |
| S3 | 4 | 38 | 8 | 236 | 58 | 11 | 70 | 806 | 12 | 13 | 52 | 589 | 44 | 396 | 5 |
| S4 | 25 | 132 | 89 | 89 | 67 | 22 | 217 | 1962 | 24 | 22 | 86 | 1142 | 63 | 600 | 0 |
| S5 | 0 | 0 | 0 | 108 | 54 | 22 | 39 | 1619 | 0 | 0 | 25 | 337 | 0 | 431 | 0 |
| S6 | 0 | 0 | 0 | 113 | 47 | 25 | 39 | 1198 | 20 | 13 | 35 | 148 | 9 | 220 | 0 |
| S7 | 0 | 0 | 0 | 142 | 60 | 29 | 46 | 795 | 14 | 12 | 36 | 631 | 56 | 969 | 7 |
| S8 | 0 | 11 | 0 | 150 | 59 | 36 | 39 | 588 | 10 | 11 | 25 | 394 | 32 | 551 | 5 |
| S9 | 0 | 4 | 0 | 98 | 60 | 31 | 30 | 436 | 17 | 14 | 26 | 287 | 19 | 428 | 4 |
| S10 | 0 | 82 | 0 | 105 | 62 | 7 | 35 | 522 | 12 | 10 | 31 | 514 | 36 | 462 | 0 |
| S11 | 0 | 0 | 35 | 88 | 42 | 22 | 187 | 794 | 4 | 12 | 21 | 1071 | 72 | 765 | 9 |
| S12 | 0 | 0 | 49 | 267 | 218 | 32 | 203 | 1115 | 109 | 91 | 214 | 341 | 29 | 584 | 0 |
| S13 | 0 | 0 | 38 | 234 | 241 | 30 | 100 | 840 | 169 | 151 | 266 | 96 | 0 | 173 | 0 |
| S14 | 0 | 0 | 10 | 139 | 159 | 11 | 33 | 1048 | 11 | 11 | 43 | 293 | 16 | 194 | 0 |
| S15 | 0 | 0 | 17 | 50 | 95 | 12 | 98 | 440 | 17 | 23 | 48 | 614 | 39 | 336 | 9 |
| S16 | 0 | 0 | 0 | 25 | 73 | 30 | 68 | 281 | 9 | 19 | 31 | 445 | 0 | 190 | 5 |
| S17 | 0 | 0 | 0 | 73 | 97 | 33 | 71 | 831 | 17 | 24 | 60 | 799 | 72 | 1002 | 14 |
| S18 | 0 | 0 | 23 | 27 | 98 | 41 | 67 | 545 | 25 | 36 | 46 | 637 | 42 | 390 | 7 |
| S19 | 0 | 0 | 64 | 59 | 210 | 116 | 24 | 780 | 21 | 23 | 115 | 316 | 78 | 1507 | 16 |
| S20 | 0 | 0 | 0 | 31 | 38 | 17 | 24 | 354 | 6 | 7 | 16 | 243 | 15 | 187 | 0 |
| S21 | 0 | 0 | 76 | 40 | 168 | 180 | 23 | 704 | 97 | 100 | 59 | 344 | 13 | 447 | 18 |
| S22 | 0 | 0 | 0 | 16 | 5 | 43 | 15 | 135 | 5 | 8 | 15 | 83 | 13 | 203 | 0 |
| S23 | 0 | 0 | 0 | 58 | 14 | 0 | 9 | 238 | 0 | 0 | 9 | 235 | 18 | 342 | 0 |
| S24 | 0 | 0 | 0 | 15 | 29 | 34 | 6 | 147 | 8 | 8 | 5 | 77 | 12 | 352 | 0 |
| S25 | 0 | 0 | 0 | 50 | 30 | 90 | 40 | 313 | 0 | 0 | 14 | 125 | 11 | 183 | 0 |
| S26 | 0 | 0 | 0 | 72 | 26 | 119 | 30 | 661 | 24 | 27 | 35 | 223 | 34 | 190 | 0 |
| S27 | 0 | 0 | 10 | 43 | 131 | 64 | 72 | 204 | 22 | 25 | 35 | 1023 | 33 | 1092 | 0 |
| S28 | 0 | 0 | 0 | 18 | 0 | 0 | 13 | 328 | 15 | 10 | 21 | 217 | 14 | 245 | 0 |
| S29 | 0 | 0 | 0 | 54 | 62 | 22 | 26 | 628 | 0 | 14 | 24 | 506 | 22 | 625 | 0 |
| S30 | 0 | 0 | 0 | 48 | 39 | 8 | 17 | 457 | 8 | 10 | 8 | 320 | 9 | 165 | 0 |
| S31 | 0 | 0 | 0 | 18 | 27 | 10 | 25 | 588 | 13 | 17 | 17 | 204 | 25 | 304 | 0 |
| S32 | 0 | 0 | 0 | 30 | 31 | 43 | 30 | 128 | 9 | 17 | 44 | 196 | 18 | 711 | 0 |
| S33 | 0 | 0 | 0 | 17 | 5 | 24 | 12 | 161 | 8 | 5 | 10 | 120 | 11 | 343 | 0 |

| Site | C21 | C22 | C23 | C24 | 16:1 | 18:1 | Phytol | 20:1 | 22:1 | 24:1 |
|------|-----|-----|-----|-----|------|------|--------|------|------|------|
| S1 | 93 | 700 | 29 | 6 | 15 | 12 | 78 | 142 | 8 | 10 |
| S2 | 105 | 997 | 39 | 10 | 14 | 5 | 57 | 156 | 14 | 18 |
| S3 | 44 | 228 | 9 | 36 | 5 | 0 | 55 | 117 | 7 | 7 |
| S4 | 74 | 400 | 8 | 75 | 26 | 16 | 102 | 215 | 11 | 0 |
| S5 | 67 | 1192 | 78 | 250 | 0 | 0 | 95 | 27 | 0 | 16 |
| S6 | 39 | 622 | 39 | 202 | 0 | 0 | 8 | 17 | 0 | 0 |
| S7 | 139 | 1917 | 78 | 214 | 0 | 0 | 144 | 83 | 15 | 8 |
| S8 | 51 | 655 | 24 | 112 | 0 | 0 | 66 | 24 | 7 | 16 |
| S9 | 66 | 183 | 27 | 92 | 5 | 0 | 94 | 42 | 4 | 0 |
| S10 | 14 | 122 | 8 | 14 | 0 | 4 | 142 | 87 | 6 | 17 |
| S11 | 42 | 478 | 26 | 156 | 26 | 9 | 372 | 114 | 10 | 0 |
| S12 | 101 | 1500 | 80 | 298 | 0 | 0 | 147 | 51 | 0 | 0 |
| S13 | 33 | 1548 | 29 | 357 | 76 | 108 | 45 | 23 | 29 | 23 |
| S14 | 63 | 1827 | 31 | 449 | 0 | 0 | 26 | 55 | 0 | 0 |
| S15 | 25 | 522 | 20 | 180 | 0 | 10 | 149 | 69 | 5 | 5 |
| S16 | 18 | 570 | 26 | 169 | 0 | 0 | 306 | 49 | 0 | 0 |
| S17 | 172 | 1985 | 80 | 592 | 0 | 0 | 257 | 118 | 0 | 0 |
| S18 | 32 | 707 | 69 | 289 | 0 | 0 | 381 | 125 | 0 | 0 |
| S19 | 124 | 1005 | 21 | 264 | 0 | 0 | 180 | 48 | 0 | 0 |
| S20 | 39 | 391 | 24 | 140 | 0 | 0 | 87 | 8 | 0 | 0 |
| S21 | 92 | 1206 | 71 | 444 | 0 | 0 | 57 | 32 | 0 | 0 |
| S22 | 11 | 193 | 25 | 109 | 0 | 0 | 104 | 21 | 0 | 0 |
| S23 | 30 | 335 | 9 | 251 | 0 | 0 | 83 | 57 | 0 | 0 |
| S24 | 12 | 107 | 11 | 92 | 0 | 0 | 133 | 9 | 0 | 0 |
| S25 | 77 | 502 | 13 | 152 | 0 | 0 | 141 | 34 | 0 | 0 |
| S26 | 53 | 2216 | 84 | 329 | 0 | 0 | 1055 | 20 | 0 | 0 |
| S27 | 41 | 1808 | 91 | 326 | 0 | 0 | 345 | 21 | 0 | 0 |
| S28 | 36 | 437 | 25 | 163 | 0 | 0 | 123 | 44 | 0 | 0 |
| S29 | 76 | 1128 | 30 | 208 | 0 | 0 | 194 | 85 | 0 | 0 |
| S30 | 37 | 1069 | 27 | 141 | 0 | 0 | 563 | 13 | 0 | 0 |
| S31 | 73 | 305 | 22 | 195 | 0 | 0 | 173 | 11 | 0 | 0 |
| S32 | 93 | 1093 | 45 | 196 | 0 | 0 | 117 | 14 | 0 | 0 |
| S33 | 21 | 564 | 3 | 102 | 0 | 0 | 93 | 5 | 0 | 0 |

## Appendix 9: Concentration (ng g$^{-1}$ DW) of sterols from the Clyde Sea sediment samples

| Site | st1 | st2 | cop | epicop | st3 | st4 | chol | cholest | brass | st5 | st6 | camp |
|------|-----|-----|-----|--------|-----|-----|------|---------|-------|-----|-----|------|
| S1 | 2553 | 237 | 1903 | 436 | 5413 | 625 | 15861 | 1473 | 5737 | 1921 | 1739 | 2161 |
| S2 | 2972 | 455 | 3888 | 337 | 7305 | 943 | 24911 | 1729 | 6778 | 2667 | 3482 | 4813 |
| S3 | 1984 | 135 | 1821 | 274 | 5665 | 132 | 30471 | 149 | 10410 | 1391 | 683 | 356 |
| S4 | 613 | 226 | 4026 | 6242 | 2350 | 397 | 27476 | 3816 | 6066 | 1262 | 597 | 2193 |
| S5 | 964 | 273 | 1916 | 589 | 1447 | 363 | 13869 | 1076 | 2013 | 1022 | 780 | 1066 |
| S6 | 738 | 0 | 2600 | 1186 | 1816 | 756 | 27169 | 1879 | 2739 | 1412 | 1197 | 1318 |
| S7 | 2993 | 557 | 3124 | 1019 | 3850 | 1461 | 26550 | 1678 | 7224 | 3466 | 2742 | 3909 |
| S8 | 1184 | 180 | 1366 | 369 | 1246 | 471 | 9551 | 282 | 2872 | 1120 | 764 | 1656 |
| S9 | 1270 | 202 | 1028 | 440 | 2043 | 683 | 18235 | 1147 | 4497 | 1693 | 1728 | 2619 |
| S10 | 4773 | 587 | 2933 | 818 | 5186 | 1739 | 39687 | 755 | 10894 | 4458 | 4670 | 6005 |
| S11 | 1223 | 0 | 6859 | 731 | 1191 | 298 | 20388 | 612 | 2443 | 1680 | 2550 | 2934 |
| S12 | 1558 | 358 | 9833 | 1770 | 2885 | 877 | 29223 | 1312 | 2722 | 0 | 3387 | 5887 |
| S13 | 460 | 520 | 6594 | 6945 | 572 | 726 | 5929 | 3716 | 675 | 790 | 1218 | 328 |
| S14 | 498 | 0 | 3804 | 541 | 520 | 0 | 25600 | 0 | 674 | 374 | 600 | 874 |
| S15 | 577 | 0 | 2416 | 575 | 434 | 189 | 9388 | 376 | 550 | 506 | 552 | 914 |
| S16 | 230 | 0 | 1581 | 123 | 132 | 0 | 6759 | 0 | 410 | 201 | 224 | 458 |
| S17 | 2206 | 0 | 7485 | 848 | 1505 | 0 | 19479 | 2522 | 1972 | 783 | 1994 | 2522 |
| S18 | 537 | 0 | 5959 | 570 | 951 | 541 | 12878 | 1779 | 1105 | 1067 | 1349 | 1987 |
| S19 | 2234 | 346 | 7939 | 1965 | 2345 | 662 | 28195 | 2911 | 4155 | 2887 | 4329 | 6792 |
| S20 | 418 | 99 | 801 | 246 | 233 | 162 | 3337 | 167 | 726 | 337 | 149 | 426 |
| S21 | 605 | 0 | 5576 | 480 | 958 | 0 | 13908 | 2926 | 1341 | 951 | 1455 | 1436 |
| S22 | 790 | 735 | 1560 | 191 | 734 | 1316 | 5618 | 317 | 897 | 863 | 1192 | 180 |
| S23 | 454 | 0 | 1827 | 240 | 540 | 0 | 7042 | 234 | 1229 | 473 | 619 | 824 |
| S24 | 744 | 692 | 1469 | 180 | 691 | 1239 | 5288 | 298 | 844 | 813 | 1122 | 169 |
| S25 | 1703 | 0 | 4072 | 1402 | 1769 | 261 | 17429 | 36 | 2786 | 1513 | 2194 | 3066 |
| S26 | 1747 | 0 | 4116 | 1469 | 3397 | 94 | 34955 | 1527 | 4756 | 2433 | 2272 | 6139 |
| S27 | 1083 | 0 | 3544 | 4746 | 993 | 185 | 21974 | 1929 | 1300 | 3304 | 2043 | 11765 |
| S28 | 457 | 0 | 830 | 250 | 1025 | 147 | 14553 | 1139 | 1135 | 727 | 680 | 2999 |
| S29 | 565 | 0 | 798 | 418 | 2371 | 93 | 5786 | 2373 | 1642 | 985 | 1572 | 3523 |
| S30 | 2107 | 0 | 2943 | 1329 | 2505 | 67 | 17425 | 203 | 4168 | 356 | 462 | 4128 |
| S31 | 1188 | 0 | 1193 | 752 | 1088 | 170 | 8633 | 989 | 2405 | 1295 | 730 | 2645 |
| S32 | 1706 | 0 | 2024 | 622 | 2368 | 54 | 16751 | 607 | 3697 | 605 | 1657 | 1986 |
| S33 | 699 | 0 | 976 | 348 | 547 | 61 | 2735 | 226 | 1102 | 378 | 911 | 925 |

| Site | st7 | stig | st8 | st9 | sito | st10 | dino | C30tri |
|------|-----|------|-----|-----|------|------|------|--------|
| S1 | 575 | 569 | 184 | 252 | 360 | 8529 | 23649 | 0 |
| S2 | 727 | 502 | 405 | 307 | 408 | 13495 | 25014 | 147 |
| S3 | 122 | 182 | 171 | 0 | 228 | 5024 | 8368 | 0 |
| S4 | 0 | 646 | 409 | 0 | 887 | 9353 | 11268 | 0 |
| S5 | 420 | 328 | 294 | 274 | 395 | 2119 | 39940 | 0 |
| S6 | 0 | 381 | 376 | 0 | 1265 | 3837 | 20461 | 0 |
| S7 | 779 | 893 | 1228 | 271 | 1674 | 7676 | 34423 | 73 |
| S8 | 327 | 285 | 310 | 0 | 387 | 2518 | 6250 | 0 |
| S9 | 499 | 2243 | 388 | 170 | 4577 | 3568 | 11584 | 0 |
| S10 | 1265 | 1996 | 908 | 626 | 1803 | 10818 | 39746 | 0 |
| S11 | 283 | 1139 | 0 | 0 | 3361 | 10237 | 14447 | 0 |
| S12 | 402 | 926 | 1343 | 0 | 5648 | 7513 | 16253 | 0 |
| S13 | 510 | 498 | 997 | 863 | 3435 | 11372 | 2895 | 654 |
| S14 | 0 | 355 | 0 | 0 | 7338 | 2683 | 1949 | 292 |
| S15 | 219 | 301 | 0 | 0 | 1307 | 12502 | 4593 | 302 |
| S16 | 0 | 221 | 0 | 0 | 3637 | 5040 | 2592 | 462 |
| S17 | 0 | 1071 | 0 | 0 | 16904 | 32618 | 26947 | 425 |
| S18 | 488 | 526 | 290 | 0 | 10052 | 27830 | 17734 | 232 |
| S19 | 254 | 670 | 148 | 0 | 12082 | 58724 | 6109 | 9125 |
| S20 | 0 | 244 | 0 | 0 | 5324 | 6537 | 3349 | 99 |
| S21 | 0 | 779 | 312 | 0 | 13726 | 3982 | 7644 | 0 |
| S22 | 0 | 383 | 0 | 0 | 3395 | 45322 | 2208 | 2217 |
| S23 | 0 | 813 | 0 | 0 | 9168 | 2270 | 2988 | 307 |
| S24 | 0 | 360 | 0 | 0 | 3196 | 42661 | 2078 | 2087 |
| S25 | 662 | 3506 | 324 | 206 | 8151 | 5660 | 6014 | 683 |
| S26 | 0 | 5619 | 757 | 0 | 11405 | 10045 | 7934 | 1003 |
| S27 | 1406 | 3797 | 1111 | 344 | 9258 | 12515 | 7308 | 624 |
| S28 | 187 | 2110 | 193 | 0 | 5646 | 1166 | 2905 | 126 |
| S29 | 259 | 2044 | 575 | 0 | 6985 | 8683 | 3286 | 1467 |
| S30 | 830 | 1878 | 504 | 383 | 8810 | 7672 | 8091 | 1058 |
| S31 | 532 | 2350 | 217 | 171 | 8378 | 8370 | 4624 | 144 |
| S32 | 578 | 2133 | 468 | 134 | 6473 | 4807 | 11218 | 0 |
| S33 | 197 | 1170 | 1098 | 0 | 3864 | 9876 | 2045 | 360 |

Appendix 10: Concentration (ng g$^{-1}$ DW) of fatty acids from the Loch Riddon core

| Depth(cm) | br12:0 | 12:0 | iso-13:0 | ante-13:0 | 13:0 | br14:0 | 14:0 | 14:1 | iso-15:0 | ante-15:0 | 15:0 | i-16:0 | 16:0 | 16:1w9 | 16:1w7 | iso-17:0 | 16:1w5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-5 | 4 | 83 | 13 | 11 | 16 | 70 | 1306 | 20 | 585 | 1081 | 550 | 272 | 3966 | 57 | 1639 | 365 | 532 |
| 5-8 | 52 | 726 | 154 | 103 | 142 | 491 | 1712 | 96 | 918 | 1141 | 740 | 497 | 3412 | 93 | 1205 | 847 | 578 |
| 8-11 | 83 | 770 | 235 | 151 | 204 | 620 | 1474 | 124 | 962 | 1261 | 904 | 722 | 3604 | 121 | 1029 | 483 | 449 |
| 11-14 | 78 | 719 | 219 | 142 | 189 | 583 | 1384 | 128 | 927 | 1143 | 830 | 647 | 3499 | 107 | 1175 | 624 | 660 |
| 14-17 | 87 | 795 | 268 | 184 | 222 | 711 | 1546 | 91 | 700 | 1362 | 1015 | 1033 | 4057 | 109 | 1060 | 702 | 465 |
| 17-20 | 72 | 693 | 240 | 163 | 189 | 602 | 1324 | 63 | 923 | 1165 | 906 | 913 | 3682 | 132 | 893 | 624 | 379 |
| 20-23 | 43 | 503 | 213 | 157 | 165 | 449 | 1052 | 76 | 656 | 810 | 627 | 557 | 2611 | 98 | 764 | 360 | 226 |
| 23-26 | 35 | 461 | 211 | 158 | 171 | 473 | 1195 | 73 | 714 | 876 | 696 | 635 | 2800 | 108 | 804 | 385 | 243 |
| 26-29 | 66 | 629 | 227 | 170 | 192 | 607 | 1311 | 154 | 842 | 1060 | 864 | 804 | 3216 | 119 | 788 | 444 | 292 |
| 29-32 | 67 | 515 | 233 | 184 | 190 | 588 | 1131 | 120 | 653 | 1139 | 948 | 930 | 2383 | 89 | 629 | 500 | 275 |
| 32-35 | 56 | 770 | 221 | 192 | 195 | 716 | 1821 | 95 | 931 | 1139 | 927 | 986 | 2661 | 105 | 646 | 572 | 494 |
| 35-40 | 68 | 627 | 235 | 170 | 191 | 647 | 1508 | 160 | 884 | 1105 | 871 | 845 | 3388 | 96 | 617 | 446 | 361 |
| 40-45 | 44 | 559 | 209 | 167 | 207 | 510 | 1323 | 101 | 717 | 882 | 661 | 587 | 2821 | 101 | 746 | 348 | 269 |
| 45-50 | 70 | 662 | 194 | 141 | 153 | 560 | 1314 | 104 | 748 | 908 | 698 | 657 | 2859 | 68 | 698 | 367 | 249 |
| 50-55 | 86 | 628 | 189 | 185 | 195 | 607 | 1428 | 98 | 757 | 932 | 759 | 748 | 2195 | 76 | 649 | 367 | 301 |
| 55-60 | 73 | 860 | 246 | 210 | 270 | 804 | 1598 | 135 | 938 | 1060 | 898 | 928 | 3534 | 116 | 740 | 449 | 493 |
| 60-65 | 47 | 524 | 280 | 186 | 186 | 656 | 1659 | 134 | 941 | 1029 | 830 | 986 | 3660 | 99 | 889 | 480 | 448 |
| 65-70 | 70 | 706 | 213 | 157 | 183 | 639 | 1394 | 105 | 840 | 930 | 814 | 799 | 3223 | 98 | 733 | 425 | 350 |
| 70-75 | 102 | 731 | 215 | 160 | 177 | 590 | 1173 | 91 | 730 | 829 | 669 | 600 | 2800 | 70 | 712 | 332 | 262 |
| 75-80 | 30 | 471 | 272 | 176 | 211 | 372 | 1532 | 53 | 544 | 1005 | 788 | 742 | 2248 | 86 | 761 | 207 | 324 |
| 80-85 | 98 | 856 | 285 | 222 | 281 | 797 | 1779 | 52 | 1004 | 961 | 870 | 1082 | 3921 | 80 | 764 | 603 | 418 |
| 85-90 | 106 | 815 | 251 | 188 | 226 | 669 | 1400 | 39 | 522 | 782 | 821 | 829 | 3226 | 54 | 823 | 447 | 297 |
| 90-95 | 96 | 802 | 212 | 161 | 200 | 645 | 1358 | 35 | 763 | 919 | 782 | 722 | 3301 | 79 | 602 | 460 | 294 |
| 95-100 | 36 | 664 | 172 | 131 | 221 | 631 | 1428 | 96 | 870 | 949 | 906 | 803 | 3579 | 97 | 687 | 364 | 313 |
| 100-105 | 17 | 394 | 112 | 86 | 122 | 552 | 1365 | 26 | 806 | 905 | 799 | 679 | 3239 | 44 | 678 | 416 | 279 |
| 105-110 | 22 | 741 | 186 | 132 | 172 | 584 | 1413 | 23 | 797 | 824 | 805 | 617 | 3278 | 40 | 742 | 362 | 253 |
| 110-115 | 25 | 765 | 205 | 147 | 191 | 645 | 1279 | 39 | 817 | 923 | 886 | 734 | 3251 | 62 | 805 | 387 | 303 |
| 115-120 | 25 | 322 | 92 | 156 | 185 | 428 | 1287 | 22 | 936 | 662 | 825 | 766 | 2877 | 50 | 739 | 295 | 315 |
| 120-125 | 27 | 422 | 106 | 81 | 100 | 442 | 1145 | 67 | 606 | 667 | 733 | 433 | 2641 | 55 | 733 | 211 | 156 |
| 125-130 | 48 | 643 | 175 | 127 | 162 | 624 | 1429 | 30 | 788 | 938 | 785 | 67 | 3322 | 38 | 508 | 371 | 209 |
| 130-135 | 36 | 586 | 169 | 137 | 193 | 707 | 1437 | 67 | 948 | 637 | 841 | 743 | 2830 | 50 | 609 | 279 | 392 |
| 135-140 | 54 | 696 | 187 | 148 | 175 | 701 | 1403 | 78 | 880 | 1061 | 933 | 783 | 2921 | 58 | 337 | 405 | 238 |
| 140-145 | 64 | 742 | 185 | 151 | 171 | 580 | 1566 | 62 | 742 | 822 | 747 | 646 | 2067 | 47 | 290 | 311 | 201 |
| 145-150 | 70 | 643 | 166 | 129 | 160 | 556 | 1322 | 92 | 655 | 725 | 635 | 519 | 2841 | 39 | 377 | 261 | 207 |

| Depth(cm) | ante-17:0 | 17:0 | 16:2 | 17:1 | 18:0 | 18:1w9 | 18:1w7 | 18:2w6 | 19:0 | 20:0 | 20:1 | 21:0 | 22:0 | 24:0 | 25:0 | 26:0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-5 | 230 | 317 | 12 | 74 | 2380 | 792 | 11 | 347 | 97 | 748 | 28 | 3 | 345 | 212 | 17 | 26 |
| 5-8 | 241 | 300 | 6 | 92 | 2193 | 592 | 635 | 315 | 69 | 1081 | 14 | 3 | 411 | 169 | 11 | 36 |
| 8-11 | 371 | 440 | 5 | 130 | 2537 | 816 | 758 | 467 | 182 | 1436 | 50 | 5 | 491 | 363 | 34 | 125 |
| 11-14 | 330 | 392 | 1 | 121 | 2457 | 706 | 598 | 349 | 179 | 1492 | 35 | 6 | 709 | 614 | 61 | 136 |
| 14-17 | 546 | 637 | 1 | 164 | 2490 | 1325 | 885 | 665 | 324 | 2818 | 63 | 13 | 1226 | 1019 | 112 | 294 |
| 17-20 | 485 | 593 | 0 | 142 | 2214 | 1235 | 710 | 513 | 224 | 2684 | 67 | 13 | 1153 | 767 | 84 | 297 |
| 20-23 | 267 | 358 | 1 | 91 | 2380 | 805 | 708 | 290 | 139 | 1827 | 33 | 8 | 819 | 601 | 64 | 259 |
| 23-26 | 319 | 413 | 1 | 96 | 2835 | 940 | 368 | 335 | 181 | 2202 | 43 | 11 | 989 | 694 | 81 | 259 |
| 26-29 | 374 | 495 | 2 | 108 | 2858 | 781 | 429 | 390 | 210 | 2431 | 44 | 11 | 904 | 700 | 83 | 212 |
| 29-32 | 483 | 534 | 17 | 121 | 2627 | 775 | 491 | 542 | 281 | 2828 | 50 | 12 | 915 | 622 | 69 | 77 |
| 32-35 | 437 | 562 | 2 | 141 | 2223 | 832 | 461 | 545 | 230 | 2637 | 51 | 12 | 815 | 424 | 42 | 92 |
| 35-40 | 379 | 504 | 1 | 113 | 2312 | 801 | 443 | 346 | 250 | 2768 | 45 | 13 | 1080 | 834 | 105 | 350 |
| 40-45 | 284 | 402 | 1 | 77 | 3534 | 1091 | 353 | 305 | 280 | 3335 | 59 | 14 | 1223 | 906 | 135 | 397 |
| 45-50 | 293 | 380 | 2 | 77 | 2167 | 745 | 313 | 278 | 180 | 2302 | 31 | 11 | 878 | 732 | 74 | 231 |
| 50-55 | 319 | 410 | 3 | 104 | 1878 | 581 | 407 | 315 | 202 | 2442 | 35 | 16 | 969 | 721 | 72 | 170 |
| 55-60 | 382 | 512 | 4 | 108 | 2275 | 873 | 464 | 397 | 288 | 3672 | 82 | 37 | 1634 | 1452 | 94 | 198 |
| 60-65 | 478 | 506 | 18 | 30 | 1840 | 300 | 465 | 492 | 257 | 2631 | 52 | 17 | 1770 | 559 | 145 | 180 |
| 65-70 | 333 | 507 | 16 | 102 | 3424 | 860 | 372 | 289 | 314 | 2707 | 49 | 16 | 1111 | 845 | 143 | 330 |
| 70-75 | 244 | 358 | 1 | 70 | 1996 | 363 | 318 | 221 | 258 | 3079 | 34 | 25 | 1406 | 868 | 119 | 354 |
| 75-80 | 352 | 273 | 2 | 53 | 2114 | 557 | 328 | 196 | 261 | 3036 | 19 | 18 | 1524 | 1051 | 145 | 448 |
| 80-85 | 507 | 733 | 7 | 153 | 2583 | 954 | 466 | 290 | 452 | 3818 | 129 | 24 | 1296 | 1065 | 150 | 399 |
| 85-90 | 358 | 556 | 1 | 115 | 3498 | 924 | 297 | 335 | 415 | 3785 | 56 | 22 | 1267 | 877 | 140 | 460 |
| 90-95 | 320 | 551 | 2 | 101 | 2438 | 829 | 245 | 302 | 531 | 4436 | 52 | 44 | 1947 | 1496 | 225 | 616 |
| 95-100 | 345 | 584 | 5 | 119 | 3704 | 794 | 354 | 344 | 275 | 2543 | 43 | 32 | 1900 | 1578 | 178 | 650 |
| 100-105 | 294 | 543 | 1 | 109 | 3817 | 841 | 240 | 343 | 426 | 3706 | 52 | 3 | 1421 | 1734 | 191 | 471 |
| 105-110 | 247 | 502 | 1 | 91 | 2492 | 645 | 271 | 216 | 329 | 3069 | 57 | 15 | 1102 | 611 | 187 | 747 |
| 110-115 | 306 | 620 | 2 | 111 | 2673 | 656 | 361 | 295 | 450 | 3680 | 43 | 20 | 1249 | 786 | 193 | 791 |
| 115-120 | 199 | 686 | 0 | 91 | 2569 | 759 | 294 | 320 | 428 | 3734 | 70 | 10 | 1615 | 1314 | 210 | 605 |
| 120-125 | 180 | 322 | 1 | 58 | 2844 | 354 | 308 | 183 | 204 | 2425 | 23 | 12 | 1011 | 747 | 186 | 593 |
| 125-130 | 286 | 481 | 0 | 100 | 3455 | 705 | 265 | 223 | 300 | 3145 | 40 | 19 | 1261 | 1100 | 163 | 595 |
| 130-135 | 290 | 710 | 1 | 93 | 2365 | 869 | 248 | 209 | 499 | 4015 | 61 | 11 | 1545 | 1217 | 246 | 726 |
| 135-140 | 316 | 574 | 1 | 120 | 2723 | 597 | 245 | 253 | 321 | 3488 | 36 | 23 | 1403 | 1284 | 213 | 734 |
| 140-145 | 195 | 400 | 2 | 67 | 2364 | 551 | 200 | 201 | 317 | 2898 | 43 | 17 | 1125 | 957 | 143 | 386 |
| 145-150 | 201 | 344 | 1 | 33 | 2669 | 533 | 165 | 156 | 185 | 2405 | 323 | 15 | 1109 | 940 | 145 | 398 |

# Appendix 11: Concentration (ng g$^{-1}$ DW) of fatty alcohols from the Loch Riddon core

| Depth(cm) | C12 | C14 | iso-C15 | ante-C15 | C15 | C16 | iso-C17 | ante-C17 | C17 | C18 | C19 | C20 | C21 | C22 | C23 | C24 | Phy | 20:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-5 | 7 | 303 | 73 | 34 | 63 | 1372 | 21 | 21 | 36 | 755 | 25 | 352 | 12 | 107 | 0 | 5 | 133 | 184 |
| 5-8 | 0 | 195 | 38 | 13 | 33 | 932 | 4 | 6 | 17 | 492 | 12 | 225 | 5 | 55 | 0 | 0 | 60 | 95 |
| 8-11 | 6 | 239 | 43 | 14 | 40 | 1179 | 10 | 10 | 26 | 834 | 18 | 198 | 6 | 51 | 0 | 0 | 77 | 81 |
| 11-14 | 5 | 177 | 35 | 10 | 29 | 874 | 4 | 4 | 18 | 603 | 21 | 163 | 33 | 253 | 0 | 0 | 65 | 89 |
| 14-17 | 0 | 173 | 30 | 8 | 25 | 817 | 3 | 4 | 15 | 657 | 28 | 100 | 35 | 410 | 0 | 8 | 57 | 77 |
| 17-20 | 0 | 104 | 18 | 6 | 21 | 571 | 0 | 0 | 11 | 542 | 28 | 101 | 26 | 246 | 0 | 11 | 47 | 63 |
| 20-23 | 0 | 126 | 16 | 47 | 18 | 410 | 0 | 0 | 8 | 337 | 13 | 90 | 30 | 308 | 0 | 10 | 22 | 35 |
| 23-26 | 0 | 133 | 17 | 3 | 20 | 583 | 0 | 0 | 9 | 475 | 23 | 61 | 57 | 501 | 0 | 8 | 22 | 36 |
| 26-29 | 0 | 116 | 19 | 4 | 19 | 550 | 0 | 0 | 9 | 384 | 15 | 378 | 32 | 300 | 0 | 10 | 12 | 34 |
| 29-32 | 0 | 145 | 22 | 5 | 23 | 647 | 0 | 0 | 12 | 465 | 23 | 561 | 55 | 554 | 0 | 10 | 19 | 33 |
| 32-35 | 0 | 109 | 16 | 4 | 21 | 525 | 0 | 0 | 7 | 303 | 11 | 268 | 16 | 131 | 0 | 5 | 9 | 19 |
| 35-40 | 0 | 154 | 20 | 3 | 23 | 692 | 0 | 0 | 13 | 430 | 17 | 449 | 40 | 354 | 0 | 8 | 10 | 22 |
| 40-45 | 0 | 227 | 29 | 2 | 30 | 870 | 0 | 0 | 13 | 402 | 25 | 495 | 37 | 233 | 0 | 7 | 16 | 18 |
| 45-50 | 0 | 169 | 21 | 1 | 24 | 647 | 0 | 0 | 11 | 386 | 19 | 375 | 32 | 208 | 0 | 11 | 11 | 15 |
| 50-55 | 0 | 113 | 18 | 1 | 20 | 545 | 0 | 0 | 9 | 284 | 13 | 200 | 12 | 166 | 0 | 0 | 15 | 14 |
| 55-60 | 0 | 108 | 16 | 4 | 17 | 508 | 0 | 0 | 9 | 305 | 16 | 225 | 20 | 196 | 0 | 3 | 9 | 14 |
| 60-65 | 0 | 98 | 17 | 1 | 19 | 462 | 0 | 0 | 8 | 295 | 12 | 234 | 25 | 153 | 0 | 7 | 5 | 7 |
| 65-70 | 0 | 168 | 25 | 1 | 29 | 669 | 0 | 0 | 12 | 324 | 15 | 296 | 25 | 157 | 0 | 4 | 6 | 12 |
| 70-75 | 0 | 132 | 24 | 1 | 27 | 694 | 0 | 0 | 12 | 303 | 15 | 204 | 16 | 106 | 0 | 5 | 6 | 12 |
| 75-80 | 0 | 140 | 27 | 1 | 33 | 882 | 0 | 0 | 14 | 384 | 21 | 212 | 23 | 185 | 4 | 11 | 6 | 14 |
| 80-85 | 0 | 150 | 33 | 1 | 37 | 842 | 0 | 0 | 14 | 347 | 19 | 260 | 26 | 166 | 5 | 13 | 6 | 16 |
| 85-90 | 0 | 155 | 44 | 1 | 44 | 1246 | 0 | 0 | 15 | 447 | 22 | 293 | 25 | 162 | 4 | 8 | 10 | 12 |
| 90-95 | 0 | 115 | 44 | 1 | 39 | 1181 | 0 | 0 | 12 | 380 | 32 | 270 | 42 | 244 | 7 | 9 | 9 | 15 |
| 95-100 | 0 | 116 | 36 | 1 | 38 | 800 | 0 | 0 | 13 | 259 | 12 | 168 | 27 | 257 | 0 | 9 | 6 | 18 |
| 100-105 | 0 | 59 | 25 | 1 | 27 | 616 | 0 | 0 | 12 | 278 | 16 | 195 | 15 | 251 | 0 | 11 | 4 | 11 |
| 105-110 | 0 | 69 | 31 | 1 | 29 | 597 | 0 | 0 | 11 | 280 | 15 | 198 | 19 | 215 | 0 | 10 | 5 | 10 |
| 110-115 | 0 | 76 | 29 | 1 | 29 | 614 | 0 | 0 | 12 | 244 | 12 | 242 | 21 | 264 | 0 | 16 | 3 | 8 |
| 115-120 | 0 | 80 | 33 | 1 | 29 | 737 | 0 | 0 | 12 | 263 | 22 | 532 | 21 | 387 | 4 | 25 | 3 | 16 |
| 120-125 | 0 | 57 | 32 | 1 | 31 | 655 | 0 | 0 | 12 | 238 | 25 | 495 | 37 | 364 | 8 | 27 | 4 | 14 |
| 125-130 | 0 | 73 | 23 | 1 | 22 | 677 | 0 | 0 | 12 | 229 | 24 | 444 | 49 | 374 | 9 | 49 | 3 | 11 |
| 130-135 | 0 | 57 | 31 | 1 | 18 | 659 | 0 | 0 | 12 | 235 | 22 | 521 | 53 | 412 | 4 | 22 | 2 | 16 |
| 135-140 | 0 | 76 | 34 | 1 | 19 | 636 | 0 | 0 | 13 | 319 | 34 | 588 | 58 | 479 | 10 | 28 | 2 | 7 |
| 140-145 | 0 | 56 | 26 | 1 | 19 | 516 | 0 | 0 | 10 | 219 | 26 | 612 | 40 | 381 | 11 | 26 | 1 | 16 |
| 145-150 | 0 | 62 | 30 | 1 | 17 | 570 | 0 | 0 | 11 | 233 | 32 | 509 | 39 | 312 | 11 | 31 | 2 | 20 |

## Appendix 12: Concentration (ng g$^{-1}$ DW) of sterols from the Loch Riddon core

| Depth(cm) | St1 | Cop | Epicop | St2 | St3 | Chol | Cholest | Brass | St4 | St5 | Camp | St6 | Stig | Sito | St7 | Dino | C30trien |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-5 | 416 | 833 | 384 | 548 | 93 | 4586 | 242 | 934 | 395 | 316 | 832 | 0 | 206 | 2822 | 2557 | 1811 | 99 |
| 5-8 | 436 | 843 | 265 | 582 | 0 | 5667 | 308 | 1038 | 415 | 283 | 886 | 0 | 224 | 2945 | 1709 | 2517 | 660 |
| 8-11 | 318 | 698 | 244 | 342 | 0 | 5535 | 293 | 941 | 498 | 362 | 858 | 131 | 204 | 2544 | 4137 | 2405 | 297 |
| 11-14 | 269 | 830 | 193 | 418 | 0 | 4945 | 227 | 806 | 394 | 415 | 782 | 0 | 168 | 2052 | 4523 | 2592 | 438 |
| 14-17 | 269 | 686 | 170 | 260 | 0 | 4034 | 184 | 642 | 236 | 392 | 704 | 0 | 160 | 2589 | 4176 | 2624 | 188 |
| 17-20 | 236 | 613 | 175 | 302 | 0 | 4938 | 182 | 593 | 225 | 329 | 663 | 97 | 130 | 2380 | 5640 | 3392 | 212 |
| 20-23 | 116 | 337 | 87 | 102 | 0 | 2614 | 0 | 308 | 88 | 98 | 606 | 0 | 0 | 2602 | 3331 | 1738 | 105 |
| 23-26 | 135 | 326 | 0 | 135 | 0 | 2875 | 0 | 388 | 125 | 217 | 645 | 0 | 143 | 2015 | 3788 | 1675 | 522 |
| 26-29 | 122 | 276 | 0 | 86 | 0 | 1888 | 0 | 243 | 0 | 113 | 533 | 0 | 0 | 3191 | 2366 | 1079 | 151 |
| 29-32 | 148 | 228 | 0 | 120 | 0 | 2208 | 0 | 328 | 77 | 159 | 534 | 0 | 0 | 4122 | 3523 | 1144 | 115 |
| 32-35 | 0 | 74 | 0 | 0 | 0 | 2273 | 0 | 244 | 0 | 0 | 289 | 0 | 0 | 2945 | 2220 | 714 | 450 |
| 35-40 | 124 | 108 | 0 | 119 | 0 | 2213 | 0 | 307 | 101 | 157 | 346 | 165 | 174 | 3634 | 3663 | 1018 | 406 |
| 40-45 | 0 | 0 | 0 | 0 | 0 | 1524 | 0 | 114 | 0 | 0 | 430 | 0 | 172 | 3624 | 5977 | 1158 | 89 |
| 45-50 | 0 | 0 | 0 | 0 | 0 | 832 | 0 | 103 | 0 | 0 | 308 | 0 | 100 | 2620 | 3890 | 951 | 138 |
| 50-55 | 0 | 0 | 0 | 0 | 0 | 570 | 0 | 0 | 0 | 0 | 242 | 0 | 0 | 2379 | 2310 | 695 | 109 |
| 55-60 | 0 | 0 | 0 | 0 | 0 | 592 | 0 | 0 | 0 | 0 | 227 | 0 | 0 | 2436 | 2489 | 695 | 108 |
| 60-65 | 0 | 0 | 0 | 0 | 0 | 621 | 0 | 110 | 0 | 0 | 395 | 0 | 0 | 2538 | 1974 | 1056 | 95 |
| 65-70 | 0 | 0 | 0 | 0 | 0 | 708 | 0 | 118 | 0 | 0 | 413 | 0 | 187 | 3081 | 3601 | 839 | 138 |
| 70-75 | 0 | 0 | 0 | 0 | 0 | 565 | 0 | 96 | 0 | 0 | 334 | 0 | 133 | 2465 | 2651 | 727 | 468 |
| 75-80 | 0 | 0 | 0 | 0 | 0 | 615 | 0 | 99 | 0 | 0 | 239 | 0 | 136 | 2770 | 2677 | 696 | 318 |
| 80-85 | 0 | 0 | 0 | 0 | 0 | 617 | 0 | 127 | 0 | 0 | 344 | 0 | 157 | 2797 | 2037 | 499 | 1014 |
| 85-90 | 0 | 0 | 0 | 0 | 0 | 699 | 0 | 157 | 0 | 0 | 424 | 0 | 152 | 3111 | 2294 | 596 | 366 |
| 90-95 | 0 | 0 | 0 | 0 | 0 | 701 | 0 | 146 | 0 | 0 | 402 | 0 | 188 | 3464 | 2526 | 516 | 557 |
| 95-100 | 0 | 0 | 0 | 0 | 0 | 529 | 0 | 95 | 0 | 0 | 450 | 0 | 108 | 3565 | 1345 | 556 | 1030 |
| 100-105 | 0 | 0 | 0 | 0 | 0 | 504 | 0 | 89 | 0 | 0 | 418 | 0 | 113 | 3738 | 1192 | 342 | 353 |
| 105-110 | 0 | 0 | 0 | 0 | 0 | 451 | 0 | 92 | 0 | 0 | 437 | 0 | 0 | 3621 | 1228 | 350 | 355 |
| 110-115 | 0 | 0 | 0 | 0 | 0 | 460 | 0 | 0 | 0 | 0 | 413 | 0 | 84 | 2972 | 1126 | 275 | 308 |
| 115-120 | 0 | 0 | 0 | 0 | 0 | 995 | 0 | 239 | 0 | 0 | 606 | 0 | 246 | 4647 | 2785 | 614 | 543 |
| 120-125 | 0 | 0 | 0 | 0 | 0 | 1013 | 0 | 261 | 0 | 0 | 501 | 0 | 169 | 3545 | 2111 | 586 | 2766 |
| 125-130 | 0 | 0 | 0 | 0 | 0 | 1003 | 0 | 205 | 0 | 0 | 445 | 0 | 145 | 4106 | 1875 | 570 | 1588 |
| 130-135 | 0 | 0 | 0 | 0 | 0 | 1001 | 0 | 240 | 0 | 0 | 479 | 0 | 146 | 3941 | 1834 | 624 | 4253 |
| 135-140 | 0 | 0 | 0 | 0 | 0 | 1083 | 0 | 270 | 0 | 0 | 538 | 0 | 182 | 5382 | 3555 | 1022 | 451 |
| 140-145 | 0 | 0 | 0 | 0 | 0 | 800 | 0 | 125 | 0 | 0 | 404 | 0 | 211 | 4689 | 3606 | 909 | 820 |
| 145-150 | 0 | 0 | 0 | 0 | 0 | 696 | 0 | 268 | 0 | 0 | 658 | 0 | 135 | 5266 | 2652 | 848 | 610 |